EFFECT OF SELECTED CHEMICAL CONTAMINANTS ON GROWTH, BIOCHEMICAL COMPOSITION, DNA DAMAGE AND SUPEROXIDE DISMUTASE ACTIVITY IN FOUR MARINE ALGAE

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THESIS SUBMITTED IN FULFILMENT OF
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ABSTRACT

One of the important tasks of environmental monitoring is the detection of potentially hazardous compounds such as chemical contaminants that can induce oxidative damage in aquatic organisms. Our objective is to investigate the effect of selected chemical contaminants (metals, textile dyes and organophosphate pesticides) on growth (chlorophyll a, carotenoid) , biochemical composition (carbohydrate, protein and lipid), DNA damage ([Random Amplified Polymorphic DNA (RAPD) and AP-site (Abasic-site)] and Superoxide dismutase (SOD) enzyme activity in four tropical marine algae, *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa* after short term (four days) and long term (ten days) exposure under laboratory conditions. Results will be used to assess the suitability of the different end-points (biomarker) and algal species for use in development of bioassays. The algae were exposed to different concentrations of twelve chemical contaminants including seven metals [Cadmium (Cd); Cobalt (Co); Chromium (Cr); Copper (Cu); Iron (Fe); Manganese (Mn) and Zinc (Zn)], three textile dyes [Supranol Br. Red 3Bur (Acidic dye); Astrazon Red FBL (Basic dye) and Lanaset Red 2GA (Metal complex dye)] and two organophosphate pesticides [Malathion and Dichlovos] in a range of concentrations (0.01, 0.1, 1, 10, 100 and 500 mg/L) in Provasoli medium without EDTA using conical flask (for microalgae: *Chlorella vulgaris* and *Tetraselmis tetrahele*) and multiwell plates (for macroalgae: *Boergesenia forbesii* and *Ventricaria ventricosa*) with test volumes of 500mL and 10mL respectively, for 4 and 10 days under controlled conditions. The growth rate and IC_{50} values (concentration of toxicants estimated to inhibit 50% algal growth relative to control) were determined on day 4 and day 10 based on Chl *a* content. The biochemical composition (carbohydrate, protein and lipid) and SOD activity were

also assessed. RAPD and AP-site content analysis was conducted on DNA extracted from the treated samples. The results showed that, the carbohydrate, protein, lipid and AP-site content decreased with increasing concentration of chemical contaminants. The SOD activity in the treated algae increased with increasing chemical contaminants concentration until the threshold, beyond which the cell lost their resistance and died. There are changes occurring in RAPD profiles (variation in band intensity as well as gain or loss of bands) in all treated samples compared with control. The results showed that, of the end-points, Genomic template stability was the most sensitive for *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa. Chlorella vulgaris,* appears to be the most useful bioassay organism because it was the most sensitive to toxicants. Finding from this study will contribute towards the development of bioassays for the detection and monitoring of metal, textile dye and organophosphate pesticide contamination based on DNA damage detection, growth, biochemical composition and stress enzyme response in tropical marine microalgae and macroalgae.

ABSTRAK

Salah satu tugas penting dalam pemonitoran alam sekitar ialah untuk mengesan bahan-bahan berpotensi berbahaya seperti bahan pencemar kimia yang boleh mengakibatkan kerosakan oksidatif pada organisma akuatik. Tujuan kami ialah untuk menyiasat kesan bahan pencemar kimia (logam, bahan pewarna pencelup kain dan bahan ubat pembunuh serangga organofosfat) ke atas pertumbuhan (klorofil *a*, karotenoid), komposisi biokimia (karbohidrat, protein dan lemak), kerosakan DNA [Amplifikasi Polimorfik DNA Secara Rawak (RAPD) dan Tapak-AP (Tapak Abasik)] dan aktiviti enzim Superoxide dismutase (SOD) di dalam empat alga marine tropika, *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa* selepas didedahkan untuk jangka pendek (empat hari) dan jangka panjang (sepuluh hari) di dalam makmal. Keputusan akan digunakan untuk menilai kesesuaian titik-akhir (petunjuk biologi) berbeza, dan spesis alga untuk digunakan dalam pembangunan ujian biologi. Alga didedahkan kepada dua belas jenis bahan pencemaran kimia termasuk tujuh logam [Cadmium (Cd); Cobalt (Co); Chromium (Cr); Kuprum (Cu); Ferum (Fe); Manganese (Mn) dan Zink (Zn)], tiga bahan pewarna pencelup kain [Supranol Br. Red 3Bur (Acidic dye); Astrazon Red FBL (Basic dye) dan Lanaset Red 2GA (Metal complex dye)] dan dua bahan ubat pembunuh serangga organofosfat [Malathion dan Dichlovos] dalam julat kepekatan berbeza (0.01, 0.1, 1, 10, 100 and 500 mg/L) di dalam medium Provasoli tanpa EDTA menggunakan kelalang konikal (untuk mikroalga: *Chlorella vulgaris* dan *Tetraselmis tetrahele*) dan piring pelbagai lubang (untuk makroalga: *Boergesenia forbesii* dan *Ventricaria ventricosa*) dengan isipadu ujian, 500mL and 10mL setiap satu, selama empat dan sepuluh hari di dalam keadaan terkawal. Kadar pertumbuhan, nilai IC_{50} (kepekatan bahan toksik dianggarkan merencat 50%

pertumbuhan alga dibandingkan dengan kontrol) telah ditentukan pada hari keempat dan kesepuluh berdasarkan kandungan klorofil *a*. Komposisi biokimia (karbohidrat, protein dan lemak) dan aktiviti enzim SOD juga dinilai. Analisis RAPD dan kandungan Tapak-AP dijalankan ke atas DNA yang telah diekstrak daripada sampel yang telah dirawat. Keputusan menunjukkan bahawa kandungan karbohidrat, protein, lemak dan tapak-AP menurun mengikut peningkatan kepekatan bahan pencemar kimia. Aktiviti SOD di dalam alga yang telah dirawat meningkat dengan peningkatan kepekatan bahan pencemar kimia sehingga peringkat ambang, melepasi keadaan dimana, sel kehilangan pertahanan dan mati. Terdapat perubahan ditunjukkan pada profil RAPD (variasi di dalam intensiti band, dan juga perolehan atau kehilangan band) di dalam sampel yang telah dirawat dibandingkan dengan sampel kontrol. Keputusan menunjukkan, diantara titik-akhir (petunjuk-bio), Kestabilan Templat Genomik adalah paling sensitif untuk *Chlorella vulgaris*, *Tetraselmis tetrahele*, *Boergesenia forbesii* dan *Ventricaria ventricosa*. *Chlorella vulgaris*, muncul sebagai organisma ujian biologi paling berguna kerana ia paling sensitif kepada bahan toksik. Penemuan daripada kajian ini akan menyumbangkan kearah pembangunan ujian biologi untuk mengesan dan memonitor pencemaran logam, bahan pewarna pencelup kain dan bahan ubat pembunuh serangga organofosfat berdasarkan pengesanan kerosakan DNA, pertumbuhan, komposisi biokimia dan tindakan balik enzim yang tertekan, didalam mikroalga dan makroalga tropika marin.

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDICES

LIST OF SYMBOLS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometer
Acidic dye	Supranol Br. Red 3Bur
AP-site	Abasic-site
AP-PCR	Arbitrary Primed PCR
ARP	Aldehyde Reactive Probe
Basic dye	Astrazon Red FBL
BER	Base Excision Repair
CCAP	Culture Collection of Algae and Protozoa
CAR	Carotenoid
Cd	Cadmium
Chl a; CHL	Chlorophyll a
CHO	Carbohydrate
Ca	Calcium
Cl	Chloride
Co	Cobalt
COD	Chemical Oxygen Demand
CO_2	Carbon dioxide
Cr	Chromium
Cu	Copper
DAF	DNA Amplification Fingerprinting
DHA	Docosahexaenoic Acid
DO	Dissolved Oxygen
DW	Dry weight
EQS	Environmental Quality Standard
Fe	Iron
HDPE	High Density Polyethlene
H_2O_2	hydrogen peroxide
$HO^{\bullet}$	hydroxyl radicals
K	Potassium
LIP	Lipid
MUACC	Murdoch University Algal Culture Collection
Metal complex dye	Lanaset Red 2GA
Mn	Manganese
NAPFPRE	National Prawn Fry Production and Research Centre
Na	Sodium
NH_4-N	Ammoniacal-nitrogen
(NO_3-N)	Nitrate
OD_{620nm}	Optical Density at 620 nm
O_2^-	superoxide anion
$^1O_2$	singlet molecular oxygen
PCR	polymerase chain reaction
PO_4	Phosphate
PRO	Protein
PUFAs	Polyunsaturated Fatty Acids
RAPD	Random Amplified Polymorphic DNA
ROS	oxygen species
SCGE	Single-cell gel electrophoresis
SCES	sister chromatid exchanges

SCP	Single Cell Protein
SiO_2	Silica
SO_4	Sulphate
SOD	Superoxide dismutase
UMACC	University of Malaya Algae Culture Collection
Zn	Zinc

1.0 INTRODUCTION

One of the important tasks of environmental monitoring is the detection of potentially hazardous compounds in water. The detection and monitoring of toxic contaminants in the aquatic environment becomes expensive and complex when contaminants exist in low undetectable levels. The toxic contaminants which originate from land-based uncontrolled industrial discharge and also sea-based sources such as heavy metals, textile dye and pesticides are present in the aquatic system as the consequence of human population growth and industrial development. The production, consumption and disposal of anthropogenic chemicals and wastes continue to increase. In Malaysia, based on the Malaysian Environmental Quality Report (DOE, 2010) surface waters such as rivers, lakes and seas, receive large quantities of pollutants from sewage treatment plants (49.27%), manufacturing industries (44.57%), animal farms (3.70%) and agro-based industries (2.46%). The report also showed that 40.68% of the scheduled wastes generated in 2010 were from chemical industry (765,208.44 metric-tones/year), 8.88% metal/engineering industry (166,938.06 metric-tones/year), 0.06% textile industry (1197.97 metric-tones/year) and 1.71% shipping industry (32,248.85 metric-tones/year wastes).

A large amount of these toxic contaminants are potentially carcinogenic and mutagenic substances. Exposure to these substances has potentially short and long-term consequences for the survival of organisms at the individual and population level, offering an exciting area of research. Aquatic organisms such as phytoplankton, aquatic plants, shrimp, fish, crustaceans and mussel are linked directly or indirectly to the human food chain. This is an important reason why we should be concerned about their

exposure to environmental mutagens and carcinogens, particularly as many of these organisms have the capacity to (i) transform these agents to biologically active metabolites and (ii) accumulate toxicants in their cells and tissues at concentrations several orders of magnitude above that found in the environment (David, *et al*., 2010).

Algae are particularly good indicator organisms to be used as the tool for the detection and monitoring of toxic contaminants since they are typically the most abundant life forms in aquatic environments and occupy the base of the food chain. Algae have been widely used as bioindicators for pollution due to toxicants such as metals, pesticides, textile dye and xenobiotics (Ismail, *et al*., 2002; Phang and Murugadas, 2004; Hong, *et al*., 2010; Lim, *et al.*, 2010; Chen, *et al*., 2012; Piotrowska-Niczyporuk, *et al*., 2012). Most of the current studies, focus on the effects of the toxic contaminants on growth or physiological response in algae (Nam and An, 2010; Carrera-Martínez, *et al*., 2010; Lim, *et al*., 2010; Chen, *et al*., 2012; Mellado, *et al*., 2012; Piotrowska-Niczyporuk, *et al*., 2012). However recently there has been increased interest in investigating the effects of such pollutants at enzymatic and molecular levels. This includes the effects on biochemical composition, DNA damage, gene expresion and activities of stress enzymes.

Genotoxic pollutants like ionizing radiations (X-rays, gamma rays); nonionizing radiation (Ultraviolet light), chemicals (metals, pesticides, oleochemicals, organotins) and endogenous agent are described as the DNA damaging agents. A large proportion of these environmental agents are potentially genotoxic and carcinogenic in nature. Many contaminants present in marine waters not only endanger survival and physiology of the organisms (Giesy and Hook, 1989; Handy, 1994; Hagger, *et al*., 2005; Zhou *et al*., 2011)

but also induce genetic alterations (Fabacher, *et al*., 1991; West, 1989) which may lead to mutations (De Flora, *et al*., 1991; De Flora, *et al*., 2000; Maccubin, *et al.*, 1991) and/or carcinogenesis (Folmar, *et al.*, 1993). The effects of the mutations can either be silent throughout many generations or have significant impact on the gene pool of a population.

The genotoxicity of pollutants is directly related to their effects on the structure and function of DNA molecules. Various compounds in polluted water were reported to be capable of interacting with the DNA of living cells and therefore cause genotoxic effects (Bolognesi, *et al*., 2004; Buschini, *et al.*, 2004; Klobucar, *et al.*, 2003; Ding, *et al.*, 1999). The impact of genotoxic materials on the integrity and functioning of cellular DNA has been investigated in many marine organisms (Atienzar and Jha, 2004; Rong and Yin, 2004; Castano and Becerril, 2004; Zhou, *et al*., 2011). Several biomarkers have been utilised as tools in the detection of genotoxin endpoints. DNA damage may be assessed by various techniques including RAPD analysis, Single-cell gel (SCG) electrophoresis or Comet assay, alkaline elution assay, chromosomal aberrations, sister chromatid exchanges (SCES), microsome assay, micronucleus assay, etc.

The random amplified polymorphic DNA (RAPD) technique is a useful assay for the detection of genotoxin-induced DNA damage and mutations. The RAPD is based on the polymerase chain reaction (PCR). It employs a short random primer and yields a number of PCR products of varying lengths. These DNA segments are separated by gel electrophoresis to generate a DNA-fingerprint (Welsh, *et al*., 1990; Williams, *et al*., 1990). In the detection of genotoxicity, the amplification products generated by control (unexposed) and exposed samples are compared. An advantage of the RAPD assay is

that, no prior knowledge of the genome under investigation is required. The assay requires very little source material and the analysis can also be performed non-destructively which can be useful for the screening of rare or valuable samples. These technologies are relatively cheap and do not require the use of special and expensive equipment (Atienzar, *et al*., 2000a).

AP-site counting or abasic-site (apurinic/apyrimidinic site) assay is one of the assays used to detect DNA damage in genotoxicity studies. The cellular processes that can lead to DNA damage are oxygen consumption that results in the formation of reactive oxygen species (ROS) (eg: superoxide, hydroxyl free radicals and hydrogen peroxide). The hydroxyl radical which is converted from superoxide and hydrogen peroxide by the Fenton reaction can produce a multiplicity of modifications in DNA. Oxidative attack by hydroxyl radical on the deoxyribose moiety will lead to the release of free bases from DNA, generating strand breaks with various sugar modifications and simple abasic-sites. It has been estimated that endogeneous ROS can result in about $2x10^5$ base lesions per cell per day (Lindahl, 2000). The abasic-site is the most common lesion in DNA and was suggested to be an important intermediate in mutagenesis and carcinogenesis (Wang, *et al.*, 2009; Zhao, *et al.*, 2007; Chakravarti, *et al.*, 2005; Kamiya, *et al*., 1992). In this assay, Aldehyde Reactive Probe (ARP) reacts specifically with an aldehyde group which is the open ring form of the abasic-sites. This reaction makes it possible to detect DNA modifications that result in the formation of an aldehyde group. After treating DNA containing abasic-sites with ARP reagent, abasic-sites are tagged with a biotin residue. By using an excess amount of ARP, all abasic-sites can be converted to biotin-tagged abasic-sites. Therefore, abasic-sites can be quantified using avidin-biotin assay followed by a colorimetric detection of peroxidase or alkaline

phosphatase conjugated to the avidin (Dojindo, 2009). In the detection of genotoxin-induced DNA damage, the quantity of the abasic-site per 1 x 10^5 bp generated by control (unexposed) and exposed samples are compared.

The exposure of algae to environmental stress like genotoxic agents (heavy metals, UV light, pesticide, oleochemicals, etc) can increase the cellular levels of reactive oxygen species (ROS), such as the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), singlet molecular oxygen ($^1O_2$) and hydroxyl radicals ($HO^\bullet$), that disturb the steady state balance of prooxidants and antioxidants in the cells (Bowler, *et al*., 1992). Against this oxidative stress, plants have developed enzymatic defenses such as superoxide dismutase, peroxidases and catalase to limit lipid peroxidation, DNA damage and protein degradation, (Geoffroy, *et al*., 2002) where these enzymes like superoxide dismutase (SOD) catalyze the disproportionation of $^2O_2^-$ to O_2 and H_2O (Wolfe-Simon, *et al.,* 2005). Superoxide dismutase (SOD) is one of the most important enzymes in the front line of defense against oxidative stress. In marine environments, oxidative stress in algae is often caused by high light intensity and increased pH of the seawater as a result of algal photosynthesis (Falkowski and Raven, 1997). It is an imbalance between light uptake and carbon fixation in the algal cells that results in the production of oxygen radicals around photosystem I and increases the activity of superoxide dismutase (Collen, 1994; Mtolera, 1995; Asada, 1999; Falkowski and Raven, 1997). Therefore, anti-oxidative enzyme activities (superoxide dismutase) can be used as one of the biomarkers to evaluate the toxic effect of chemicals contaminants on the marine algae.

In Malaysia, metals, textile dye and organophosphate pesticides are among the dominant contaminants which originate from the land-based uncontrolled industrial

discharge and also sea-based source and can potentially produce a wide range of consequences to the marine biota including the algae. However, the physiological response, biochemical composition, DNA damage and oxidative enzymatic mechanisms in response to these toxic chemicals remain generally unknown. In this study, two microalgae *Chlorella vulgaris* Beijerinck and *Tetraselmis tetrahele,* (West) Butcher and two macroalgae *Boergesenia forbesii* (Harvey) Feldman and *Ventricaria ventricosa* (Agardh) Olsen & West are exposed to a diversity of chemical contaminants, and their responses in terms of growth rate, biochemical composition (carbohydrate, protein, and lipid), DNA damage and superoxide dismutase enzyme activity are determined. The aim is to identify suitable biomarkers in algae for use in detection and monitoring of chemical contamination in the marine environment. With this information, bioassays can be developed.

1.1 OBJECTIVES AND RESEARCH QUESTIONS

1.1.1 Objectives of Study

The main objective of this project is to investigate the effect of selected chemical contaminants on growth, biochemical composition, DNA damage and Superoxide dismutase (SOD) enzyme activity in four marine algae. This is done by:

a) Correlating the effects of selected chemical contaminants (metals, textile dyes and organophosphate pesticides) to growth of *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa* after short term (four days) and long term (ten days) exposure under laboratory conditions.

b) Correlating the effects of selected chemical contaminants (metals, textile dyes and organophosphate pesticides) to biochemical composition (carbohydrate, protein and lipid) of *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa* after short term (four days) and long term (ten days) exposure under laboratory conditions.

c) Correlating the effects of selected chemical contaminants (metals, textile dye & organophosphate pesticides) to DNA (DNA damage) in *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa* after short term (four days) and long term (ten days) exposure under laboratory conditions using Random Amplified Polymorphic DNA (RAPD) and AP-site (Abasic-site) count assay.

d) Correlating the effects of selected chemical contaminants (metals, textile dye & organophosphate pesticides) on the activity of anti-oxidant-defense enzyme such as Superoxide dismutase (SOD) in *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa* after short term (four days) and long term (ten days) exposure under laboratory conditions.

e) Assessing the suitable these bioassay end-points (growth rate, biochemical composition, DNA damage and SOD activity) for detection of toxicity in *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii and Ventricaria ventricosa.*

f) Assessing the possible mechanism (s) of the effect of these chemical contaminants on growth, biochemical composition, DNA damage and Superoxide dismutase (SOD) enzyme activity of *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii and Ventricaria ventricosa.*

1.1.2 Research Questions

The Research Questions addressed in this thesis are:

Q1: Do selected chemical contaminants (metals, textile dye and organophosphate pesticides) produce effects on the growth, biochemical composition, DNA and Superoxide dismutase enzyme activity in the *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii and Ventricaria ventricosa* cultures after exposure for short term (four days) and long term (ten days) duration?

Q2: Are there differences in the effects of the selected chemical contaminants (metals, textile dye and organophosphate pesticides) on growth, biochemical composition, DNA and Superoxide dismutase enzyme activity in the *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa* cultures after exposure for short term (four days) and long term (ten days) duration?

Q3: What is the possible mechanism (s) of the effect of these contaminants on the selected algae?

Q4: Can the bioassays based on the four end-points be ranked in terms of sensitivity to the selected chemical contaminants (metals, textile dye and organophosphate pesticides)?

Figure 1.1 illustrates the research approach used in this study.

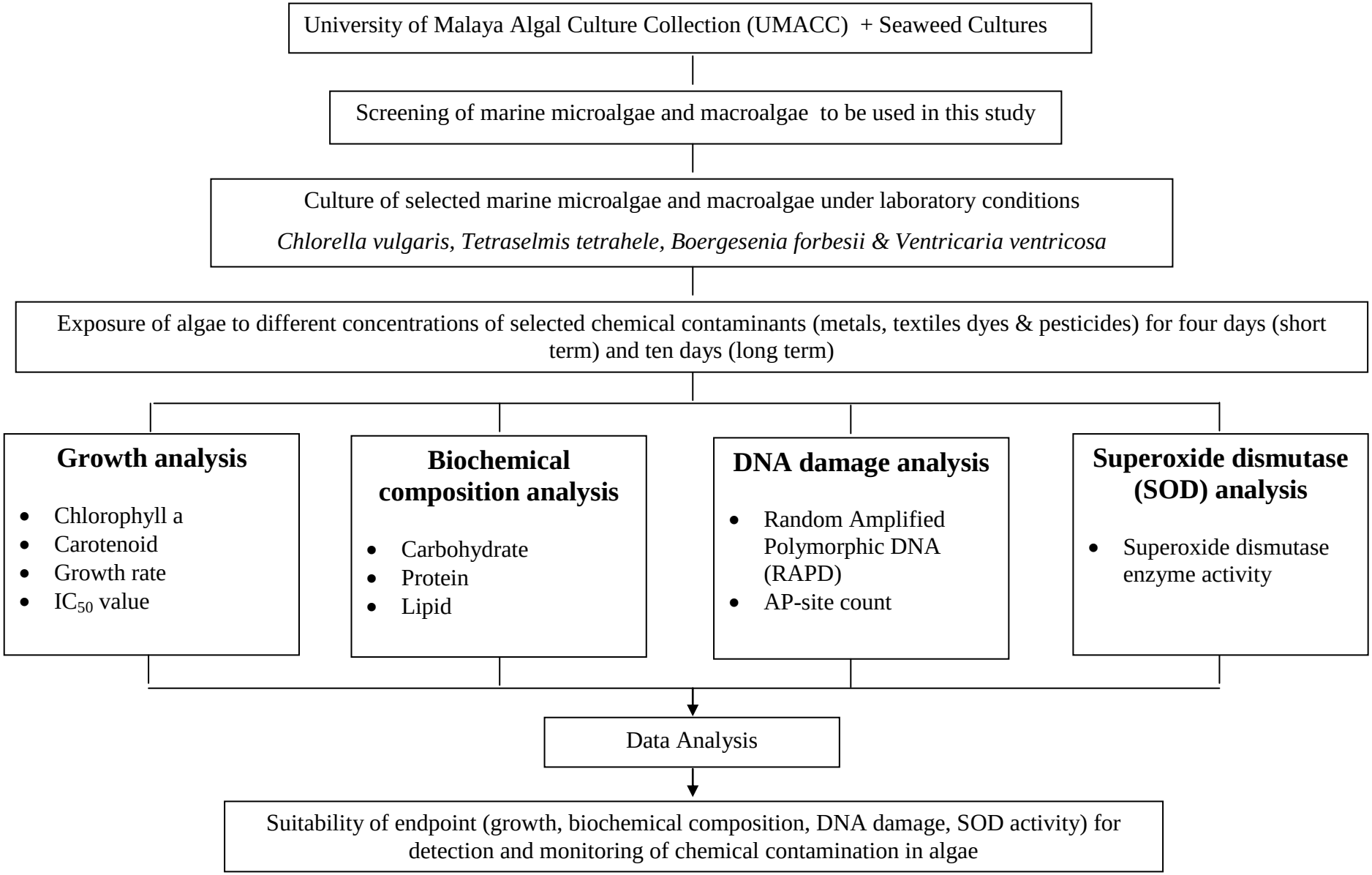


Figure 1.1: Research approach used in this study

2.0 LITERATURE REVIEW

2.1 CHEMICAL CONTAMINANTS IN THE MARINE SYSTEM

Contaminants are defined in the European legislation as: *"substances (i.e. chemical elements and compounds) or groups of substances that are toxic, persistent and liable to bio-accumulate and other substances or groups of substances which give rise to an equivalent level of concern"* [Water Framework Directive, Article 2(29)] (http://ec.europa.eu/environment/marine/good-environmental-status/descriptor-8/index_en.htm).

Chemical contaminants in the marine system occur due to the entry of the chemical contaminants from natural and anthropogenic land-base and sea-based activities, into the marine environment. These chemical contaminants can degrade the state of marine waters and can cause serious damage to its functioning. Chemical contaminant inputs to marine waters may be diluted with the absolute size and volume of the ocean. Therefore pollution incidents may not be understood immediately after release and the changes may not be detected until the appearance of the effects on the biota occur due to chronic exposure.

A consequence from the contamination of the marine waters is that organisms themselves or biological processes may be adversely affected. The toxic effects of an individual or mixture of chemicals on marine organisms depend on the toxicity profile of the chemicals, their synergetic or antagonistic effects, bioavailability and persistence, and also the ability of marine organisms to take up, accumulate and metabolize the

chemicals. In addition, chemical contaminants may be partly responsible in outbreaks of diseases or endocrine effects, which adversely affect individuals, or populations of marine organisms.

2.2 SOURCE OF CHEMICAL CONTAMINANTS AND THEIR PATHWAY IN THE MARINE SYSTEM

The sources of chemical contamination in a marine environment are varied. Reports of abundant studies have identified the sources of these contaminants and their pathway into the marine system (Storey, *et al*., 2011). Most contaminants enter the marine environment from land-based activities. There are many different ways of inputs of chemical contaminants into the marine environment. Generally there are three main types of inputs of chemical contaminants into the marine waters: (i) direct discharge of chemical contaminants into the ocean; (ii) runoff into waters due to rain and (iii) contaminants that are released from the atmosphere. Sometimes, chemical contaminants can flow out into water, soil and air during their manufacture, use or disposal process. These are not intentionally dumped but are the results of accident leaks, farm fertilizing or combustion of product containing these chemicals. Once they escape into the environment, they can travel for long distance in air, soil and water. Table 2.1 summarizes the source of chemical contaminants in marine water and their pathway into the marine system.

Table 2.1: Source of chemical contaminants and their pathway in marine system

No	Source	Description
1	Industrial discharge	● Discharges of contaminated effluent from industry into the marine aquatic environment via river system; represent major sources of aquatic pollution. The complex nature of these effluents makes assessment of their potential biological impact difficult. Whole Effluent Toxicity Testing (WETT) has been widely used to assess the potential toxicity of effluents. The results of these tests can be used for a variety of functions including resource consent monitoring and compliance, toxicity identification evaluations and evaluation of effluent treatment processes.
2	Industrial gases emission (Atmospheric deposition)	● Air pollutants from industrial include CO, SOx and NOx. These air pollutants can contribute to acidification of soils and water via precipitation. Some of these compounds are carcinogens and will influence the health of humans, flora, fauna and aquatic organism.
3	Agricultural farm discharge and run off	● A variety of pesticides used in agriculture farm including herbicides, insecticides, fungicides,rodenticides and mollussicides and other organic compounds have been detected in marine water systems. The pesticides used in agricultural farm, can affect both target and nontarget organisms when discharged into a waterbody, thus destroying the structure of the aquatic ecosystem. Organophosphate pesticides is one of the widely used insecticides nowadays, is very efficient in agricultural and sanitary pest control. After use, organophosphate pesticides is released directly into the drainage and river system by runoff and finally to marine environment.
4	Road runoff	● Heavy metals, hydrocarbons (eg: PAHs), suspended solids, pesticides and nutrients are the main contaminants found in road runoff. These contaminants can accumulate on road surfaces due to the road maintenance operations and wear of vehicle components. ● Metals content (Zinc, lead, copper, cadmium and nickel) in runoff is of concern because of their potential toxicity and persistence in the environment. High levels of suspended solids can also have adverse impacts if they enter streams, rivers and oceans. Vehicle component wear is a source of chromium, zinc, iron and aluminium deposition on the road surface. Wear of car brakes deposits copper, lead, chromium, manganese and zinc. Engine wear and fluid leakage is also a source of aluminium, copper, nickel and chromium deposition on roads. The discharges from vehicles can be emitted into the air, directly onto the road and they are washed off the road surface by the combination of rain and traffic movement. ● Pesticides and herbicides which are toxic to organisms, are usually spray along a road and railway tracks, banks and terraces over long distances for transport safety reasons. The track drainage is therefore contaminated with pesticides and herbicides and present threat to the environment. ● Suspended solids, from runoff can act as a carrier of contaminants. Many contaminants in runoff tend to associate with small particle substrate and therefore can become accumulated in freshwater and marine sediments and water.
5	Human waste and servicing	● Solid and liquid wastes produced from human activity along the coastal area are not unique to our marine environment. Wastes from houses, hotels, shops, leisure boats, in habours and marinas including organic and solid wastes are disposed of along the coastal area and they are considered as a serious ecological problem. In developing countries, and also in rural areas, solid wastes, including plastics and other non-degradable wastes, are still dumped along the coastal area. Even though the organic wastes will be decomposed eventually, contaminants are still released directly into the marine environment.

References: Buckler and Grenato, (1999); Cooper, (2003); Fu and Wang, (2011); Hall, *et al*., (1979); Isakson, *et al*., (2001); Legret and Pagotto, (1999); Li *et al.*, (2005); Sansalone, *et al*., (1996); Sriyaraj and Shutes, (2001); Perrodin, *et al.*, (2011); Scholz, (2004); Storey, *et al*., (2011)

Table 2.1: Source of chemical contaminants and their pathway in marine system (continued)

No	Source	Description
6	Emissions from the combustion of fossil fuels from ships, ferries and boat	● Fossil fuels are hydrocarbons that, if they were pure and burnt in a abundantly supply of oxygen, would produce carbon dioxide and water. However, naturally occurring pollutants and artificial additives are also present in fuels and these, combined with incomplete combustion (due to inefficient engines), cause other pollutants and contaminants to be released into the atmosphere. Air pollutants from ships include SO_2 and NOx. Both of them can contribute to acidification of soils and water via precipitation. In addition, ships in ports, which are often located around the urban area, generate emissions that can significantly decrease urban marine water quality.
7	Oil pollution from land-based activities	● Oil consists of a mixture of hydrocarbons, including chained and cyclic (aromatic) molecules. About 20-25% of non-hydrocarbons, containing metals and sulphur; and vanadium. The composition and size of hydrocarbons and non-hydrocarbons depend on the origin of the oil and the degree to which it has been processed. Large, long-chained molecules are not easily broken down by bacteria and are therefore very persistent in the environment. ● Small quantities of oil are spilled on land and enter the marine system through river system inputs. Hydrocarbons accumulated in sediments can result in the slow release of hydrocarbons over long periods. In many cases, mitigation procedures and clear-up processes using toxic chemicals as dispersing agents have caused more ecological damage than the oil. ● In busy tourist regions with high leisure boat activity, small oil spills may be common, often in combination with sewage-driven eutrophication due to high population densities in the tourist season.
8	Oil pollution from sea-based activities	● Majority amounts of oil pollution enter the marine system directly through oil spillages from tanker and other shipping accidents, tanker cleaning, discharge of bilge and ballast water, and also indirect incidents such as spillages during regular production procedures at off-shore or coastal refineries. In Malaysia, shipping incidents and accidents that occur due to collisions, fires and storms are the greatest sources of oil pollution in marine environment.
9	Shipping accident	● Shipping accidents usually occur in the ocean, near the river estuary or around the islands due to the domestic movement, rough weather, hydrographic condition and traffic density. Besides oils and fuels, other pollutants originating from shipping accidents and incidents include toxic chemicals, pesticides, herbicides, fertilizers, acids, alkalines and solid wastes. Large quantities of waste materials, as well as oil remains, are dumped and washed out to the ocean. Fuels or toxic cargo leaching from wrecks potentially have long-term impacts to the marine environment.
10	Antifouling agents	● Marine organisms attached to ships, ferries and boats cause drag, increase weight, directly decrease speed, increase fuel consumption and require regular expensive removal. To avoid such adverse effects, antifouling agents are used to kill and remove bacteria, eukaryotic uni- and multicellular algae, barnacles and other sessile animals from ships hulls. In the 1960s organo tin-containing paints (e.g: tributyl tin, TBT) were introduced worldwide. This chemical killed marine fouling organisms more effective than any other antifouling agent. However, there is a worldwide ban on the application of TBT-containing paints to vessels under 25m, and all vessels in the European Union, the US, Australia and New Zealand. Other organometals have been used, in particular copper-based paints. However, copper-containing compounds are also like tin, can accumulated and therefore will biomagnificated, in the marine food chain. ● Piers and ferry terminals are regularly sprayed with pesticides and herbicides that are slowly released into the marine water to facilitate easy and safe access for vehicles and foot passengers.

References: Buckler and Grenato, (1999); Cooper, (2003); Fu and Wang, (2011); Hall, *et al*., (1979); Isakson, *et al*., (2001); Legret and Pagotto, (1999); Li *et al*., (2005); Sansalone, *et al*., (1996); Sriyaraj and Shutes, (2001); Perrodin, *et al.*, (2011); Scholz, (2004); Storey, *et al*., (2011)

2.3 CHEMICAL CONTAMINANTS AND HAZARD IDENTIFICATION

In order to assess toxicity of the contaminants, information on four aspects is used: (i) structure-activity relationship, (ii) in-vitro or short term studies, (iii) in-vivo animal bioassay and (iv) information for human epidemiological properties (Klaassen, 2008). In many cases, toxicity information for chemical contaminants is limited. Data requirement for specific chemicals can vary greatly by compound type and applicable regulatory statutes. In 2003, the European Union released a regulatory framework for Registration, Evaluation and Authorisation of Chemicals (REACH) (http://ec.europa.eu/environment/chemicals/reach/reach_intro.htm). Under the REACH framework, starting in 2007, all stakeholders must submit physical, chemical and toxicological data as well as risk assessment for all chemicals in use (Hulzebos, *et al*., 2010; Klaassen, 2008).

2.3.1 Structure-Activity Relationship

Structure-activity relationships (SARs) have been used for assessment of single and/or complex mixture of structurally related compounds. Initial decisions on whether to continue development of a chemical, to submit pre-manufacturing notice (PMN) or to require additional testing maybe based largely on results from structure-activity relationship (SARs) and limited short-term assay. A chemical's structure, solubility, stability, pH sensitivity, electrophilicity, volatility and chemical reactivity can be important information for hazard identification. Historically, certain key molecular structures have provided regulators with some of the most readily available information on the basis of which to assess hazard potential (Faustmann and Omenn, 2008).

2.3.2 In-Vitro and Short-Term Test

The biological information of chemical and their hazard identification process can be obtained via assessment of the test chemical in in-vitro or short-term test (Faustman and Omenn, 2008). For example EPA mutagenicity guidelines call for assessment of reverse mutations using the Ames *Salmonella typhimurium* assay; forward mutation using mammalian cells, mouse lymphoma L5178Y, Chinese hamster ovary or Chinese hamster lung fibroblast and in vivo cytogenetic assessment (bone marrow metaphase analysis or micronucleus test) and other various assays of genetic and mutagenic endpoints (Faustman and Omenn, 2008).

The validation and application of short-term assay is particularly important to risk assessment because such assay can be designed to provide information about mechanism of effects, they are fast and inexpensive compared with lifetime bioassay (McGregor, *et al.,* 1999). Validation of in vitro assay requires determination of their sensitivity (ability to identify true carcinogens), specificity (ability to recognize noncarcinogen as noncarcinogen) and predictive value for the toxic endpoints under evaluation (Faustmann and Omenn, 2008).

2.3.3 Animal Bioassay

Animal bioassays are a key component of the chemical and hazard identification process. A basic assumption of risk assessment is that chemicals that cause tumors in animals can cause tumors in humans. All human carcinogens that have been adequately tested in animals produce positive results in at least one animal model. The USEPA cancer guideline (USEPA, 2005) also assumes relevance of animal bioassay, unless lack

of relevance for human assessment is specially required. In general, the most appropriate rodent bioassay is those that test exposure pathway of most relevance to predicted or known human exposure pathways (Faustmann and Omenn, 2008).

2.3.4 Use of Epidemiologic Data.

Epidemiologic study is the most convincing line of evidence for human risk in which a positive association between exposure and disease is observed. Epidemiologic studies begin with known or presumed exposures, comparing exposed versus nonexposed individuals, or with known cases, compared with person lacking the particular diagnosis. Human epidemiology studies provide very useful information for hazard assessment and can provide quantitative information for data characterization (Faustmann and Omenn, 2008).

There are three major types of epidemiology study design: cross-sectional studies, cohort studies and case-control studies. Cross-sectional studies, survey groups of humans to identify risk factors (exposure) and disease but are not useful for establishing cause and effect. Cohort studies evaluate individuals selected on the basis of their exposure to a chemical under study. Therefore, based on exposure status, these individuals are monitored for development of disease. These potential studies monitor over time individuals who are at the begining are disease-free, to determine the rates at which they develop disease. In case-control studies, subjects are selected on the basis of disease status: disease cases and matched cases of disease-free individuals. Exposure histories of the two groups are compared to determine key consistent features in their

exposure histories. All case-control studies are retrospective studies (Faustmann and Omenn, 2008).

2.4 CONSEQUENCES OF CHEMICAL CONTAMINANTS IN THE MARINE SYSTEM

The consequences of chemical contaminants that have been discharged from land-based and sea-based activity on marine organisms and environment are varied. Many studies have been carried out worldwide, which have examined the relationship between chemical contamination with aquatic organism health and ecosystems (Perrodin *et al*., 2011).

Chemical contamination affected the freshwater and marine water ecosystems. Through groundwater and land-based run-off, all contaminants released into terrestrial or freshwater systems potentially reach coastal and marine environments. Therefore, coastal water environment represent the largest sink for contaminants. These conditions are due to the increased application of products that produce chemical contaminants that are used for human settlements, industries, agricultural and other practices (Giulio and Newman, 2008). Table 2.2 summarizes the consequences of chemical contaminants to the marine environment.

Table 2.2: Consequences of chemical contaminants in the marine system

No	Consequences	Description
1	Contamination in food chains due to industrial and agricultural discharges containing metals	● Metals that are bioavailable can be accumulated by primary producers, like algae, higher plants, as well as filter and deposit feeders and organisms at higher trophic levels. Algae absorb metals mainly in their ionic form directly from the water; rooted plants take up metals from the sediments, and animals accumulate metals bound to particles or through consumption of algae and plants. Although most metals (e.g. copper, cobalt, iron, manganese, vanadium and zinc) are essential micronutrients, essential in enzyme reactions, photosynthesis, respiration and nutrient uptake, at higher concentrations they become toxic. Other metals, such as lead, mercury, chromium are usually not required by aquatic organisms can cause toxic effects even at low concentrations. Toxicity is related to metal types and species specific, usually affecting germination, photosynthesis and reproduction. In addition to metals taken up through the consumption of grazers, metals are also actively bioacummulated by deposit and filter-feeding invertebrates through consumption of particles.
2	Human contamination	● Humans become contaminated directly from chemical household products, eating heavily processed food flavour with chemical preservatives or eating contaminated seafood and animal fats.
3	Acidification in soil and water bodies due to emission of gases from land-based and sea-based activity	● Both SOx and NOx inputs from land based activities (eg: industrial, transport, etc) enter soils and water bodies, precipitation (acid rain) and run-off. They can cause acidification of water bodies, as both NOx and SO are precursors for strong acids. Acidic water bodies are characterised by pH below 5.5 and acid neutralising capacity (ANC) near or below 0μeq/L. ● Low pH leads to physical and chemical alterations of the water body such as the availability of nutrient and metals in the water body, and increased light penetration and temperature. All of these affect aquatic species. ● Changes in pH also directly affect species and communities by reducing photosynthesis, respiration and growth of most organisms. Therefore, changes of important communities have been observed, such as decreases in species richness of both algal and macrophytic flora, and decreases in biomass. ● Productivity is reduced which has effects on the aquatic food chain, directly decreasing fish stocks including those of potential commercial value. In addition, herbivorous and carnivorous invertebrates are directly influenced by changes in the chemical composition of the water. Effects of water acidification are complex, but generally cause a shift in species abundance and result in reduced standing crop of both primary producers and consumers. ● In combination of NO_2 with volatile organic carbons and other pollutants, they can act as a precursor for ozone (eg: ground-level ozone), which is harmful to humans and vegetation, including commercial crops and phytoplankton. ● Other chemicals such as benzene, PAHs and gases emitted from the combustion of fossil fuels from ships, ferries and boat, are reported as toxic and genotoxic compounds. ● Ships in ports, which are often located around the urban area, generate emissions that can significantly decrease urban air and marine water quality.

References: Beasley and Kneale, (2002); Buckler and Grenato, (1999); Langston, (1996); Law, *et al., (*1998); Planas, (1996); Smith, (1981); Stengel and Dring, (2000).

Table 2.2: Consequences of chemical contaminants in the marine system (continued)

No	Consequences	Description
4	Give advers impact on freshwater ecosystem due to road runoff	● The biological impact of runoff from roads is likely to be greatest on freshwater ecosystems, as chemical contaminants are washed into rivers before its end up in the marine environment. The potential of toxicity depends on the concentration and the physical and chemical properties of each contaminant present. The impact also depends on the sensitivity of organisms to these runoff contaminants and the ability of the ecosystem to assimilate a given contaminant or mixture of contaminants. ● Benthic macro-invertebrates living in contaminated sediments receive prolonged exposure to contaminants via gill cell osmosis and ingestion. As macro-invertebrates are an important food sources for many aquatic consumers, this can then result in the biomagnification of pollutants through the food chain. High loads of suspended solids from maintenance and construction work can also cause aquatic habitat disturbance and cause a decrease in aquatic plant and animal populations.
5	Human waste and servicing contamination on-shore area	● Effects of contaminants on shore regions have been widely described. Plastics are not degradable and may act as substrata for settlement of marine organisms. Litter and plastics derived from houses, resorts and shops along the coastal area and from ships, may be ingested by fish, marine mammals, turtles or seabirds and this may lead to choking or indeed malnutrition or starvation.
6	Oil contamination due to oil spill	● The environmental effects of oil spills depend on a number of factors including the nature of the oil (e.g. light or crude oil) and the composition of different hydrocarbons; spread and destination; management procedures and treatment. ● Wind speed and weather conditions can delay physical removal from the sea surface, but hot weather may increase evaporation and therefore reduce the volume of oil. However, we should remember that hydrocarbons evaporated from the sea surface do not just disappear, but enter the atmosphere, and this condition may reduce the immediate severity of oil pollution on nearby shores, but the ecological effects are shifted to a different level and location. ● Addition of detergents to disperse the oil technique, to recover the affected shorelines, is time-consuming. Oil is then further driven into sediments that then act as long-term sources of hydrocarbon pollution, long after the surface layers may have recovered. Dispersants are not always effective in removing the oil, and can be toxic to biota. Hydrocarbons and dispersants can cause fish and shellfish mortality. Many PAHs are carcinogenic and mutagenic to most organisms. Physical removal of oil from the sea surface appears the most suitable procedure, but this may not be possible due to bad weather or the sheer volume of the spill. In consequence, not all oil will be removed from the sea or the shore, no matter which methods, or combination of methods, are used. Natural bacteria decomposition of small amount, in particular small droplets is possible but slow. ● Chronic exposure to oil will change the tolerant species flora, and many factors determine the effects of oil spills on intertidal fauna and flora in acute exposure. The physical coverage of shorelines by oil has several effects on plants and animals. Light is limited for photosynthesis, and this will reduce productivity. Also, bacterial decomposition of oil has a high biological oxygen demand (BOD) and can further reduce O_2- availability for sessile animals. Bleaching can occur and if covered by heavy oil deposits, plants are physically damaged and likely to break. Long-term effects are greatest in fine-grained sediments which naturally support high productivity and diverse fauna, and are critical systems at the base of the marine food web. Humans may directly be affected by oil spills when aquaculture installations and local fish stocks are under threat.

References: Beasley and Kneale, (2002); Buckler and Grenato, (1999); Langston, (1996); Law, *et al.,* (1998); Planas, (1996); Smith, (1981); Stengel and Dring, (2000).

Table 2.2: Consequences of chemical contaminants in the marine system (continued)

No	Consequences	Description
7	Antifouling agents contamination	● TBT which have been banned as biocides on ships and boats in 1987, is toxic to marine and freshwater organisms, in particular molluscs, even at concentrations as low as 0.1µg/L. Oysters and other shellfish show shell deformation and reproductive abnormalities. Natural and farmed populations of at least 70 species of gastropods are known to be affected by what is known as imposex/intersex, and female sterility, caused severe damage to mollusc populations near ports and marinas. For marine animals, organotins are accumulated in fatty tissues. Effects on fish, birds and humans appear less severe as TBT is degraded in the body, however it can potentially give adverse impacts on human health through the consumption of seafood. ● Copper-based paints are still used, and also leach into freshwater or marine systems and are toxic to organisms. Even though, it is thought to be less bioavailable than copper which readily forms organic complexes, copper accumulation in algae and other aquatic biota and copper contamination near harbours and marinas has been observed.
8	Contamination of toxic sediment	● Coastal and estuarine sediments are continuous sinks for contamination. Toxic sediments can be physically removed and dumped elsewhere. However, both the method of the removal and the destination of toxic sediments are unfavourable. Physical disruption of the sediments, re-suspension and oxygenation of toxins may increase their bioavailability to biota. Dumping of toxic marine sediments on land can lead to the leaching of contaminants into groundwater. Regular dredging of sediments which may or may not be toxic occurs near entrances to ports, have often been dumped in designated marine (coastal or offshore) dump sites. Although these are generally away from busy shipping routes, dredging can cause disturbance of (toxic) sediments, and stimulate further release of nutrients or toxins into the marine systems where they may become available for uptake by marine biota.

References: Beasley and Kneale, (2002); Buckler and Grenato, (1999); Langston, (1996); Law, *et al.*, (1998); Planas, (1996); Smith, (1981); Stengel and Dring, (2000).

A summary of the long-term ecological and socio-economic impacts of pollutants in aquatic systems are:

(i) Biodiversity reduction or loss.

(ii) Change in standing crop and species composition.

(iii) Loss of key species resulting in decrease in ecosystem stability and function.

(iv) Reduce facility value of coastal systems for human settlement and tourism.

(v) Losses for the fishing and shellfish industry affecting income for local communities.

2.5 POTENTIAL MITIGATION AND SOLUTION

The simplest action to reduce marine contamination problems is to reduce the source of contamination, that is reduced the number and quantities of compounds used in anthropogenic activities. A range of chemical contaminants that threaten aquatic, in particular coastal systems, are derived from land-base activities through riverine or atmospheric inputs. Reduced inputs into groundwater, streams and rivers will also improve water quality and environmental health of estuarine, coastal and marine systems. Although, marine contamination is a global problem, local environment protection plan have an important role to play in securing safe drinking water supplies, species diversity, agricultural crops and improving local facilities and finally preserving human health.

Many Toxic Chemicals Laws (legislations) have been implemented worldwide by responsible agencies that lay down strategies to fight against chemical contaminantion in the aquatic environment. The EU REACH Regulation (Registration, Evaluation,

Authorisation and Restriction of Chemical Substances), which entered into force in 2007, is one way of achieving a better environmental status. The aim of REACH is to improve the protection of human health and the environment through the better and earlier identification of the intrinsic properties of chemical substances, and the environmental risk they pose. Under REACH regime, manufacturers and importers are required to gather information on the properties of chemical substances, which will allow their safe handling, and to register the information. The regulation also calls for the progressive substitution of the most dangerous chemicals when suitable alternatives have been identified (Hulzebos, *et al*., 2010; Klaassen, 2008).

Another European legislation is the Water Framework Directive (Directive 2000/60/EC), which lays down a strategy to fight against the contamination of water, including adopting specific measures against contamination by individual contaminants/pollutants or groups of contaminants/pollutants presenting a significant risk to or via the aquatic environment. For those contaminants/pollutants, action should aim at the progressive reduction and, for priority hazardous substances, as defined in Article 2(30) of the Directive, at the cessation or phasing-out of discharges, emissions and losses (Hulzebos, *et al*., 2010).

In Malaysia, the Malaysia Environmental Quality Report (DOE, 2010), which is required under Section 3(1)(i) of the Environmental Quality Act 1974, has established requirements for the chemical status of surface water including rivers and marine water. Environmental Quality Standard (EQS) can be defined as the maximum allowable concentration of the contaminants not causing harm. The EQS is based on the lowest toxic effect observed for aquatic organisms during testing in the laboratory with standard

organisms. The National Water Quality Standard for Malaysia can be found in Annex of Malaysia Environmental Quality Report 2010 (DOE, 2010) (Appendix 1-Appendix 3).

Table 2.3 summarises the potential mitigations and solutions that can be adopted to reduce the chemical contamination in marine environment.

2.6 CHEMICAL CONTAMINANTS USED IN THE STUDY

For the present study, twelve chemical contaminants including seven metals [Cadmium (Cd); Cobalt (Co); Chromium (Cr); Copper (Cu); Iron (Fe); Manganese (Mn) and Zinc (Zn)], three textile dyes [Supranol Br. Red 3Bur (Acidic dye); Astrazon Red FBL (Basic dye) and Lanaset Red 2GA (Metal complex dye)] and two organophosphate pesticides [Malathion and Dichlovos] were used.

2.6.1 Metals

Heavy metal pollution has become a serious environmental problem as a result of the toxicity and non-susceptibility to the environment (Lesmana, *et al.*, 2009). Heavy metals can be present in the wastewater of several industrial processes, such as electroplating, metal finishing, metallurgical works, tanning, chemical manufacturing, mining and battery manufacturing (Kang *et al*., 2007; Al-Rub, 2006; Aksu and Acikel, 2000). It also can be obtained in the road runoff, which may contain chromium, zinc, iron, lead, aluminium, copper, manganese, cadmium and nickel, originating from the vehicle components' wear and tear (Scholz, 2004; Sriyaraj and Shutes, 2001).

Table 2.3: Potential mitigations and solutions for chemical contaminants in the marine system

	Action	Potential mitigation and solution
1	Reduced the emission of gases from land-based activities	● Industries require new energy source and technological change, where low impact fuel and greener technology are required. ● There has been little development in the use of biofuels, to reduce the application of fossil fuel. ● Hybrid vehicles that combine fossil fuel and electric technologies, electric vehicles or the introduction of fuelcell technology are probably the cleanest transport mode (assuming that the energy used is produced without causing environmental damage). ● Stricter guidelines on emissions from industrial sources and transportation may be required to reduce the emission.
2	Reduce the emission of gases (SOx, NOx) from ship.	● The 1997 protocol to MARPOL 73/78 (Annex VI) which regulates the emission of SOx and NOx from ship exhausts came into operation in May 2005. It is monitored by the International Maritime Organisation (IMO). Reduction in SOx emissions can be achieved by the use of low-sulphur fuel or the use of exhaust gas-cleaning systems. The incineration of contaminated packaging and PCB-releasing materials on board ships is also banned under the new Annex. ● Multi-storage facilities on ships may be required when ships need to refuel outside the EU with high-sulphur fuels, but the burning of low-sulphur fuels is required on entry into EU territorial waters because of current EU legislation (Directive 1999/32/EC). ● Stricter emission controls from ships, introduction of cleaner fuels or biodiesels, should be included in the idling restriction to prevent pollution at the ports. Emissions from ships need to be regulated and included in global emission restrictions and guidelines. The implementation of this, possibly through the introduction of taxes.
3	Treatment of runoff from road and rail	● A variety of road drainage systems are used to treat runoff and these can vary in their effectiveness. The main systems consist of infiltration technologies, porous pavement, stormwater ponds, wetland systems, oil/grit separator, filtration systems, vegetated swales and filter strips. The principle behind most systems is to collect runoff as it drains from the road surface, then either filter or infiltrate it through soil, removing particulate and soluble matter before it is discharged into waterways.
4	Recycle wastes	● Use of environmentally friendlier materials (and their recycling) in construction work, industrial and household component materials. Therefore in the future, these materials can continuously recycle or reused.
5	Proper waste management along the coastal area and waste from the ships	● Solid and liquid wastes from shoreline area, along the road, from the ferries and ships need to be collected frequently and appropriately disposed at landfill. ● The dumping of solid waste including waste and harmful substances is restricted under MARPOL 73/78 (Annex V and III). The disposal of plastics is prohibited anywhere in the sea, while dumping other rubbish is restricted only in coastal and protected areas. Record books logging waste disposed and incinerated are mandatory for large ships. ● Better education and trainning of crew and passengers is required to reduce the waste disposal into marine environment.

References: Annon, (2004); Bailey and Solomon, (2004); European Commission, (2002); Everall and Lees, (1996); Farmer, (2001); Mikkelson, *et al*., (1996); O'Reilly, *et al*., (2004); Owen,(1999); Pagotto, *et al*., (2000); Schramm, (1991); Sriyaraj and Shutes, (2001)

Table 2.3: Potential mitigations and solutions for chemical contaminants in the marine system (continued)

	Action	Potential mitigation and solution
6	Reduce pesticides and fertilizer application	● Improved management and maintainance of the track. ● Use or introduce the non-persistent organic pesticide (eg: glyphosphate imazapyr-containing substances). ● Reduce the amount of pesticides (use smaller quantities at lower concentraration). ● Use foliar pesticides and not soil pesticides. ● Use pesticides that have a half-life of less than 2-6 months and that can be totally degradable within a year of application. ● Restriction of pesticide application during adverse weather conditions. ● Spraying in environmentally sensitive area (eg: protected groundwater catchment region) should be avoided. ● Alternative weed removal method should be identified and applied (eg: removal of weed by hot steam, use of cloth sealing layer). ● Use mechanical clearance in environmental sensitive areas.
7	Prevent oil-spills from tankers	● Oil spillages occur during normal operation when tankers; after discharging the oil, fill cargo tanks with ballast water for stability and cleaning, and then release dirty ballast water (a mixture of oil and water) into the sea. ● To reduce the spillage during ballast water exchange or cleaning operations; separators in hulls of modern tankers is used, where ballast water is kept separate from oil compartments, and can be released into the water without having been in contact with oil. ● Other methods include the consecutive cleaning of different compartments with high-pressure jets and maintenance of water in compartments until the oil floats on top of the water. Instead of being released into the sea, the dirty ballast water is pumped into separate tanks and stored until the oil forms a layer on top; the oil-free water underneath can be pumped out into separate tanks and eventually be released into the sea. The oil can be recovered and transferred into a slop tank, and fresh oil is loaded on top ('Load on Top'method). ● More effective in preventing oil release into the sea is the 'crude-oil washing' developed in the 1970s which involves the cleaning of cargo compartments with oil (instead of water) which makes the process more effective and reduces the amount of oil-water mixtures produced. Crude oil washing was made mandatory for new tankers in 1978 by the MARPOL Convention protocol, and revised specifications regarding design, operation and controls were adopted by IMO in 1999.

References: Annon, (2004); Bailey and Solomon, (2004); European Commission, (2002); Everall and Lees, (1996); Farmer, (2001); Mikkelson, *et al*., (1996); O'Reilly, *et al*., (2004); Owen, (1999); Pagotto, *et al*., (2000); Schramm, (1991); Sriyaraj and Shutes, (2001).

Table 2.3: Potential mitigations and solutions for chemical contaminants in the marine system (continued)

	Action	Potential mitigation and solution
8	Prevent shipping accident to reduce oil spill	● Improved tanker route management by strict legislation at the local port which managing high traffic density especially at the environmentally sensitive and protected marine areas. ● Improved tanker route management by using single traffic routes along busy tanker route and increase navigational communication could reduce the frequency of accidents. ● Use the tug boats (including emergency tug boats) is recommended, to reduced the accident. ● Increase environmental consideration by port authorities should be enforced by stricter regulations and implemented by national governments. ● To improve tanker safety, and ultimately reduce the number and size of oil spills; better training of pilots and crew is recomended; it is thought that 80% of accidents are due to human error caused by poor crew performance. ● Improved policy for design, construction, technical condition and maintenance of ships, where stricter safety regulations is required. ● Specific information, training and emergency response centres have been set up to allow more effective prevention and response to oil spills in different seas. ● Support oil spillage related research and development. ● Provide guidelines for governments and individuals involved in oil transfer and transport, and those involved in the planning, conduct and administration of salvage operations.
9	Use new types of antifouling paints	● Use new antifouling paints, containing active ingredients (eg. 4,5-dichlor-2-n-octyl-4-isothiazolin-3-on (DCOI) in Sea-Nine 211, and zinc pyrithion) that are thought to be biodegradable. ● Use non-stick foulant release substances including silicon- and ceramicepoxy coatings. Non-adhesive surfaces can deter microbial and algal settlement, which is usually the first stage in biofouling succession. ● Use electric sterilization of ships' hulls or the use of non-adhesive surface structures such as microscopic prickles. ● Use coatings containing natural antifouling compounds; some of these are derived from marine bacteria, algae, corals and sponges. Natural biocides may be enzyme-based that prevent the settlement of bacteria by breaking down attachment mucilages. ● Routine mechanical removal of fouling organisms from large ships and aquatic surfaces. However it is considered impractical as it is inefficient and labour intensive because it involves the physical removal by divers or extended dry-docking.

References: Annon, (2004); Bailey and Solomon, (2004); European Commission, (2002); Everall and Lees, (1996); Farmer, (2001); Mikkelson, *et al*., (1996); O'Reilly, *et al*., (2004); Owen, (1999); Pagotto, *et al*., (2000); Schramm, (1991); Sriyaraj and Shutes, (2001).

Table 2.3: Potential mitigations and solutions for chemical contaminants in the marine system (continued)

	Action	Potential mitigation and solution
10	Remediation of fresh water systems due to acidification	● To decrease pH in acidified water bodies, lime ($CaCO_3$) can be added to lakes, streams and or whole catchments. In Scandinavian lakes, large-scale additions of lime from boats, planes and helicopters were undertaken to neutralise waters acidified by dry and wet deposition of sulphuric and nitric acids. Lime is relatively inexpensive, it dissolves readily in water, at low concentrations is non-toxic to biota, and also reduces the toxicity of heavy metals, in particular aluminium, copper, cadmium, lead and zinc. ● Fast-growing large (floating) plants can be introduced that take up nitrogen quickly and can easily be removed from the water.
11	Remediation of toxic sediment	● Seaweeds that naturally absorb nutrients, either as particulates or in a dissolved form, from the water may be cultured in the disturbed sediments. Seaweed biomass can be processed into an energy-rich, valuable biofuel.

References: Annon, (2004); Bailey and Solomon, (2004); European Commission, (2002); Everall and Lees, (1996); Farmer, (2001); Mikkelson, *et al*., (1996); O'Reilly, *et al*., (2004); Owen, (1999); Pagotto, *et al*., (2000); Schramm, (1991); Sriyaraj and Shutes, (2001).

The discharges from industries and vehicles can be emitted into the air, directly onto the road and then washed by the rain into streams, rivers and finally to the oceans.

Contamination of soils, groundwater, sediments, surface water and air with hazardous and toxic metals poses significant adverse effects for both human health and the environment (Ansari and Malik, 2007). For human health impacts, each metal shows different effects and symptoms. For example, in the case of minor zinc (Zn) exposure, muscular stiffness, irritability, nausea and loss of appetite are common (Bhattacharya, *et al*., 2006). Cadmium (Cd) can provoke cancer, kidney damage, mucous membrane destruction, bone damage, vomiting, diarrhea and itai-itai disease, as well as affect the production of progesterone and testosterone (Godt, *et al.,* 2006). Chromium (Cr) can cause cancer in the digestive tract and lungs (Martins, *et al*., 2006; Kiran, *et al*., 2007). Copper (Cu) consumption in high doses can cause serious toxicological concerns since it can be deposited in the brain, liver, pancreas, skin, and myocardium (Liu, *et al.*, 2008a); It also can initiate intestinal distress, kidney damage and anemia (Al-Rub, *et al*., 2006). Manganese (Mn) triggers neurotoxicity, gastrointestinal accumulation and low hemoglobin levels (Parvathi, *et al*., 2007). Lead (Pb) has been cited as one of the most toxic metals that have long-term negative impacts on health, causing encephalopathy, anemia, hepatitis and nephritic syndrome (Deng, *et al.*, 2006). The presence of nickel exceeding its critical level may cause serious lung and kidney problems, pulmonary fibrosis, gastrointestinal distress and skin dermatitis (Borba, *et al.*, 2006). Mercury has been identified for its nervous system deterioration, including protoplasm poisoning (Alluri, *et al.*, 2007).

2.6.2 Textile Dye

The textile processing industry generates many wastes streams, including water-based effluents as well as air emissions, solid wastes, and hazardous wastes. On average, 165 liters of water is required to process 1 kilogram of textile and therefore this industrial sector is a significant source of water pollution (Aksu, 2005). Another major problem in textile effluent is the color. A dye usually used in the textile processing industry is a coloured substance that has an affinity to the substrate to which it is being applied. The dye is generally used in an aqueous solution. The dye used in the textile procesing industry can enter the marine system via uncontrolled discharge of effluent into the river system and directly to the sea. Textile dyes are acutely toxic by all routes of exposure. Some of these compounds bind strongly to essential enzymes and inactivating them (Aksu, 2005).

2.6.3 Organophosphate Pesticide

Pesticides are compounds or mixture of compounds used to kill pests (insect, plant patogent, weeds, mollusks, birds, mammals, fish, nematodes and microbes) that compete with humans for food, destroy property, spread or are a vector for disease or cause nuisance. Organophosphate pesticides refers to a group of insecticides (used against insect) containing organic phosphorus (V) compound.

Organophosphate pesticides are strong nerve agents that inhibit the enzymes acetylcholinesterase and other cholinesterases in nerve cells, which are essential to nerve function in insects, humans and other animals. Organophosphate pesticides affect these enzymes in wide-ranging ways and therefore have potential to be a toxicant.

Organophosphate pesticides are usually applied in agricultural farms and have potential to give adverse toxic effects on non-target orgamisms. Organophosphate pesticides can degrade rapidly by hydrolysis on exposure to sunlight, air and soil. Although organophosphate pesticide can degrade fast, they have greater acute toxicity, present risks to the person who may be exposed to a large amount of this toxicant (USEPA, 1992; Costa, 2006). It also has accumulative toxic effect to wildlife; therefore multiple exposures to the chemicals amplify the toxicity (Palmer, *et al*., 2007). The organophosphate pesticides used in the agriculture farm can enter the marine system via runoff and discharge of effluent into the river system and directly to the sea.

Table 2.4 summarizes the characteristic of the chemical contaminants used in this study.

2.7 ALGAE

Algae are eukaryotic organisms, very large and diverse group of simple, typically autotrophic organisms, ranging from unicellular to multicellular forms. The term algae refer to both macroalgae and microalgae. The number of algal species has been described by the AlgaeBase, as more than 35 000 species and most of them are microalgae (http://www.algaebase.org; Guiry, 2012).

The current classification system known as the three domain (Bacteria, Archea and Eukarya), the algae have been segerated into different kingdom within the domain Eukarya (Glaucophyta, Rhodophyta, Heterokontophyta, Haptophyta, Cryptophyta, Dinophyta, Euglenophyta, Chlorarachniophyta and Chlorophyta) and Bacteria (Cyanophyta and Prochlorophyta) (Solomon, *et al*., 2008).

Table 2.4: Characteristics of chemical contaminants used in this study

Chemical contaminants	Characteristic	Application	Toxic effects on aquatic organism	References
Metals				
Cadmium	● Symbol: Cd ● Atomic no: 48 ● Cd is soft, bluish-white, malleable, ductile, toxic and is present in the free ionic state in water as a divalent cation. Cd metal is insoluble in water, but its chloride and sulfate salts are freely soluble. ● Cd and its compounds enter the environment only from geological (distributed in the Earth's crust, but usually associated with zinc and copper) or from human activities (metal mining, by-product of zinc smelting, vehicle tyres and engine exhausts, and fossil fuel combustion).	● Cd is used largely in batteries (predominantly rechareable nickel-cadmium) and pigments (for plastic products), coatings and plating, as stabilizer for plastic. ● Cd is important for the marine diatom (which use cadmium-dependent carbonic anhydrase) which live in environment with very low zinc concentration used cadmium rather than zinc to grow.	● Cd and its compounds are black-listed materials, which by international agreement may not be discharged or dumped into the environment. ● Cd has toxicity effects on phytoplankton, seaweed, mussel, etc. Cd taken up by aquatic organisms is strongly dependent on water pH. Accumulation of Cd in the food chain appears to be significant and cause ecological damage.	Emmanouil *et al*., (2007); Lane, *et al*., (2000); Lane *et al*., (2005); Morrison *et al*., (2008); Piotrowska-Niczyporuk *et al*., (2012); Sabater, (2000); Turner *et al*., (2008)
Cobalt	● Symbol: Co ● Atomic no: 27 ● Co is a ferromagnetic metal. Pure Co is not found in nature, but compounds of Co (e.g. sulfidic cobaltite, safflorite and skutterudite) are common. Co is produced as a by-product of copper and nickel mining.	● Co is used in the preparation of magnetic, wear-resistant, and high-strength alloys; batteries, catalyst, pigment and colouring, electroplating, etc. ● Mammals require small amounts of Co (basis of vitamin B12), Co-60 a radioactive metal, is an important radioactive tracer and cancer treatment agent.	● Co is toxic to seaweed and phytoplankton, etc. High concentrations of Co can inhibit algal growth, chlorophyll synthesis and induce changes in photosynthetic activity.	Barceloux and Barceloux, (1999); Hawkins, (2001); Michel, *et al*., (1991); Morrison, *et al*., (2008); Rehren, (2003); Vijayaraghavan, *et al*., (2005); Wang, (2006)

Table 2.4: Characteristics of chemical contaminants used in this study (continued)

Chemical contaminants	Characteristic	Application	Toxic effects on aquatic organism	References
Metals				
Chromium	● Symbol: Cr ● Atomic no: 24 ● Cr is a steely-gray, lustrous, hard metal. It is also odourless, tasteless, and malleable. Cr is mined as chromite ($FeCr_2O_4$) ore. ● Cr exists in the oceans primarily in two valency states: particle-active trivalent chromium, Cr (III), and more soluble hexavalent chromium, Cr (VI).	● Used in metallurgy, to impart corrosion resistant, create a shiny finish or increase hardness, as dye and paints, as a catalyst, chemical reagent, Cr salts are used in the tanning of leather ● Trivalent chromium (Cr^{3+}) is required in trace amounts for sugar metabolism in human.	● Cr has toxicity in microalgae, seaweed, mussel, fish, etc	Atli, *et al*., (2010); Atli & Canli, (2010); Ferreira, *et al.*, (2007); Emmanouil, *et al.*, (2007); Morrison, *et al.*, (2008); Nguyen, *et al*., (2005); Stearns, *et al*., (1995); Vincent, (2007).
Copper	● Symbol: Cu ● Atomic no: 29 ● Cu is a ductile metal with very high thermal and electrical conductivity. Pure copper is soft and malleable and a freshly-exposed surface has a pinkish or peachy color. ● Cu has several oxidation states, usually 2^+, where they often impart blue or green colors to natural minerals.	● Cu is used in piping, alloy, electrical application (electric conductor,thermal conductor), Architecture/industry (building material), household products, coinage, biomedical application, chemical application (catalyst, anti-fouling paints, wood preservative), etc ● Cu is essential trace nutrient in all human, plants and animals.	● Cu has a wide range of toxicities in algae, fish, mussel, crustacean, etc ● High concentration of Cu in water has been found to damage marine life causing damage to gills, liver, kidneys, and the nervous system, inhibit photosynthesis, etc. Cu readily taken up by algae and invertebrates can accumulate in the food chain.	Gant, *et al*.,(2007); Noyce, *et al*., (2006); Noyce, *et al*., (2007); Pease, *et al*., (2010); Trevisan, *et al*., (2011).

Table 2.4: Characteristics of chemical contaminants used in this study (continued)

Chemical contaminants	Characteristic	Application	Toxic effects on aquatic organism	References
Metals				
Iron	● Symbol: Fe ● Atomic no: 26 ● Fe is lustrous and silvery in color. It is soft, and one of the ferromagnetic elements. ● Fe is extracted mainly from the iron ore hematite. It oxidises in air and water to form Fe_2O_3 and is rarely found as a free element.	● Fe is the most widely used in engineering applications (construction of machinery and machine tools, automobiles, the hulls of large ships), structural components for building, used in the form of steel, etc ● Fe is essential for all known organism. In cells, Fe is generally stored in the centre of metalloproteins.	● Fe has a wide range of toxicities in microalgae, zooplankton, fish, etc ● Excessive Fe can be toxic, because free ferrous iron reacts with peroxidase to produce free radicals, which are highly reactive and can damage DNA, protein, lipids and other cellular components.	Atli, *et al*., (2010); Sato, *et al*., (2007)
Manganese	● Symbol: Mn ● Atomic no: 25 ● Mn is the most abundant element in the earth's crust and is found as a free element in nature (often in combination with iron), and in many minerals. Mn is a hard gray-white metal, and is very brittle, fusible with difficulty, but easily oxidized.	● Mn is used in objects made of steel, in portable batteries, or in aluminum beverage cans, plays a vital role in improving the properties of the alloys, as pigment and as oxidation chemicals. ● Mn is required as an essential element (trace mineral) for all living organism. Mn (II) ions function as cofactor for a number of enzymes.	● High concentrations of Mn can inhibit the photosynthesis process in the algae and plants.	Dismukes, *et al*., (2006); Elsner, *et al.*, (2005)
Zinc	● Symbol: Zn ● Atomic no: 30 ● Zn is reactive bluish grey metal. Zn can be extracted from sphalerite, smithsonite, hemimorphite and franklinite minerals. The common oxidation state of Zn is +2.	● Zn is most widely used as base for white pigment in watercolour and paint, as activator in rubber industry, deodorant, anti-dandruff shampoos, wood preservative, luminescent pigment, etc ● Zinc is an essential trace element, necessary for sustaining all organisms. Zn is used as cofactor for many enzymes	● Zn has toxicity in algae, fish, moss etc ● Excess content of Zn can inhibit the photosynthesis process in the algae and plants and alter the gene expression in the cell.	Atli, *et al*., (2010); Wang, *et al*., (2009); Rau, *et al*., (2007); Sabater, (2000)

Table 2.4: Characteristics of chemical contaminants used in this study (continued)

Chemical contaminants	Characteristic	Application	Toxic effects on aquatic organism	References
Textile dye				
Supranol Br. Red 3Bur (Acidic dye)	● IUPAC name: 6-[[4-(Acetylcyclohexylamino)phenyl]azo]-4-(benzoylamino)-5-hydroxy-1,7-naphthalenedisulfonic acid disodium salt ● Formula: $C_{31}H_{28}N_4Na_2O_9S_2$ ● Mol. Weight: 710.68g/mol ● Acidic dye are water-soluble anionic dyes	● Used to dye fibers such as wool, silk, nylon, jute and leather	● Acidic dye has toxicity in microalgae, fish,etc	Aksu and Tezer, (2005); Balter, (2009); Hunger, (2003); Parikh and Madamwar, (2005) Sumathi, *et al.*, (2001); Zollinger, (2003)
Astrazon Red FBL (Basic dye)	● IUPAC name: 3(or5)-[[4-[benzylmethylamino]phenyl]azo]-1,2(or1,4)-dimethyl-1H-1,2,4-triazolium bromide; ● Formula: $C_{18}H_{23}BrN_6$ ● Mol. Weight: 403.3194g/mol ● Basic dye are water-soluble cationic dyes	● Used to dye acrylic fibers, wool and silk. ● Basic dye also used in the colourization of paper	● Basic dye has toxicity in microalgae, fish,etc	Aksu and Tezer (2005); Balter, (2009); Hunger, (2003); Parikh and Madamwar (2005); Sumathi, *et al.*, (2001); Zollinger, (2003)
Lanaset Red 2GA (Metal complex dye)	● IUPAC name: Chromat (2-), (2,4-Dihydro-4-((hydroxy-4- nitrophenyl) azo)-5-methyl- 2-phenyl-3H-pyrazol-3-onato (2-))(3-((4,5-dihydro- 3-methyl- 5-oxo-1-phenyl-1Hpyrazol- 4-yl)azo)- 2-hydroxy- 5-nitrobenzol sulfonato(3-))-, Dinatrium ● CAS No: 70209-87-9 ● Metal complex dye (pre-metallized dyes) has great affinity towards protein fibres. It can be classified into two classes. 1:1 metal-complexes (one dye molecule gets co-ordinated with a single metal atom) and 1:2 metal complexes (one metal atom is co-ordinated with double dye molecules). The dye molecules are typically a monoazo structure which can contain additional groups like hydroxyl, carboxyl or amino groups. They can form strong co-ordination complexes with transition metal ions, like Nickel, Chromium, Cobalt and Copper.	● Used for a variety of applications like wood stains, leather finishing, stationery printing inks, inks, coloring for metals, plastic etc.	● Metal complex dye has toxicity in microalgae, fish,etc	Aksu and Tezer (2005); Parikh and Madamwar (2005); Sumathi, *et al.*, (2001)

Table 2.4: Characteristics of chemical contaminants used in this study (continued)

Chemical contaminants	Characteristic	Application	Toxic effects on aquatic organism	References
Organophosphate pesticide				
Dichlovos	● IUPAC name: 2,2-dichlorovinyl dimethyl phosphate ● Formula: $C_4H_7C_{l2}O_4P$ ● Mol. Weight: 220.98 g/mol ● Dichlorvos is an OP insecticide that occurs as an oily colorless to amber liquid that is slightly soluble in water. It has an aromatic chemical odor (sweetish smell). ● Dichlorvos act by interfering with the activities of cholinesterase, an enzyme that is essential for the proper working of the nervous systems of both humans and insects.	● Dichlorvos used in pest control is diluted with other chemicals and used as a spray. It can also be incorporated into plastic that slowly releases the chemical. ● Dichlorvos is used as an agricultural insecticide on crops, stored products, and animals. It is also used as an insecticide for slow release on pest-strips for pest control in homes. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips, and white flies in greenhouse, outdoor fruit, and vegetable crops ● Therapeutically, Dichlorvos is used as an anthelmintic (worming agent) for dogs, swine, and horses, as a botacide (agent that kills fly larvae) for horses, and in flea collars for dogs	● UV light makes dichlorvos more toxic to aquatic life by 5-150 times. ● Dichlovos has a wide range of toxicities in shrimp, fish, eel, arthropod, etc ● EPA has classified dichlorvos as a Group B2, probable human carcinogen. In 1995, EPA proposed cancellation of dichlorvos for all home uses, and for many commercial and industrial uses	ATSDR, (1997); USEPA, (1999); Meister, (1992).
Malathion	● IUPAC name: 2-(dimethoxyphosphinothioylthio) butanedioic acid diethyl ester ● Formula: $C_{10}H_{19}O_6PS_2$ ● Mol. Weight: 330.36g/mol ● Malathion is an OP insecticide that occurs as a clear clear colourless liquid when pure; yellow to deep brown liquid that is soluble in water (145 mg/l at 25°C). It has a skunk-like odor. ● It is available in emulsifiable concentrate, wettable powder, dustable powder, and ultra low volume liquid formulations. ● Malathion is an OP parasympathomimetic which binds rreversibly to cholinesterase.	● Malathion is widely used in agriculture, residential landscaping, public recreation areas and in public health pest control. ● Malathion is effective againts sucking and chewing insects on fruits and vegetables, mosquitoes, flies, household insects, animal parasites (ectoparasites), and head and body lice. ● Malathion may also be found in formulations with many other pesticides.	● Malathion has a wide range of toxicities in phytoplankton, zooplankton, fish; crustaceans; marine benthic, and to the aquatic stages of amphibians etc.	Bonner, *et al.*, (2007); Edwards, *et al*., (2007)

Most of the algae are photosynthetic like plants and simple because their tissues are not organized into the many distinct organs found in plants. Algae do not have roots, stems, leaves or well-defined vascular tissue. Even though many seaweed are plant-like in appearance and some of them show specialization and differentitiation of their vegetative cells, they do not form embryos, their reproductive structures consist of cells that are potentially fertile and lack sterile cells covering or protecting them (Barsanti and Gualtieri, 2006).

Algae occur in dissimilar forms such as microscopic single cells, macroscopic multicellular loose or filmy aggregations, matted or branched colonies, or more complex leafy or blade forms. The diversity of algal size ranges from picoplankton only 0.2-2.0µm in diameter, to giant kelps with fronds up to 60m (Barsanti and Gualtieri, 2006).

The oceans cover about 71% of the earth's surface and contain more than 5000 species of planktonic microscopic algae, the phytoplankton, which forms the base of the marine food chain.Through photosynthesis algae combine carbon dioxide from the atmosphere along with water and light within the chloroplasts to produce energy and biomass, and to emit oxygen. (Anastasakis, *et al*., 2011). Through photosynthesis and respiration, carbon dioxide and oxygen are cycled and recycled in marine ecosystems by microalgae and seaweed and they produce approximately 50% of the oxygen we inhale (Barsanti and Gualtieri, 2006). Algae also will uptake and store nutrients which can later be cycled in the ecosystem by decomposers. They also accumulate high quantities of metals such as lead, zinc, copper, cadmium, chromium and manganese; acting as a sink for these pollutants (Anastasakis, *et al.*, 2011).

Microalgae provide both benefits as well as negative impacts to humans, even causing death. When the microalgae population becomes too large and uncontrolled, in response to pollution containing nutrients such as nitrogen and phosphate, the algae blooms can reduce the water transparency, causing the death of other marine organisms. They are often responsible for massive fish and bird kills, producing poisons and toxins through resulting harmful algal blooms (Liu, *et al*., 2007).

2.71 Uses of Algae

Microalgae and macroalgae (seaweed) have been used by man for hundreds of years as food, fodder, medicine and fertilizer. Ancient records show that people collected macroalgae for food as early as 500 B.C in China and one thousand years later in Europe (Barsanti and Gualtieri, 2006). Algae are used in many maritime countries as a source of food, for industrial application, for agriculture application, as fertilizer and etc.

Microalgae are rich sources of vitamins, essential amino acids, minerals, essential fatty acids, and carotenoid pigments for aquatic animals (Takeuchi, *et al.,* 2002). In feeding trials with fish, many types of microalgae have been found to increase growth (protein accretion), feed utilization, physiological activity, stress response, starvation tolerance, disease resistance, and carcass quality (Mustafa and Nakagawa, 1995). Table 2.5 summarizes the uses of algae to humans.

Table 2.5: Uses of Algae

Uses	Description	References
Human food	● *Nostoc* sp., *Arthrospira* sp., *Porphyra* sp., *Palmaria palmata*, *Chondrus crispus*, *Gracilaria* sp., *Alaria esculenta*, *Laminaria japonica, Undaria* sp., *Monostroma* sp., *Enteromorpha* sp., *Kappaphycus* sp., *Eucheuma* sp., *Gelidiella* sp., *Ulva* sp., *Caulerpa* sp. and others have been commerciallised as food in many East Asia country.	Barsanti and Gualtieri, (2006); Graham and Wilcox, (2000)
Agriculture fertiliser	● Large brown macroalgae such as *Ascophyllum* sp., *Ecklonia* sp., *Fucus* sp. and *Sargassum* sp. which are exposed at low tide are collected and composted (by mix seaweed with sand, let it rot and then dig it in): usual practice used by farmers to produce fertilizer in U.K, France and many countries around the world.	McHuge and Dennis, (2003)
Soil conditioner	● Maerl is commonly used as a soil conditioner.	Barsanti and Gualtieri, (2006)
Feed	● Microalgae have important role in mariculture and have been used as live feeds for larval or juvenile crustaceans and fish, for all bivalve mollusks including oysters, scallops, clams and mussels, and as feed for zooplankton, which are fed to late larval and juvenile fish and crustaceans.	Zhou, (2006).
Phycolloids	● *Kappaphycus* sp., *Eucheuma* sp., *Gelidium* sp., *Gelidiella* sp. are among the source of hydrocolloids (such as agar, alginate and carragenan) from algae which are used to thicken aqueous solution, to form gels (jellies of varying degrees of firmness), to form water-soluble film and to stabilize certain products such as ice-cream and canned food.	Partridge, (1983)
Cosmetic	● Extract of algae is often used in cosmetic product particularly in face, hand and body creams or lotions. Milled macroalgae, packed in sacket, is sold as an additive to bath water and bath salts with macroalgae meal are also sold.	Barsanti and Gualtieri, (2006)
Therapeutic supplements	● Microalgae are a unique source of high-value biochemical compounds such as carotenoids (Fucoxanthin, ß-carotene, astaxanthin), PUFA (DHA, EPA), Vitamins (A, B_1, B_2,B_6, B_{12}, niacin and C); rich in iodine, K, Fe, Mg and Ca and polysaccharides (ß-glucan) are used as therapeutic substances that usually extracted from the microalgae.	Barsanti, *et al*., (2006); Bigogno, *et al*.,(2002); Mondragon, (2003); Morton, (2008); Simoons, (1991)
Medicinal and application	● Seaweed have curative powers for tuberculosis, arthritis, colds and influenza, worm infestation and tumor; Source of iodine to prevent goiter; Carrageenan is used as anti-viral agent and *Corallina* is being used in bone-replacement therapy.	Jyonouchi, *et al*., (2000); Maeda, *et al*., (2005)
Methane, alcohol esters and acids	● By fermentation, algae can produce methane, alcohol esters and acids. Seaweeds especially those with high carbohydrate contents, may be fermented to produce bioethanol.	Barsanti and Gualtieri, (2006)

Table 2.5: Uses of Algae (continued)

Uses	Description	References
Gas, chemicals and coal-like product	● By pyrolysis, algae can produce gas, chemicals and coal-like products	Barsanti and Gualtieri, (2006)
Bioremediation	● Algae are cultured in ponds and are either harvested or used to treat effluents pumped through the ponds	Barsanti and Gualtieri, (2006); Markou and Georgakakis, (2011)
Serve as feeding, breeding and nursery grounds for marine animals,	● Seaweeds contribute to photo-oxygenation of the coastal waters and serve as feeding, breeding and nursery grounds for various marine animals, especially the fishery resources and endangered animals like turtles and dugongs.	Barsanti and Gualtieri, (2006)
Energy collector	● The mass culture of seaweeds may be manipulated to remove carbon dioxide from the atmosphere while producing alternative sources of renewable carbon neutral energy.	Barsanti and Gualtieri, (2006)
Alternative Energy source	● Algae based biofuel and biogas (which have potential to produce more biomass per unit area in a year than any other form of biomass) have a potential to be use as alternative energy source in the future.	Barsanti and Gualtieri, (2006); Douškova, *et al*., (2010)
Pollution control	● Sewage can be treated with algae, reducing the need for greater amounts of toxic chemicals than are already used; Algae can be used to capture fertiliser in runoff from farms. When subsequently harvested, the enriched algae itself can be used as fertilizer.; Aquariums and ponds can be filtered using algae, which absorb nutrients from the water in a device called an algae scrubber, also known as an ATS	Barsanti and Gualtieri, (2006); Fu and Wang, (2011)
Plastics	● Algae has been implemented in the production of biodegradable plastics by Cereplast, Inc. An agreement has also been reached with the US Military to introduce more biodegradable plastics as it attempts to move away from petroleum based plastics and utilize more environmentally friendly alternatives	Casey, (2010)

2.7.2 Microalgae and Macroalgae (Seaweed) Used in the Study

For the present study, two species of microalgae, *Chlorella vulgaris* Beijerinck (UMACC 245) and *Tetraselmis tetrahele* (West) Butcher (UMACC 144) and two species of macroalgae (seaweed), *Boergesenia forbesii* (Harvey) Feldman and *Ventricaria ventricosa* (Agardh) Olsen & West were used as tools for bioassay to detect and monitor the chemical contaminants in the marine system.

2.7.2.1 *Chlorella vulgaris* Beijerinck, 1890

Description:

Chlorella vulgaris Beijerinck is a genus of single-celled green algae, belonging to the phylum Chlorophyta. It is spherical in shape, without gelatinous envelope, about 2 to 10µm in diameter and is without flagella. Cell solitary or aggregated into small group. Cell walls smooth without acetoresistant layer, containing glucosamine (chitosan). Nuclei are single and eccentric. Chloroplast is single cup-shape and pariental. Pyrenoid is single and covered with starch envelope. Pyrenoid stroma penetrated with 2 or 3 closely adpressed thylakoids. Asexual reproduction by autospores, 2-8 per cell; released by rupture of parental cell wall. Habitat: essentially cosmopolitan in both freshwater and marine habitats (http://www.algaebase.org; Guiry, 2012).

Classification

Empire : Eukaryota
Kingdom : Plantae
Subkingdom : Viridaeplantae
Infrakingdom : Infrakingdom “Chlorophyta”
Phylum : Chlorophyta
Subphylum : Tetraphytina

Class : Trebouxiophyceae
Order : Chlorellales
Family : Chlorellaceae
Genus : *Chlorella*
Species : *vulgaris*

2.7.2.2 *Tetraselmis tetrahele* (West) Butcher, 1959

Description:

Tetraselmis tetrahele (West) Butcher is a genus of single-celled green algae, belonging to the phylum Chlorophyta. Size about 10 to 14µm in diameter. Unicellular flagellates Cell more or less compressed, often slightly curved but never twisted. Cells are cordiform, elliptical or almost spherical. Anterior end with invagination, 4 equal flagella in 2 opposite pairs. The cell contains a single more or less cup-shape chloroplast (very rarely two chloroplast), usually with a central pyrenoid. A single eyespot is present, located on one of the flattened sides of the cell. Cell surrounded by close-fitting periplast of fused scales. The flagella covered by square/diamond-shape scales in 24 rows overlaid by 24 double rows of scales. Two rows of hair-shaped scales project from opposite sides of the flagella. Asexual division is in the non-motile stage. In many species one of the daughter cells inverts within the parent periplast, the 2 daughter cell then lie in reversed position. In the non-motile stage new walls develop, old walls accumulating as cocentric ring around the cell or being polarized on one side, forming a stalk. The stalks may be long and occasionally branched. Vegetative thick walled cysts known in several species. This germinates by division into 4 cells. Habitat: marine habitats. (http://www.algaebase.org; Guiry, 2012).

Classification

Empire : Eukaryota
Kingdom : Plantae
Subkingdom : Viridaeplantae
Infrakingdom : Infrakingdom "Chlorophyta"
Phylum : Chlorophyta
Subphylum : Tetraphytina
Class : Chlorodendrophyceae
Order : Chlorodendrales
Family : Chlorodendraceae
Genus : *Tetraselmis*
Species : *tetrahele*

2.2.7.3 *Boergesenia forbesii* (Harvey) Feldman, 1938

Description:

Boergesenia forbesii (Harvey) Feldman is a genus of single-celled green algae, belonging to the phylum Chlorophyta. Thalli composed of one to too many unbranched, elongate, puriform vesicles forming incurved rosette-like clusters. Siphonous cell with 2mm to 8mm diameter at distal ends, 1cm to 5cm tall. Annular constriction prominent is on tapered basal ends of vesicles. Apical orientation maintained throughout development. Rhizoid well developed from which new uprights can arise. Adventitious rhizoids, tenacular cells and lenticular cells not present. Cell division by modified segregative division in main axes: by centripetal invagination in rhizoid. Cells multinucleate, with numerous discoid chloroplasts each with single pyrenoid surrounded with starch sheath and bisected by traversing thylakoids. Haploid and diploid chromosome counts of 14-18 and 36 reported for *Boergesenia forbesii*. Nuclear DNA amount of 2.7, 4.9 and 9.5 pg (1C, 2C and 4C) estimated for *Boergesenia forbesii*.

Boergesenia forbesii occurs only in the tropical Indo-West Pasific where it is found in sandy, coral rubble habitants. Strong water motion seems to be important although the banana-like clusters are often found in more sheltered crevices and oftem by themselves. Because of its large cell size, *Boergesenia* has been used for a wide variety of physiological, developmental and nuclear studies. The cytoskeleton and its role in cytoplasmic motility have been extensively studied (http://www.algaebase.org; Guiry, 2012).

Classification

Empire : Eukaryota
Kingdom : Plantae
Subkingdom : Viridaeplantae
Infrakingdom : Infrakingdom "Chlorophyta"
Phylum : Chlorophyta
Subphylum : Tetraphytina
Class : Siphonodadophyceae
Order : Siphonocladales
Family : Siphonocladaceae
Genus : *Boergesenia*
Species : *forbesii*

2.2.7.4 *Ventricaria ventricosa* (Agardh) Olsen & West

Description:

Ventricaria ventricosa (Agardh) Olsen & West is a genus of single-celled green algae, belonging to the phylum Chlorophyta. Thallus composed of a single, unbranched, aseptate vesicular cell, 1mm to 4 mm diameter. Plants are spherical, pyriform, lobed or irregularly deformed, depending upon substratum. Cell wall is iridescent. Annular

constriction, tenacular cells and well-developed rhizoid not present. Attachment to substratum achieved by reduced rhizoidal cells at base of cell. Cell division is by modified segregative division only. Cell is multinucleate. Chloroplasts are numerous per cell and discoid with single pyrenoid. Occasionally an adventitious rhizoid cell will expend into a minute new vesicle which detaches to become a new individual. Vegetative propagation of this type in combination with aplanospore products of segregative division in the entire thallus is common in both cultured and field specimens. *Ventricaria* is widely distributed throughout the tropics in shallow intertidal habitats, often in coral rubble areas or lodged in coral crevices. It often found in association with Dictyosphaeria. *Ventricaria* is one of the largest cells in nature and this makes it an ideal system for studies of cell physiology including electrophysiology and cell growth (http://www.algaebase.org; Guiry, 2012).

Classification

Empire : Eukaryota
Kingdom : Plantae
Subkingdom : Viridaeplantae
Infrakingdom : Infrakingdom "Chlorophyta"
Phylum : Chlorophyta
Subphylum : Tetraphytina
Class : Siphonodadophyceae
Order : Siphonocladales

Family : Valoniaceae

Genus : *Ventricaria*

Species : *ventricosa*

2.8 BIOASSAY USED IN THIS STUDY

2.8.1 Algae Toxicity Test

Algae toxicity test is carried out to determined the degree to which a substance (chemical contaminants) can damage a main producer in food chain like green algae that frequently found in aquatic system. Standard algae toxicity test are useful tools to determined the effects of pollutants on cell growth and viability over a fixed period of time. The test provides for observation of inhibition (toxic effects) as well as stimulation (trophy). Due to algae fast growth ability, it is possible to observed accute effects and also chronic effects of tested substances (APHA, 1992; ASTM, 1993; USEPA, 1978).

In the algae toxicity test, the algae is exposed to the test substances dissolved in medium or dilution water. The solubility of the test substances to the medium, under the test condition must be checked before conducting the test. In addition, a reliable method for quantifiying the test substances in the test solution must be available. Wherever possible, the test conditions should be kept constant throughout the exposure period. For instance, the test substances concentration should be maintained at least 80% of the initial concentration (concentration of substances measured at the begining of exposure). The information of the test substances is useful in establishing the test condition includes structural formula, purity, stability in light, and results of substance transformation including biodegradability in water (APHA, 1992; ASTM, 1993; USEPA, 1978).

The algae toxicity test include a measurement of algae biomass concentration growth in individual concentrations of tested toxic sample in comparison with control sample which containing algae grown in growth medium without toxicant. A test

substance is considered toxic when a statistically significant, dose-dependent inhibition of algae growth occurs (APHA, 1992; ASTM, 1993;USEPA, 1978).

Algae toxicity test provide an information on the toxicity of test substances to an important component of the aquatic biota and it maybe indicate wheather additional testing is required. The results of algae toxicity test (i) can be used to compared the sensitivity of different species of algae and the toxicities of different substances to the algae, (ii) maybe an important factor when assessing the hazards of substance/materials to aquatic organism, (iii) maybe useful for studiying biological availability of, and structure-activity relationships between test materials/substances. According to Chapman *et al*., (1996), developing a sensitive test is important for evaluating the potential impact of chemical contaminants on aquatic ecosystem, with the objective to prevent possible injuries to the biota by determined the maximum tolerance levels of toxicants. Table 2.6 summarizes the list of researchs that have been carried out to assess the green algae toxicity after being exposed to selected chemical contaminants in the marine environment.

2.8.1.1 Algae growth measurement

Growth is defined as an irreversible change in the size of a cell, organ or whole organism. It may also be the increase in cell number without changes in volume or weight. Commonly, growth is the increase in the amount of living material (protoplasm) which leads to an increase in cell size and ultimately cell division. Growth occurs only in living cells by metabolic processes involved in the synthesis of proteins, nucleic acids, lipids, and carbohydrates at the expense of metabolic energy provided by photosynthesis

Table 2.6: Summary of research on algae toxicity test in green algae exposed to selected chemical contaminants

Organism	Genotoxic agent	Toxicant Exposure time	References
Chlorococcum sp., *Chlorella* sp.	Pesticides and industrial wastes: carbophenothion, ethoprop, methyl parathion, phorate, carbaryl & neburon	96 hrs	Walsh and Alexander, (1980); Walsh, *et al.*, (1980)
Chlorella sp., Dunaliella tertiolecta	Pesticides: hexaclorocyclopentadiene, chlorpyrifos, carbonphenothion, Atrazine & Amdro	48 hrs, 72 hrs, 96 hrs	Walsh, *et al.*, (1983)
Selenastrum capricornutum	Tributyltin	24 hrs, 45 hrs	Miana, *et al*., (1993)
Tetraselmis suecica	The soluble fraction of *Solanum tuberosum*	12 days	Fibregas, *et al*., (1997)
Chlorella sp., Selenastrum capricornutum	Metronidazole (nitroimidazole)	72 hrs	Lanzky and Haning-Snrensen (1997)
Selenastrum capricornutum	Effluents from three Ontario, Canada, refineries	96 hrs	Sherry, *et al.*, (1997)
Chlorella sp.	Metal and textile effluents (23 effluent samples)	24 hrs, 48 hrs	Sponza, (2000)
Nannochloropsis gaditana, Tetraselmis suecica, Rhodomonas salina	Surfactants: linear alkylbenzene sulfonate (LAS) homologues (C10 and C13)	72 hrs	Hampel, *et al.*, (2001)
Enteromorpha intestinalis	Copper	12 hrs	Elfwing and Tedengren, (2002)
Chlorella vulgaris, Chlorella VT-1	Silver nitrate ($AgNO_3$)	12 days	Scragg and Bonnett, (2002)
Tetraselmis chui	Nutrient limitation: low phosphorus & low nitrate & recovery process	3 days	Chung, *et al.*, (2003)
Chlorella minutissima, Chlorella stigmatophora, Dunaliella tertiolecta	Cryoprotectants : methanol, dimethylsulfoxide, propylene glycol & polyvinylpyrrolidone	5 days	Tzovenis, *et al.*, (2004)
Chlorella vulgaris	Sewage treatment plants (STP) effluents	48 hrs	Aguayo, *et al.*, (2004)
Tetraselmis chuii	Therapeutic agents (diazepam, clofibrate, and clofibric acid) & a detergent (sodium dodecyl sulfate)	96 hrs	Nunes, *et al.*, (2005)
Ulva pertusa	Metals (Cd^{2+}, $Cu^{2+,}$ Zn^{2+}, Pb^{2+}) & elutriates of sludge collected from nine different locations.	5 days	Han and Choi, (2005)
Chlorella zofingiensis	Fe^{2+}, various amounts of peroxynitrite or nitryl chloride.	5 days	Ip and Chen, (2005)
Chlorella vulgaris	chloroquine (antimalarial agent and drug)	24 hrs,48 hrs, 72 hrs	Zurita, *et al.*, (2005a)

Table 2.6: Summary of research on algae toxicity test in green algae exposed to selected chemical contaminants (continued)

Organism	Genotoxic agent	Toxicant Exposure time	References
Chlorella sp.	Cd^{+2}, Ni^{+2}, Cr^{+3}, and Cu^{+2} and azo dye (methyl orange)	21 days	Doshi, *et al.*, (2006)
Chlorella vulgaris	Propyl gallate, Bromobenzene (BrB), Indium nitrate, Gemfibrozil	24 hrs, 48 hrs & 72 hrs	Zurita, *et al.*, (2007a, 2007b, 2007c)
Euglena gracilis	Chromium	96 hrs	Ferreira, *et al.*, (2007)
Chlorella pyrenoidosa, Tetraselmis chui	Phenicol antibiotics: chloramphenicol, florfenicol & thiamphenicol	4 days	Lai, *et al.*, (2008)
Pseudokirchneriella sp.	Ethyl parathion & cumene hydroperoxide	96 hrs	Andreozzi, *et al.*, (2008)
Pseudokirchneriella subcapitata and Chlorococcum sp.	OP Pesticides: fenamiphos and its metabolites (FSO, FSO_2, FP, FSOP, FSO_2P)	96 hrs	Cáceres, *et al.*, (2008)
Chlamydomonas moewusii	Paraquat (herbicide)	24 hrs	Prado, *et al.*, (2008)
Ulva pertusa, Ulva armoricana	Copper	3 days	Han, *et al.*, (2008)
Tetraselmis marina	Chlorophenols (herbicide)	6 days	Petroutsos, *et al.*, (2008)
Chlorella vulgaris	Chlorophenols & 2,4-dichlorophenol	30 days	Sahinkaya and Dilek, (2009)
Dunaliella tertiole	Oil spills samples from Santa Cristina and San Miguel de Oia	1 h, 72 h & 30 days	Carrera-Martínez, *et al.*, (2010)
Pseudokirchneriella subcapitata	Vinyl chloride (VC)	48 h	Nam and An (2010)
Chlorella pyrenoidosa	Pentachlorophenol	120 hrs	Hong, *et al.*, (2010)
Pseudokirchneriella subcapitata	Textile & tannery wastewaters	24 hrs, 48 hrs & 72 hrs	Tigini, *et al.*, (2011)
Chlorella vulgaris	Cd, Cu, Pb	24 hrs	Piotrowska-Niczyporuk, *et al.*, (2012)
Ulva compressa	Copper	7 days	Mellado, *et al.*, (2012)
Ulva pertusa	Coumaphos (organophosphate pesticides)	Day 1, 2, 3, 5 and 7, after 6 h exposure to light.	Schweikert and Burritt, (2012)

and respiration (Janick, 1979). Plant growth is often measured as a change in area, length, volume, height, wet or dry weight. Growth can be expressed as yield or growth rate (Janick, 1979).

Yield is an indication of organic production. It can be given in terms of fresh weight or dry weight of the organic mass produced over a period of time per unit volume or unit area occupied by the algae. The growth rate of a algae population is a measured of the increase in biomass over time and it is determined from the exponential phase. Growth rate is one important way of expressing the relative ecological success of a species in adapting to its natural environment or the experiment imposed upon it (Fogg and Thake, 1987).

In the algae toxicity test, for the measurement of algae biomass concentration, the algae growth in each flask is measured by one of the following methods: cell counts, fresh weight, dry weight, packed cell volume (PCV), chlorophyll content, or turbidity (light absorbance) (APHA, 1992; ASTM, 1993; USEPA, 1978). For the present study, the growth of the algae is measured using chlorophyll *a* and carotenoid measurement methods.

2.8.1.1.1 Cell counts

Cell count can be measured using counting devices like Sedgewik-Rafter, Palmer-Maloney, heamocytometer and Petrof-Hausser. Whenever feasible, 400 cells per replicate are counted to obtained $\pm$ 10% precision at 95% confidence (APHA, 1992; Reed, 2003; Anderson and Throndsen, 2003; Barsanti and Gualteri, 2006).

2.8.1.1.2 Turbidity (light absorbance)

The turbidity or absorbance of the cultures can be measured using spectrophotometer at a wavelength of 630nm to 750nm. Because of absorbance is a complex function of the volume, size and pigmentation of the algae, it would be useful to conduct a calibration curve to establish the relationship between absorbance and cell density (APHA, 1992; Reed, 2003; Barsanti and Gualteri, 2006).

2.8.1.1.3 Chlorophyll content

Chlorophylls are the primary photosynthetic pigments involved in light absorption and photochemistry in higher plants, algae and photosynthetic bacteria. They give plants their characteristic green colour. They constitute about 4% of chloroplast dry mass. There are four major in vitro algal chlorophylls (Chl) have been described: Chl *a*, Chl *b*, Chl *c* and Chl *d* (Meeks, 1974).

Chl *a* consist of a hydrophilic porphyrin head formed by four linked pyrrole rings with a magnesium atom chelated (Mg^{2+}) at the centre and a hydrophobic phytol tail. The molecular formula for Chl *a* is $C_{55}H_{72}N_4O_5Mg$ (Mr=892) (Hall and Rao, 1994). The Chl *a* content of algal cell can range from 0.3 to 2.0 percent of the dry weight (Rabinowitch, 1945).

Chlorophyll is synthesis in the chloroplast. According to Meeks, (1974), most and not all algal synthesize chlorophyll while growing heterotrophically in the dark. Algae that require light for chlorophyll biosynthesis includes strain of *Euglena*, *Ochromonas* and *Cyanidium caldarium*; mutants of *Chlamydomonas*, *Chlorella*, and

Scenedesmus. For the formation of Chl *a* molecule, Shemin in 1940 showed that the tetrapyrrole ring system in Chl structure was formed from δ-aminolevulinic acids (ALA) (Gregory, 1989). In the Shemin pathway, δALA was formed from succinyl CoA and glycine and ends with the formation of protoporphyrin IX. The protoporphyrin IX were then will incorporate Mg^{2+} and the pathway proceeds to finally form protochlorophyllide *a*, followed by Chlorophyllide *a* and lastly to become chlorophyll *a* (Hall and Rao, 1994; Beale, 2008; Tanaka and Tanaka, 2011).

Chlorophyll biosynthetic pathway in algae is assumed mainly control by of the level of δ-aminolevulinic acids (ALA) formation (Granick, 1971; Bogorad 1966; Granick 1967; Goodwin 1971; Barsanti and Gualtieri, 2006). Organisms which do not form chlorophyll in the dark accumulate only small quantities of protochlorophyllide (10^{-3} of normal Chl content) unless it was supplied with exogenous ALA (Granick, 1967). The regulation of chlorophyll biosynthesis is additionally complex since the accumulation of the pigment appears to be intimately associated with thylakoid development and photosynthetic activity. Chlorophyll biosynthesis is generally reported to be sensitive to inhibitors of both cytoplasmic (cycloheximide) and organel (chloramphenicol) ribosomes (Kirk and Tilney-Bassett, 1967).

In vitro Chl *a* is soluble in alcohols, diethyl ether, benzene and acetone and it is insoluble in the water (Hall and Rao, 1994). The pigment shows two main absorption bands in vitro: one band is in the red light region near to 660nm and 665nm and the other is in the region near to 430nm (Meeks, 1974). Chlorophyll can be extracted in 80-90% acetone or 90% methanol. The concentration of chlorophylls is generally estimated by in-vivo fluorometrically, or in-vitro either fluorometrically or spectrophotometrically

using the specific absorption (extinction) coefficient of chlorophyll in the particular solvent (Meeks, 1974).

As reported by previous researchers, the condition such as nutrition, light intensity, temperature, cell age, UV radiation, metal toxicity and other environmental stress will affect the chlorophyll content in the algae cell (Waite and Truesdale, 2003; Gurbuz, *et al*., 2004; Ferrier, *et al*., 2005; Ip and Chen, 2005). According to Soeder and Stengel, (1974) and Barsanti and Gualtieri, (2006); mineral nutrition such as nitrate, phosphate, molybdenum, selenium, Fe, Mn, Zn, Cu and Co can affects the chlorophyll content of the algae. Deficiencies of Fe, N and Mg, which were essential constituents of chlorophyll, have pronounced effects on chlorophyll synthesis and content (Kirk and Tilney-Bassett, 1967; O'Kelly, 1968).

2.8.1.1.4 Carotenoid content

Carotenoids are nutritional elements known as phytonutrient, is a group of natural, fat soluble, yellow to red pigments that are mainly found in plants, algae and photosynthetic bacteria. They play an important role in photosynthesis where it's functioning as photoreceptors and protect the cell from damage by light and oxygen. They prevent photodamage mainly by acting as physical quenchers of electronically excited molecules, (Tian and Yu, 2009; Krinsky, 1989; Woodall, *et al*., 1997).

Under normal conditions, the carotenoids of the Chlorophyceae are usually found in the chloroplast lamellae in close proximity to the chlorophyll. Like chlorophyll, carotenoid absorbs light, most strongly in the blue portion of the spectrum. They have

triple-banded absorption spectra in the region from about 400 to 550nm. The carotenoids are called the accessory photosynthetic pigments since the quanta absorbed by these pigments can be transferred to chlorophyll (Hall and Rao, 1994) for photosynthesis.

They are 750 structurally described carotenoids and more than 100 of those are found in algae (Takaichi, 2011). In vitro carotenoid is soluble in alcohols, diethyl ether, benzene and acetone and it is insoluble in the water. Carotenoid can be extracted in 80-90% acetone or 90% methanol. The concentration of carotenoid is usually estimated by in-vitro spectrophotometrically using the specific absorption (extinction) coefficient of carotenoid in the particular solvent (Goodwin, 1974).

2.8.2 Biochemical Composition Measurement

Algae contained a varying proportion of biochemical composition like proteins, carbohydrate, lipids and nucleic acids. Although there are marked differences in the composition of the algae classes and species, protein (12-35% dry weight) is regularly the major organic constituent, followed by lipid (7.2-23% dry weight) and then by carbohydrate (4.6-23% dry weight). The content of highly unsaturated fatty acids such as eicosapentaenoic acids (20:5n-3, EPA), arachidonic acid (20:4n-6, ARA) and docosahexaenoic acid (22:6n-3, DHA) is one of the major importance in the evaluation of the nutritional composition of an algae species to be used as food for marine organism. The nutritional value of algae can vary according to the culture conditions (Barsanti and Gualtieri, 2006; Stewart, 1974). Table 2.7 summarizes the biochemical composition of selected algae and Table 2.8 summarizes the research that have been conducted to evaluate the biochemical composition in green algae.

Table 2.7: Biochemical composition of algae (expressed on a dry matter basis, %)

Algae	Protein	Carbohydrates	Lipids
Chlamydomonas rheinhardii	48	17	21
Chlorella vulgaris	51-58	12-17	14-22
Chlorella pyrenoidosa	57	26	2
Chlorella ellipsoidea	5	16	84
Chroomonas salina	36	11	15
Dunaliella bioculata	49	4	8
Dunaliella salina	57	32	6
Dunaliella tertiolecta	20	12	15
Euglena gracillis	39-61	14-18	14-20
Nannochloris atomus	6.4	5.0	4.5
Nannochloropsis oculata	2	0.5	1
Scenedesmus obliquus	50-56	10-17	12-14
Scenedesmus quadricauda	47		1.9
Scenedesmus dimorphus	8-18	21-52	16-40
Tetraselmis maculata	52	15	3
Tetraselmis chui	83	33	46
Tetraselmis suecica	52	20	17
Spirogyra sp.	6-20	33-64	11-21
Ulva sp.	15-25	42-46	0.6-0.7

References: Arasaki & Arasaki, (1983); Becker, 1994; Brown (1991); Edwards, (2008); Levring, *et al*. (1969); Nisizawa, *et al*., (1987);

2.8.2.1 Carbohydrate

A carbohydrate is an organic compounds that consist of carbon, hydrogen and oxygen with the empirical formula of $Cn(H_2O)n$. The carbohydrate can be divided into four chemical grouping: monosaccharides, disaccharides, oligosaccharides and polysaccharides (Harris, 1997; Hardin, *et al.,* 2012).

In general, the monosaccharides and disaccarides, which have lower molecular weight carbohydrates, are commonly called as sugar. A sugar can be defined as an aldehyde or ketone that has two or more hydroxyl group. Thus, there are two categories of sugars: the *aldo*sugars, with a terminal carbonyl group, and the *keto*sugars, with an internal carbonyl group (Harris, 1997; Hardin, *et al.,* 2012).

Table 2.8: Summary of research on biochemical composition in green algae

Algae	Summary of papers	References
Tetraselmis suecica	Algae as feed for *Artemia*	Fibregas, *et al.*, (1996)
Tetraselmis suecica	Changes in the nutrient composition in algae- different nutrient concentrations and renewal rates	Otero and Fabregas, (1997)
Tetraselmisgracilis	Changes in biochemical profile (protein, total amino acids, carbohydrate, lipid, fatty acids) in two culture media	Lourenqo, *et al.*, (1997)
Tetraselmis sp.	Algae used in production of rotifers for the first-feed of marine fish larvae	Reitan, *et al*., (1997)
Tetraselmis suecica	As feed for juvenile clam	Caers, *et al*., (1999)
Tetraselmis suecica.	As feed for Cockles *Cerastoderma edule*	Ibarrola, *et al*., (2000)
Tetraselmis suecica	Algae as feed for tiger prawn larvae	D'Souza and Kelly, (2000)
Ulva lactuca	Nutritional composition (total dietary fiber, ash, crude lipid)	Wong and Cheung, (2000)
Ulva lactuca	The nutritional values of seaweed (in vitro protein digestibility and amino acid profiles)	Wong and Cheung, (2001)
Tetraselmis tetrahele	Algae as feed for window-pane shell, *Placuna placenta* Linnaeus	Madrones-Ladja, *et al*., (2002)
Caulerpa racemosa	Seaweed nutritive value (carbohydrate, mineral contents, lipid)	Akhtar and Sultana, (2002)
Chlorella sp.	Enhancement of fatty acid production of *Chlorella* sp. by addition of glucose and sodium thiosulphate to culture medium	Feng, *et al*., (2005)
Tetraselmis gracilis	Amino acid detection using sequential injection chromatography (SIC)	Rigobello-Masini, *et al.*, (2008)
Nannochloris oculata, Tetraselmis chui & Tetraselmis tetrahele	The effects of different diets of microalgae on the physiology of the two scallop species	Velasco, (2007)
Tetraselmis suecica	Algae as feed for mangrove oyster *Crassostrea corteziensis* juveniles	Rivero-Rodríguez, *et al.*, (2007)
Tetraselmis suecica	Algae as feed for rotifer, white sea bream and gilthead sea bream larvae	Souto, *et al.*, (2008)
Tetraselmis chuii	Algae as feed for the first days of early ornamental cleaner shrimps *Lysmata amboinensis* larvae	Cunha, *et al*., (2008)
Tetraselmis suecica	Use microalgae to improve the biochemical composition of juvenile *Artemia* as prey for *Octopus vulgaris* paralarvae,	Seixas, *et al*., (2008)
Tetraselmis suecia	Algae as feed for harpacticoid copepod *Nitocra spinipes*	Dahl, *et al*., (2008)
Tetraselmis suecica, or a commercial frozen concentrate of *Tetraselmis* sp.	Algae as feed for the copepod *Pseudodiaptomus euryhalinus*	Puello-Cruz, *et al*., (2009)
Chlorella zofingiensis	The lipid contents and fatty acid profiles of *Chlorella zofingiensis* cultured in the dark with various carbon sources	Liu, *et al*., (2010)
Tetraselmis subcordiformis	Effects of nutrient deprivation on biochemical compositions of the microalgae	Ji, *et al*., (2011)
Tetraselmis suecica	Algae as feed for euryhaline cladoceran, *Daphniopsis australis*	Ismail, *et al.*, (2011)
Tetraselmis suecica & Dunaliella tertiolecta	Algae as feed for sea urchin *Paracentrotus lividus* larvae	Carboni *et al*., (2011)

The single most common monosaccharides (in biological world) are the aldohexose D-glucose, $C_6H_{12}O_6$. Disaccarides, which consist of two monosaccharides units linked covalently. Three common dicharrides are maltose, lactose and sucrose.

Polysaccharides are long-chain polymer of sugar and sugar derivatives. It usually consists of a single kind of repeating unit, or sometimes an alternating pattern of two kinds (Hardin, *et al*., 2012). Polysaccharides served for the storage of energy (e.g: starch and glycogen) and as structural components (e.g: cellulose and chitin) in cells. Plant starch is a mixture of amylase, a linear polymer of maltose unit and amylopectin, brances of repeating maltose units (glucose-glucose in α-1,4 linkages) joined via isomaltose linkages. Cellulose in plant cell walls is a linear polymer of repeating cellobioses (glucose-glucose in ß-1,4 linkages) (Stewart, 1974; Barsanti and Gualtieri, 2006). Another important molecules which consist carbohydrate are the 5-carbon monosaccharide ribose, which is an important component of coenzyme (e.g: ATP, FAD, NAD) and backbone of the RNA. The related deoxyribose is a component of DNA (Hardin, *et al*., 2012).

Most low molecular carbohydrate such as monosaccharides, disaccharides are soluble in water, however, ethanol : water (80%v/v) is often used to extract the carbohydrate. In contrast, polysaccharides can be extracted by several methods such as boiling water, mild acids or mild alkali which can be used to solubilize storage polysaccharides (like starch in plant cell), while more vigorous treatment is required for structural polysaccharides, for instant, used 24%w/v KOH to solubilize cellulose (Hardin, *et al.*, 2012; Harris, 1997). Carbohydrate can be identified and quantified using

chemical, enzymatic, chromatographic and also capillary electrophoresis methods (Reed *et al.*, 2003).

2.8.2.2 Protein

Proteins are large biological molecules consisting of one or more chains of amino acids. Most proteins consist of linear polymers built from series of up to 20 different L-α-amino acids via peptide bonds (between the carboxyl and amino group of adjacent amino acid residues) to create polypeptide chain (Hardin, *et al*., 2012; Schultz and Liebman, 1997).

Major forces influencing polypeptide folding and stability are covalent disulfite bond formation and several types of non-covalent interactions: hydrogen bonds, ionic bonds, van der Waals interaction and hydrophobic interactions. Proteins are differ from one another mainly in their sequence of amino acids, which is controled by the nucleotide sequence of their genes and which usually results in folding of the protein into a specific three-dimensional structure that determines its activity (Hardin, *et al*., 2012; Schultz and Liebman, 1997).

Proteins are synthesis from amino acids using information encoded in gene. The DNA sequence of a gene encoded the amino acid sequence of a protein. Each protein has its own unique amino acid sequence that is specified by the nucleotide sequence of the gene encoding this protein. The genetic code is a set of three-nucleotide sets called codons and each three-nucleotide combination designates an amino acid, for example

AUG (adenine-uracil-guanine) is the code for metathionine (Hardin, *et al*., 2012; Schultz and Liebman, 1997).

Genes encoded in DNA are first transcribed into pre-messenger RNA (mRNA) by proteins such as RNA polymerase. mRNA is used as a template for protein synthesis by the ribosome. The process of synthesizing a protein from an mRNA template is known as translation. The mRNA is loaded onto the ribosome and is read three nucleotides at a time by matching each codon to its base pairing anticodon located on a transfer RNA molecule, which carries the amino acid corresponding to the codon it recognizes. The enzyme aminoacyl Trna synthetase "charges" the tRNA molecules with the correct amino acids. Proteins are always biosynthesized from N-terminus to C-terminus (Hardin *et al*., 2012; Schultz and Liebman, 1997).

There are differents methods have been developed to determine the amount of protein in an aqueous solution such as Biuret method, direct measurement of UV absorbance (Warburg-Christian method), Lowry (Folin-Ciocalteau) method and dye-binding (Bradford) method. Most assay for protein content do not give an absolute value but require standard solution, containing appropriate concentrations of a particular protein, to be analysed at the same time, so that a standard curve can be constructed. Bovine serum albumin (BSA) is commonly used as a protein standard (Reed, *et al*., 2003).

2.8.2.3 Lipid

The term lipid is used to describe a broad group of compounds with a wide variety of chemical structure, physical properties and biological function. Lipids maybe widely defines as hydrophobic or amphiphilic small molecules. Lipid can be classified to six main classes, based on their chemical structure: fatty acids, triacyglyccerols, phospholoipids, glycolipids, steroid and terpenes. Lipids in biological systems are often combined with ether proteins (e.g: in lipoproteins) or polysaccharides (e.g: in lipopolysaccharides). Biological lipids are often sub-divided into two main types (i) simple or neutral lipids and (ii) complex, compound or conjugated lipids; each of which contains fatty acids as a major structural components (Hardin, *et al*., 2012; McGarry, 1997). Functionally, lipid play at least three main roles in cells: (i) serve as form of energy storage, (ii) involved in membrane structure (iii) have specific biological function, such as the transmission of chemical signals into and within the cell.

Green algae in general seem to contain few fatty acids with more than 3 double bond or 18 carbon atoms. Minor amounts of γ-linolenic acid occur sometimes but as in higher plants, α- linolenate acid is the main 18:3 acid (Wood, 1974). The major importance of algal lipid applies in their application in biological membranes (Wood, 1974).

There are few methods were used to extract and analysed the lipids in the cell such as solvent extraction, adsorption chromatography, thin layer chromatography (TLC) of lipids and gas chromatography (GC) of lipids and lipids components (Reed, *et al.*, 2003).

2.8.3 Genotoxicity Test

Genotoxicity tests can be defined as *in vitro* and *in vivo* tests designed to detect compounds that induce genetic damage directly or indirectly through various mechanisms (Ohe, *et al.*, 2004). These tests should allow a hazard identification with respect to damage to DNA and its fixation. Fixation of damage to DNA, in the form of gene mutations, larger scale chromosomal damage, recombination and numerical chromosome changes, is generally considered to be essential for heritable effects and in the multistep process of malignancy, a complex process in which genetic changes may play only a part. Compounds which are positive in tests that detect such kinds of damage have the potential to be human carcinogens and/or mutagens, that is may induce cancer and/or heritable defects (CDER, 1997). Genotoxic agents cause primary DNA lesions such as formation of DNA-adducts, oxidation of bases, base-dimerization, or cross-link, which are either repaired or lead to irreversible alteration of the DNA or cause cell death (Preston and Hoffmann, 2008; Young, 2002; Liu, 2004).

To investigate if individual compounds induce DNA-damage, a number of standardized test systems and guidelines have been developed (OEDC, 1997; Young, 2002; Liu, 2004). Since hazardous chemicals occur in low concentrations in the environment, the detection of genotoxic effects requires the use of concentration procedures or highly sensitive indicator organisms, which can be employed for *in situ* monitoring (Liu and Cordes, 2004). Most of the indicator species have been employed in genetic research for many decades and important features include the availability of information on their genomic structure and on the sequence of the target genes, a low number of chromosomes, which is suitable for chromosomal aberration analyses, genetic

stability and fast growth that is short generation period (Liu and Cordes, 2004; Ohe, *et al.*, 2004; CDER, 1997).

For detection and monitoring purposes in the aquatic system, genotoxicity tests may be carried out using the wastewater from chemical manufacturing plants (Pavlica, *et al.,* 2001), agricultural drainage containing pesticides, textile and dye industries (Sumathi, *et al.,* 2001), rubber and palm oil mills, petroleum refineries, landfill and waste disposal sites (Deasi, *et al.*, 2010; Markou and Georgakakis, 2011). A variety of genotoxicity tests have been developed for detecting DNA damage in the aquatic organisms including Random Amplified Polymorphic DNA (RAPD) analysis, single-cell gel (SCG) electrophoresis or comet assay, detection of DNA repair synthesis, chromosomal abberations and sister chromatid exchanges (SCES), alkaline elution and micronucleus assay. In the aquatic system, the following genotoxicity tests are currently employed (Ohe, *et al.*, 2004).

2.8.3.1 Random Amplified Polymorphic DNA (RAPD) Assay

Random Amplified Polymorphic DNA (RAPD) analysis described by Williams *et al*., (1990) is a commonly used molecular marker assay in genetic diversity studies. Other related techniques include Arbitrary Primed PCR (AP-PCR) (Welsh and McClelland, 1990) and DNA Amplification Fingerprinting (DAF) (Caetano-Annoles, *et al*., 1992). These methods differ from RAPDs in primer length, the stringency conditions and the method of separation and detection of the fragments.

The principle involved in generating RAPDs is that, a single, short oligonucleotide primer, which binds to many different loci, is used to amplify random sequences from a complex DNA template. This means that the amplified fragment generated by PCR depends on the length and size of both the primer and the target genome. The assumption is made that a given DNA sequence (complementary to that of the primer) will occur in the genome, on opposite DNA strands, in opposite orientation within a distance that is readily amplifiable by PCR (Wolf, *et al*., 2004).

These amplified products (of up to 3.0 kb) are usually separated on agarose or polyacrylamide gel electrophoresis and subsequent visualized by staining with ethidium bromide. The use of a single 10-mer oligonucleotide promotes the generation of several discrete DNA products and these are considered to originate from different genetic loci. Polymorphisms result from mutations or rearrangements either at or between the primer binding sites and are detected as the presence or absence of a particular RAPD band (Figure 2.1).

The examples taken from literature shows that RAPDs are useful to infer genotoxic related population genetic effects by considering band intensity differences or gain/loss of RAPD bands (diagnostic analysis). They may also provide information on the overall genetic diversity (phenetic numerical analysis) and structure of populations (genetic numerical analysis) (Wolf, *et al.,* 2004). The RAPD assay has been widely used to detect polymorphism in different fields, such as phylogeny (Ho, *et al*., 1995), taxonomy (Wong, *et al*., 2007), epidemiology (Fancelli, *et al.,* 1998; Lin, *et al.,* 1996; Marillia and Scoles, 1996), genetic diversity (Campos, *et al*., 1994; Grayson, *et al*., 1999), pedigrees (Tinker, *et al.,* 1993), the construction of genetic maps (Binelli and

Bucci, 1994), the identification of cultivars (Koller, *et al.*, 1993), pest resistance genes (Dax, *et al.*, 1994), sex markers (Sim, *et al*., 2007; Hormaza, *et al*., 1994).

In an ecotoxicological context, the RAPD assay has been used successfully to diagnose the genotoxicity of a variety of compounds, including endocrine disruptors (4-*n*-nonylphenol and 17-estradiol) (Atienzar, *et al*., 2002a), benzo{*a*}pyrene (BaP) (Atienzar, *et al*., 1999); Benzo[a] pyrene (B[a]P) diol epoxide (BPDE) (Atienzar, *et al*., 2002b), mitomycin-C (Becerril, *et. al*., 1999), Copper (Atienzar, *et al.*, 2001), UV (Atienzar, *et al.*, 1998; Atienzar, *et al.*, 2000a) or X-rays radiation (Atienzar, *et al.*, 2002c). As reported in several publications, damage to the genomic DNA will then result in changes of the binding site and PCR product, and furthermore alter the electrophoresis pattern (Bacerril, *et al.*, 1999; Savva, 1996).

The RAPD changes such as differences in variation of band intensity as well as gain or loss of the RAPD bands (Atienzar, *et al*., 2000a) between exposed and non-exposed individuals were analysed. Indeed, the gain/loss or intensity differences of RAPD bands may be related to DNA damage, mutations or structural rearrangements induced by genotoxic agents, affecting the primer sites and/or interpriming distances (Atienzar, *et al.*, 2002a; 2002b; 2002c). These results make it possible to use this method to detect the genotoxicity of pollutants (Rong and Yin, 2004; Atienzar, *et al.*, 1999; Atienzar, *et al.*, 2000a; Conte, *et al.*, 1998). Aquatic organisms such as *Daphnia magna* (Atienzar, *et al.*, 1997; Atienzar, *et al.*, 1999; Atienzar, *et al.*, 2001; Atienzar, *et al.*, 2004); mussel (Atienzar, *et al.*, 2002b); fish (Castano and Becerril, 2004) and seaweed (Atienzar, *et al.*, 1998; Atienzar, *et al.*, 2000a) have been tested using RAPD assay.

Table 2.9 summarizes the use of RAPD assays for genotoxicity tests in aquatic organisms.

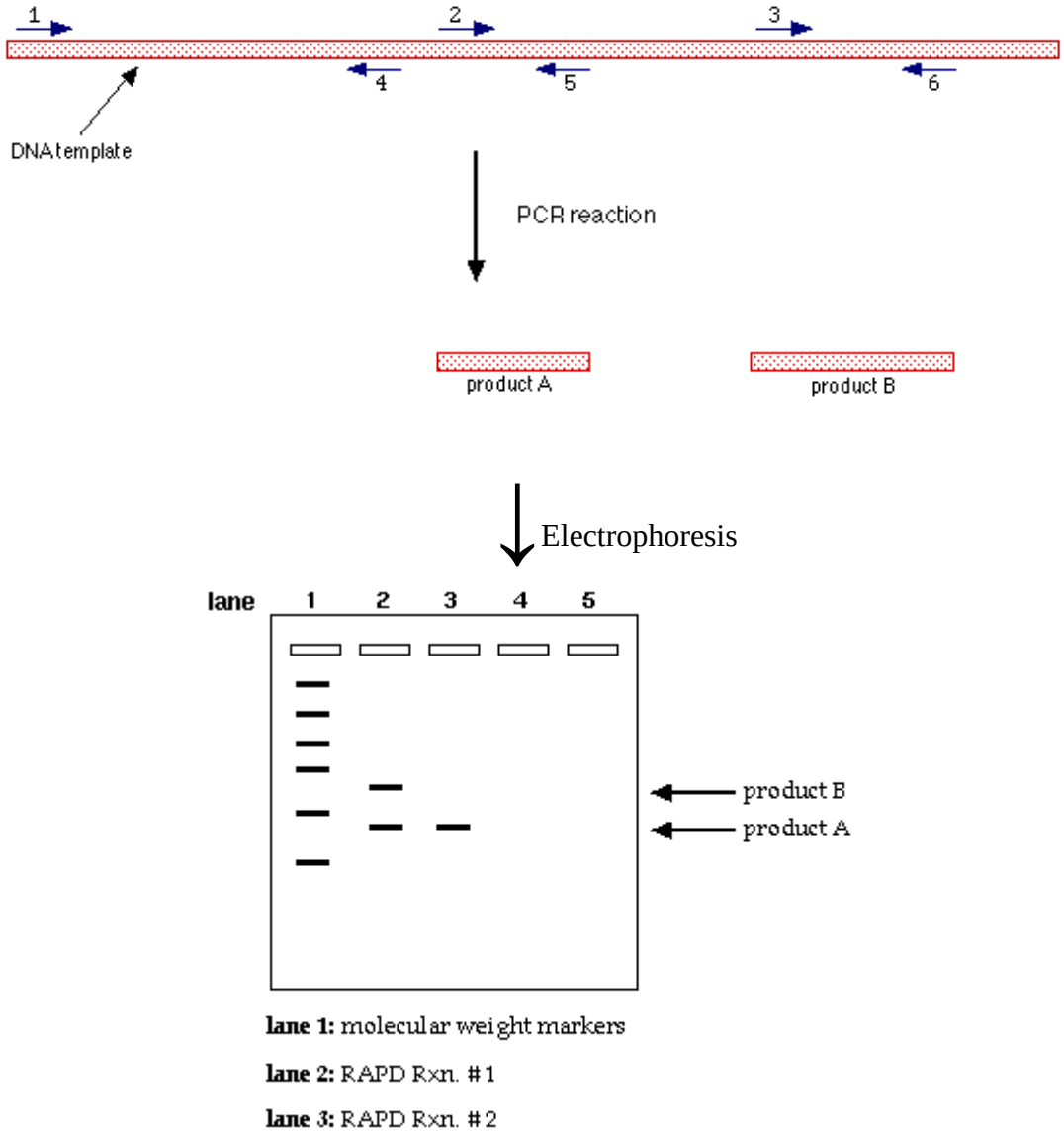


Figure 2.1: Schematic diagram of RAPD analysis

Table 2.9: Summary of environmental genotoxicity tests using RAPD assay in aquatic organisms

Organism	Cell type	Genotoxic agent	Toxicant Exposure time	References
Enteromorpha intenstinalis	Seaweed (Chlorophyta)	UVB under in vivo condition	-	Atienzar, *et al*., (1997)
Daphnia magna.	Zooplankton	UVB under in vivo condition	-	Atienzar, *et al*., (1997)
Oncorhynchus mikyss	A fibroblast-like cell line, derived from rainbow trout	Different methods of DNA extraction (chelating resin, salting out & phenolization)	-	Ferrero, *et al.*, (1998)
Daphnia magna.	Zooplankton	UV radiation	20 min	Atienzar *et al.,* (1998)
Palmaria palmata	Seaweed (Rhodophyta)	UVA, UVB (280-315nm)	20 min	Atienzar *et al.,* (1998)
clonal *Daphnia magna*	Zooplankton	Benzo{*a*}pyrene (BaP)	-	Atienzar *et al*., (1999)
Palmaria palmata	Seaweed (Rhodophyta)	UVB (280-315nm)	3 hrs	Atienzar *et al*., (2000a)
Daphnia magna	Zooplankton	Cu (15,30, 60, 90 & 120ug/L)	15 days	Atienzar *et al*., (2001)
Escherichia coli	Bacteria	The genomic DNA were cut using different restriction enzymes confirmed that *Eco*RI, *Hin*d III, *Not* I & *Pme* I	incubation for 0, 0.5, 1.5, 3 & 15 h,	Atienzar, *et al.,* (2002b)
Palmaria palmata	Seaweed (Rhodophyta)	Sonication	0, 5, 10, 20, 30, 40, 50, 60 & 90 s under continuous cycle,	Atienzar, *et al.,* (2002b)
Mytilus galloprovincialis	Mediterranean mussels	Benzo[a] pyrene (B[a]P) diol epoxide (BPDE)	1 h at 37 °C.	Atienzar, *et al.,* (2002b)
Elminius modestus	Larval barnacles	Man-made estrogens: 17b-estradiol (E2) & 4-n-nonylphenol (NP)	8 days	Atienzar, *et al.,* (2002a)
Daphnia magna	Zooplankton	Benzo{*a*}pyrene (BaP)	-	Atienzar, *et al.,* (2002c)
Palmaria palmata	Seaweed (Rhodophyta)	Sonication	-	Atienzar, *et al.,* (2002b)
Palmaria palmata	Seaweed (Rhodophyta)	Endocrine disruptors	-	Atienzar, *et al.,* (2002a)
Daphnia magna	Zooplankton	Benzo[a] pyrene (B[a]P)	-	Atienzar, *et al*., (2004)
Danio rerio	Fish	Cyclophosphamide (0.1 to 10 mg/L) & dimethoate (1.0 to 100 mg/L)	96 hrs	Rong and Yin, (2004)
Daphnia magna	Zooplankton	Benzo[a] pyrene (B[a]P); 20-50ugL	3 or 6 days & were allowed to recover in clean medium for 12 or 9 days,	Atienzar and Jha, (2004)
Mytilus edulis	Marine mollusc embryos	Tritiated water	One-hour (0.37–370 kBq/ml) of HTO	Hagger, *et al.,* (2005)
Euplotes vannus	Marine ciliate protozoa	Nitrofurazone (0, 6,15 and 24 mg/L)	24, 48, 72 & 96 h.	Zhou, *et al*., (2011)

2.8.3.2 AP-Site content assay

An AP site (apurinic/apyrimidinic site), also known as an abasic site, is a location in DNA that did not have either purine or pyrimidine base, due to DNA damages or spontaneously by a hydrolytic process (Loeb, 1989; Mol, *et al.*, 2000; Wilson and Barsky, 2001; Wang, *et al*., 2009). The chemical events that lead to DNA damage include hydrolysis, oxidation and electrophilic attack. These reactions are triggered by exposure of cells to ionizing radiation, non ionizing radiation, chemicals, exogenous agent or they can result from endogenous metabolic processes (Marnett and Plastaras, 2001). It has been estimated that under normal physiological condition at least 10,000 apurinic sites may be generated per human cell daily (Loeb, 1989; Kow, *et al*., 2000).

AP sites can be produced by spontaneous depurination but also occur as intermediates in base excision repair (Marnett and Plastaras, 2001; Nakamura, *et al*., 1998; Nakamura and Swenbergr, 1999; Lindahl and Wood, 1999). In this process, a DNA glycosylase recognizes a damaged base and cleaves the N-glycosidic bond to release the base, leaving an AP site (Fundador and Rusling, 2007). There are a variety of glycosylases that recognize different types of existing damage, including oxidized or methylated bases, or uracil in DNA. The AP site can then be cleaved by an AP endonuclerase leaving 3' hydroxyl and 5' deoxyribosephosphate. In addition, bifunctional glycosylase-lyases can cleave the AP site, leaving a 5' phosphate adjacent to a 3' α, β-unsaturated aldehyde (Melo, *et al*., 2007). Both mechanisms form a single-strand break, which is then repaired by either short-patch or long-patch base excision repair (BER) (Loeb, 1989; Marnett and Plastaras, 2001).

BER is acknowledged as the "workhorse" pathway, responsible for detect ion and repairs such as in DNA base damage, this damaged base is removed by glycosylase, and is restored to the normal base to maintain genome integrity (Hitomi, *et al*., 2007; David *et al*., 2007). The BER process consists of the following steps: (i) excision of an inappropriate base moiety (e.g. 8-OH-dG) (ii) incision at the resulting abasic site, (iii) replacement of the excised nucleotide, (iv) clean-up of the terminal end(s), and (v) sealing of the final nick by a ligase (Friedberg, *et al*., 2006; David, *et al*., 2007; Melo, *et al*., 2007). If the AP sites cannot be correctly repaired, they can lead to mutation and cytotoxic effects where the AP-sites can block DNA replication (Vidal, *et al*., 2007) which then cause replication fork stalling and are bypassed by translesion synthesis (Dianov, *et al*., 2003) and can also cause to cell death (Zhao, *et al*., 2007).

Previous reports by several researches show that the AP-site assay has been widely used to detect DNA damage in different studies, such as in the treatment of cancer (Casadevall, *et al*., 1999; Fortini, *et al*., 2003, Zhao, *et al*., 2007, Wang, *et al*., 2009), oxidative DNA base damage (Asaeda *et al*., 1998; Casadevall, *et al*., 1999; Hatahet, *et al*., 1999; Kow and Dare, 2000; Kanazawa, *et al*., 2000; Gralnick and Downs, 2003, Satoh, *et al*., 2003), and abundantly used in the base excision repair process in *Escherichia coli* (Friedberg, *et al*., 1995; Weinfeld, *et al*., 1997; Parsian, *et al*., 2002; Pfeifer and Stoffele, 2005; Cunniffe, *et al*., 2007), *Saccharomyces cerevisiae* (Boiteux and Guiliet, 2004; Sugimoto, *et al*., 2005), human cell (Mol, *et al*., 1999; Wilson and Barsky, 2001; Melo, *et al*., 2007; Hitomi, *et al*., 2007), Calf thymus DNA (David, *et al*., 2007), Herpes virus (Zhu, *et al*., 2008) and retina cell (Meira, *et al*., 2009).

In addition, the AP-site assay has also been used successfully to diagnose the genotoxicity of a variety of compounds on human cells, including antitumour drug (Casadevall, *et al*., 1999), antibiotics (Bose, *et al.*, 1980; Rabow, *et al.*, 1986), ionizing radiation (Von Somtag, 1987; Kim, *et al.*, 2003; Zhou, *et al*., 2005), UV radiation (Lloyd, 1998), alkylating agents (Loeb and Preston 1986), hydroxyl radicals (Casadevall *et al.*, 1999; Kanazawa *et al.*, 2000; Gralnick and Downs, 2003; Satoh, *et al*., 2003), Methylmethane sufonate, MMS (Asaeda *et al.*, 1998), HNO_2 or NO (Suzuki, *et al*., 2000), Styrene (Vodicka, *et al.*, 2001), Phenanthroline or para-nitrobenzamide (Martelli, *et al.*, 2002) and Dibenzo (a,1) pyrene (Chakravarti, *et al*., 2005).

As reported in few publications, damage to the bases of DNA will then result in changes to the structure of the bases as well as disrupt phosphodiester bonds leading to the fragmentation of the DNA strands in mammalian cell (Aktipis, 1997). Therefore in this study, the AP-site changes such as differences in AP-site content between exposed and non-exposed individuals were analysed. Indeed, the reduction or increment of AP-site content may be related to DNA damage and repair mechanism, mutations or structural rearrangements induced by genotoxic agents, affecting the DNA strand structure (Fundador and Rusling, 2007). The results obtained using this assay may be used to detect the genotoxicity of chemicals contaminants in the algae cell. Table 2.10 summarizes the researches that have been carried out using AP-site assays for genotoxicity studies in selected organisms.

Table 2.10: Summary of papers related to AP-Site count assay

Cell type	Genotoxic agent	References
HeLa cells	Methylmethanesulfonate (MMS).	Asaeda, *et al*., 1998
Calf thymus DNA	Potassium chromate; Glutathion (GSH) and Ascorbate (AsA)	Casadevall, *et al*., 1999
Calf thymus DNA	Tert-butyl hydroperoxide (t-BuOOH)	Kanazawa, *et al*., 2000
Salmonella enterica	Ferrous sulfate; hydrogen peroxide (H_2O_2,)	Gralnick and Downs, 2003
murine proximal tubular cell (PTC)	Cisplatin	Satoh, *et al*., 2003
Calf thymus DNA	Dibenzo[*a*,*l*]pyrene (DB[*a*,*l*]P)	Chakravarti, *et al*., 2005
plasmid DNA	γ-radiation and Peroxynitrite	Zhou, *et al*., 2005
Double-stranded (ds) salmon testes (ST) DNA	Methylmethane sulfonate (MMS)	Fundador and Rusling, 2007

2.8.4 Antioxidant Enzyme Assay: Superoxide Dismutase Activity Assay

Superoxide dismutase (SOD, EC 1.15.1.1) catalyzes the disproportion of the potentially destructive superoxide anion radical ($O_2^{\bullet-}$, a byproduct of aerobic metabolism) to molecular oxygen and hydrogen peroxide: $2O_2^{\bullet-} + 2H^+ \rightarrow H_2O_2 + O_2$. SOD was discovered by Irwin Fridovich and Joe McCord in 1969 (McCord and Fridovich, 1969).

The history of SOD started in 1938 by Mann and Keilin (Mann and Keilin, 1938) who isolated a bovine erythrocyte protein, thought to be a copper storage protein. They termed the 31.5kDa protein as hemocuprein. Subsequently, the enzyme was identified by a number of names, erythrocuprein, indophenol oxidase and tetrazolium oxidase until its catalytic function was discovered by McCord and Fridovitch, (1969). In 1969, 30 years after the initial isolation, McCord and Fridovich appropriately renamed the protein as superoxide dismutase, after discovering that the enzyme catalyzed the dismutation of superoxide radical ($O_2^{\bullet-}$) to molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) (McCord and Fridovich, 1969; Perry, *et al*., 2010).

The SODs are comprised of 150 to 220 amino acids residue subunits that form homodimeric or tetrameric protein complexes that coordinate specific metal cofactors (Bafana, *et al.,* 2011; Perry, *et al.,* 2010; Wolfe-Simon, *et al.,* 2005).

SODs are metalloproteins that can be classified into four types according to their metal cofactor: Cu/Zn (Cu/Zn-SOD), Mn (Mn-SOD), Fe (Fe-SOD) and Ni (Ni-SOD). Cu/ZnSOD (also known as SOD1 and SOD3 in humans), is found almost exclusively in eukaryotes in the cytosol and/or chloroplast and some prokaryotes. MnSOD (also known as SOD2 in humans), group is widely distributed in cytoplasm of many prokaryotes (bacteria) and in the mitochondria of eukaryotes. FeSOD (also known as SOD2 in humans), which show amino acid sequence homology with the corresponding manganese enzymes has been found in prokaryotes and within the chloroplast of some plant families. FeSOD and MnSOD have diverged significantly from each other, so that the two metals cannot functionally substitute for each other in Mn/FeSODs from most species. Ni-SOD has been found only in bacteria (*Streptomyces* and cyanobacteria) (Bafana, *et al.,* 2011; Perry, *et al*., 2010; Misra and Fridovich, 1977).

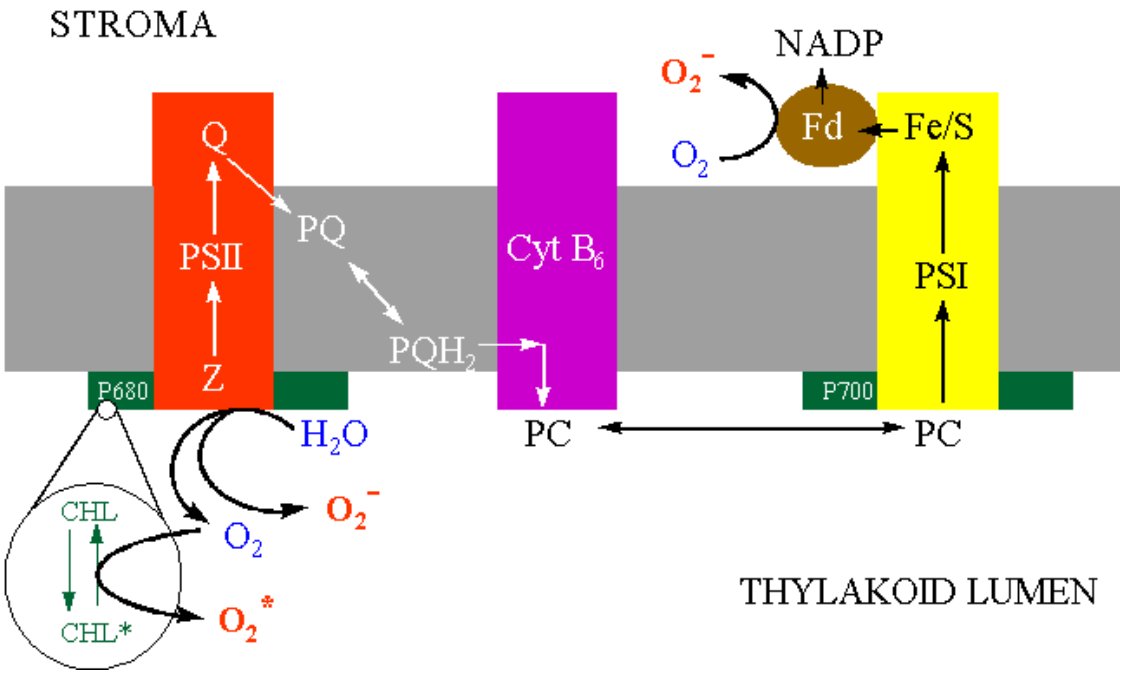
Typically for algae, the chlorophytes, haptopytes and embryophytes contain either FeSOD or multiple combinations of Fe, Mn, and Cu/ZnSODs. Cyanobacteria have either a NiSOD alone or combinations of Mn and Ni or Fe and Mn metalloforms (Cu/Zn is rare among the cyanobacteria). The bacillariophytes and rhodophytes retained an active MnSOD (Wolfe-Simon, *et al*., 2005).

SOD is one of the most important enzymatic antioxidants that are involved in detoxication of free radicals in the cell (Klaassen, 2008). Free radicals such as

superoxide anion and hydroxyl radicals are the main reactive oxygen species (ROS) that are involved in oxygen toxicity (Perry, *et al*., 2010, Klaassen, 2008). Superoxide anion is formed as a by-product of many oxidative processes in biological system including aerobic metabolism, respiration, oxidative phosphorylation and photosynthesis (Perry, *et al.*, 2010; Perelman, *et al*., 2006; Frodovich, 1972) that usually occurr in the chloroplast, mitochondria, endoplasmic reticulum, microbodies, plasma membrane and cell wall of the plants (Perelman, *et al*., 2006; Rhee, *et al*., 2005).

According to Schmid (1975), in photosynthesis of green plants including algae, superoxide anion ($O_2^{\bullet-}$) act as an intermediate in the reduction sequence of photosystem I. This was inferred from ascorbate oxidation (Epel and Neumann, 1973), from ascorbate mediated photophosphorylation (Elstner and Kramer, 1973) and from sulfite oxidation (Asada and Kiso, 1973) by chloroplast. In the zig-zag arrangement of the electron transport chain in the chloroplast membrane, the reducing site of photosystem I, is located at the outer side of the membrane (Junge and Auslander, 1973; Brzborn, 1969; Trebst, 1974) as shown in Figure 2.2. The formation of superoxide anion occurs at that site. In the absence of Ferredoxin-NADP, superoxide anion is formed directly by reduction of oxygen (Schmid, 1975).

In mitochondria, most oxygen is consumed by the cytochrome oxidase enzyme in the mitochondrial electron transport system (Rich and Bonner, 1978). According to Kowaltowski and Vercesi, (1999); up to 2% of the oxygen consumed by the mitochondrial respiratory chain received one electron reduction, typically by the semiquinone form of coenzyme Q, to produce the supeoxide radical and subsequently other ROS such as hydrogen peroxide and hydroxyl radicals as shown in Figure 2.3.



a) Singlet oxygen may be produced from triplet chlorophyll in the light harvesting complex.
b) Superoxide and hydrogen peroxide may "leak" from the oxidizing (water-splitting) side of PSII.
c) Triplet oxygen may be reduced to superoxide by ferredoxin on the reducing side of PSI.

Figure 2.2: Schematic representation of the electron transport system in the thylakoid membrane showing three possible sites of activated oxygen production (Elstner, 1991).

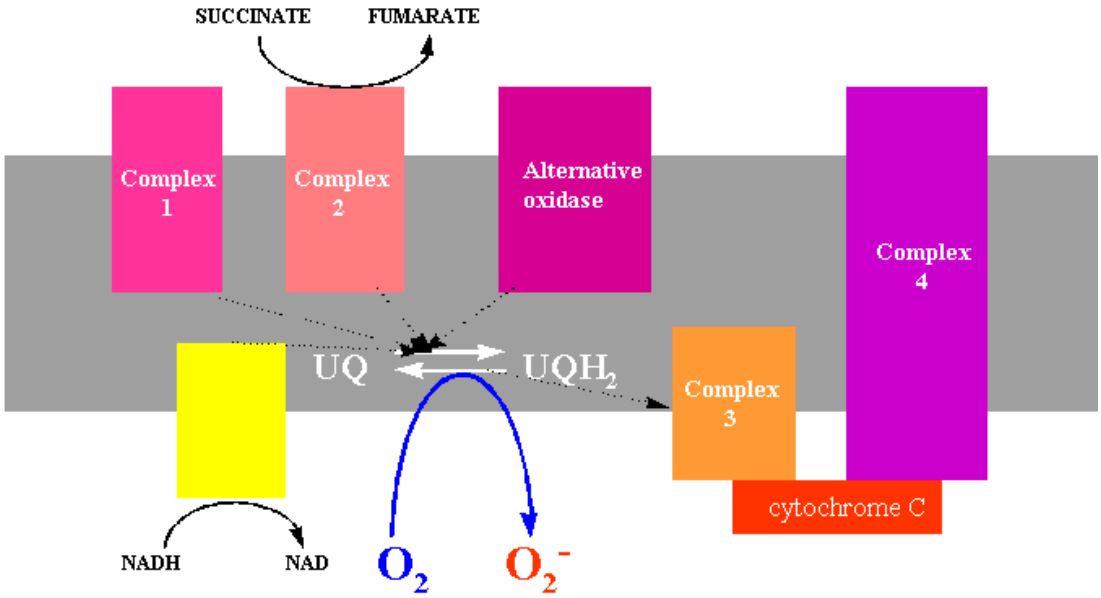


Figure 2.3: Schematic representation of the electron transport system in the mitochondrial membrane showing a possible site of superoxide production by reduced ubiquinones (Elstner, 1991).

ROS are generally indisciminant molecules and can potentially damage any cellular component. Biochemical impacts of ROS include: (i) Oxidations of unsaturated lipid components of membranes (lipid peroxidation); (ii) Oxidation of amino acids and proteins (resulting in,e.g: the addition of carbonyl groups); (iii) DNA oxidation resulting in products such as 8-hydroxy-guanosine and thyme glycol (Halliwell and Gutteridge, 1999) and (iv) Perturbed redox status (Schafer and Buettner, 2001).

SODs have a function as master keys controlling cellular ROS level in the cell (Perry, *et al*., 2010). SODs are enzymes that disproportionate superoxide anion radicals at the fastest enzyme rates ever known. According to Abdallah, *et al*., (2009); Peixoto, *et al.,* (2009) and Shopova *et al.*, (2009), SODs and SOD mimetics may have potential application as therapeutic agents in oxidative stress-related diseases. Furthermore, upregulation of SOD expression can suppress the malignant phenotype of human melanoma cells (Church, 1993).

In algae ROS play an important role in signaling of pathways that respond to biotic and abiotic stress. ROS are also activated as defense molecules when algae are attacked by pathogens, or to reduce competition by killing bacteria, reducing Fe availability and other biological ROS producer. Because ROS can indiscriminately cause damage in algae, the function of SOD is very important in protecting algae (Bafana, *et al.,* 2011). Increased level of SOD can also protect algae against physical (salinity, UV radiation, high light intensity, temperature, desiccation, etc) and chemical (metal ions, pesticides, PAH, allelochemical) stresses. Table 2.11 summarizes the list of SOD assays studies in the algae that have been reported by researchers.

Table 2.11: Summary of SOD assay in algae exposed to chemical contaminants

Organism	Genotoxic agent	Toxicant Exposure time	References
Porphyridium cruentum	Cyanide, hydrogen peroxide (H_2O_2), azide	-	Misra and Fridovich, (1977)
Chlorella sorokiniana	Paraquat	-	Rabinowitch, *et al.*, (1983)
Dunaliella salina	Paraquat	-	Rabinowitch, *et al.*, (1987)
Synechococcus sp. strain with no functional FeSOD	Methyl viologen (paraquat), norflurazon (NF).	-	Thomas, *et al.*, (1998)
Gloeocapsa sp.	Cyanide, azide.	-	Hammouda, (1999)
Gonyaulax polyedra	Hg^{2+}, Cd^{2+}, Pb^{2+}, Cu^{2+}.	48 hrs	Okamoto, *et al*., (2001)
Kappaphycus alvarezii	Clofibrate (CFB)	4 & 72 h.	Barros, *et al*., (2003)
Karenia brevis	Temperatures, H_2O_2, Pb, light intensities	4 hrs	Miller-Moreya, *et al.*, (2004)
Scenedesmus armatus	Anthracene & Phenanthrene with different PAR irradiance & CO_2 level	24 hrs	Aksmann and Tukaj, (2004)
Scenedesmus sp.	Cu, Zn	4 hrs	Tripathi and Gaur, (2004).
Scenedesmus obliquus	Cypermethrin		Li, *et al.*, (2005)
Scenedesmus sp. *Scenedesmu subspicatus,* *Scenedesmu obliquus,* *Scenedesmu microspina*	Anthracene (aromatic hydrocarbon), $CdCl_2$, chloridazone (herbicide)	12 - 48 h	Zbigniew and Wojciech, (2006)
Scenedesmus sp.	Cu, Zn	6 hrs, 7 days	Tripathi, *et al*., (2006)
Chattonella marina	Nutrients: N, P, Fe; salinity, pH & light:dark cycle	8 days	Liu, *et al*., (2007)
Chlorella vulgaris	Glufosinate (herbicides)	12 - 96 h	Qian, *et al*., (2008)
Gracilaria lemaneiformis	Nitrogen & phosphorus (N/P)	15 days	Yu and Yang, (2008)
Chlorella vulgaris	N-phenyl-2-naphthylamine (allelochemical)	-	Qian, *et al.*, (2009)
Scenedesmus vacuolatus *Chlorella kessleri.*	Cu	7 days	Sabatini, *et al.* , (2009)
Gracilaria corticata	Desiccation	4 h	Kumar, *et al*., (2011)
Chlorella vulgaris	Phytohormon containing Cd, Cu, Pb	24 hrs	Piotrowska-Niczyporuk, *et al*., (2012)
Ulva compressa	Cu	7 days	Mellado, *et al*., (2012)
Anabaena sp. *Microcystis viridis*	Combination of UV-B & herbicides (DCMU) & ascorbic acid	2 hrs, recovery exp 24 hrs	Chen, *et al*., (2012)
Ulva pertusa	Coumaphos (organophosphate insecticides)	6 h, 1-7 days after exposure to light.	Schweikert and Burritt, (2012)

2.9 POSSIBLE MECHANISMS OF CHEMICAL TOXICITY IN THE ALGAE

Increased distribution of chemical contaminants (toxicants) in marine environment, especially through anthprogenic activities, raises increasing concern for ecotoxicological effects. Reports of toxicity are common in marine organisms (Zhou, *et al*., 2011; Tigini, *et al.,* 2011; Carrera-Martinez, *et al.*, 2010) including in algae which are the primary producers in the marine food chain (Schweikert and Burritt, 2012; Mellado, *et al*., 2012; Piotrowska-Niczyporuk, *et al*., 2012). Majority of the toxicants occurring in the environment comprise metals and metal compounds. Chemically, metals in their ionic form can be very reactive and can interact with biological systems in a large variety of ways.

(i) Ionic metals can bind with other macromolecules in the cell (metal-binding ligands). For example, metals like cadmium and mercury can attach to sulfur in proteins as a preferred bio-ligand. Such binding is an important chemical mechanism, where exogenous metals which are toxic, can result in steric re-arrangement that impairs the function of biomolecules (Kasprzak, 2002). For example in inhibition of enzyme activity by metal interaction at sites other than the active centre.

(ii) The toxic metal may act as a mimic of essential metals, binding to physiological sites that normally are reserved for an essential element (Kasprzak, 2002; Cousins, *et al*., 2006; Liu, *et al.,* 2008; Klaassen, 2008). Through mimicry, the toxic metal may gain access to, and potentially disrupt a variety of important metal-mediated cellular functions. For example, mimicry for, and replacement of zinc, is a mechanism of toxicity for cadmium, copper and nickel.

(iii) Ionic metal can also mediate oxidative damage which involves the formation of reactive oxygen species (ROS) like superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical ($^{\bullet}OH$) in the cell. Mechanisms by which chemicals can enhance ROS production include redox cycling, interactions with electron transport chains (especially in mitochondria, microsomes or chloroplast) and photosensitization.

Redox cycling is maybe the most common mechanism by which a diverse array of chemicals including many environmental pollutants, in generation of intracellular ROS. In redox cycle, the parent compounds accept an electron from a reduced cofactor, such as NADH or NADPH. This reaction is usually catalyzed by a reductase such as xanthine oxidase or cytochrome P450 reductase (Kappus and Sies, 1981; Kappus, 1986). In the presence of O_2, the unpaired electron of the radical metabolite is donated to O_2, yielding O_2^- and regenerating the parent compound. Most importantly, the parent compound can repeat this cycle until it is cleared or metabolized to an inactive product. In the course of each redox cycle, two potentially events that can cause damage occur; a high energy reducing equivalent is expended (e.g: the oxidation of NADPH to $NADP^+$), and an oxygen radical is produced. A generalized redox cycle that includes association with cellular toxicities and antioxiodant defenses are shown in Figure 2.4.

Many metals can directly act as catalytic centres for redox reaction with molecular oxygen or other endogenous oxidants, producing oxidative modification of biomolecules such as protein and DNA (Kasprzak, 2002). Alternatively, metals may displace redox active essential elements from their normal cellular ligands, which in turn, may result in oxidative cellular damage. For example, cadmium which is not redox

active may cause oxidative stress through the release of endogenous iron, an element with high redox activity (Valko, *et al.,* 2006).

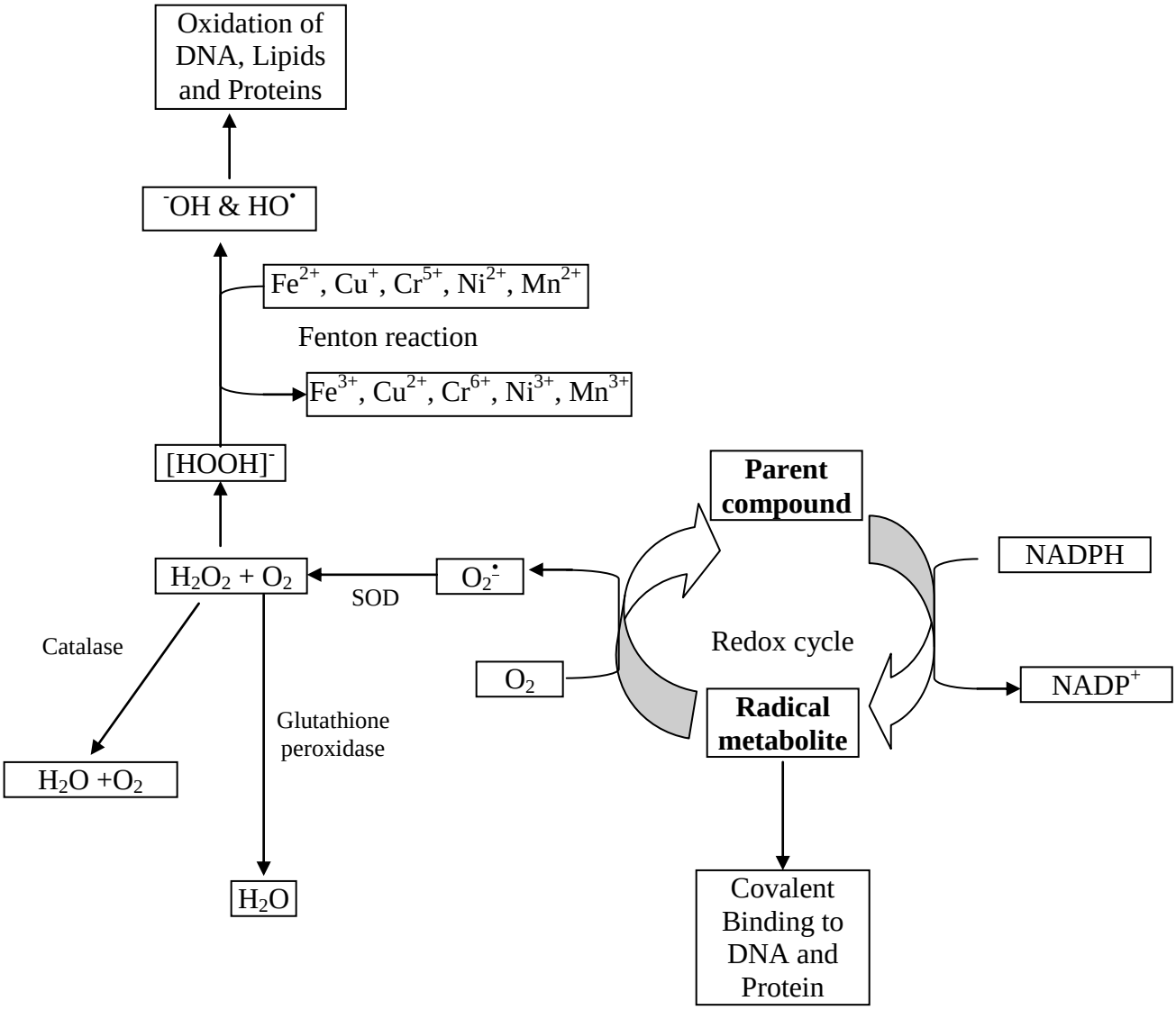


Figure 2.4: Overview of oxidative stress, including reactive oxygen species stimulation initially by redox cycling, key antioxidant defenses and potential deleterious biochemical effects (Giulio and Newman, 2008)

Some ROS, including superoxide anion ($O_2^{\bullet}$) and hydroxyl radicals ($HO^{\bullet}$) are free radicals. A free radical is a molecule or molecular fragment that contains one or more unpaired electrons in its outer orbital. Radicals are formed by accepting an electron (redox cycling) or losing an electron or by homolytic fission of a covalent bond. Free radicals formed by homolytic bond fission, can be induced by electron transfer to the molecule (reductive fission). For example, reductive hemolytic fission of hydrogen peroxide (H_2O_2) to $OH^{\bullet}$ and HO^- is called the Fenton reaction. This is catalyzed by transition metal ions, typically Fe(II), Cu(I), Cr (VI), Ni(II), Mn(II) (Gregus, 2008).

Therefore, based on the previous reports, toxicity (especially metals) has been shown to be linked to oxidative stress. Oxidative stress directly or indirectly leads to physiological dysfunction in most organisms, from single-celled organisms to mammals and plants (Sabatini, *et al.,* 2009; Anjum, *et al.,* 2012; Lushchak, 2011). With respect to toxicants- induced cellular damage to macroalgae and microalgae cells, many studies have reported that metals or compounds containing metals is an inducer of oxidative stress in unicellular algae (Schweikert and Burritt, 2012; Piotrowska-Niczyporuk, *et al*., 2012; Sabatini, *et al*., 2009).

In the algae cell, oxidative stress can occurr in the chloroplast and mitochondria (Okamoto, *et al*., 2001; Pospisil, (2009) as shown in Figure 2.2 and Figure 2.3. Chloroplasts are highly harmed by oxidative stress due to elevated oxygen concentration, electron flux, and presence of metal ions in their microenvironment (Foyer, 1996). Electron transport processes in chloroplasts and mitochondria are the potential source of ROS (Apel and Hirt, 2004). The major generation site of ROS in the thylakoid membrane of chloroplast is photosystem I (PSI) and photosystem II (PSII). Although

ROS are mainly produced by PSI, the light-induced production of various types of ROS was shown in PSII (Asada, 2006). In this respect, PSII itself is unique: (i) it is the source of molecular oxygen and (ii) it performs redox chemistry with extremely reducing and oxidizing potentials. Its reducing components are capable of reducing molecular oxygen, whereas the oxidizing components are capable of oxidizing water (Klimov, *et al*., 1979; Rappaport, *et al*., 2002).

In addition, PSII contains a number of chlorophyll molecules that can act as a photosensitizer for the production of singlet oxygen ($^1O_2$). It has been shown that electron-hole recombination chemistry can produce a triplet state of chlorophyll that converts triplet molecular oxygen into its reactive singlet form (Krieger-Liszkay, 2005; Vass and Styring, 1993; Telfer, *et al*, 1994) which then can oxidize other molecules.

Furthermore, superoxide anion ($O_2^{\bullet -}$) can be produced by the reduction of oxygen in photosystem I (Mehler reaction). Diffusion of superoxide anion ($O_2^{\bullet -}$) into stroma is followed by its dismutation to oxygen and hydrogen peroxide (H_2O_2). Reaction of H_2O_2 with reduced metal ions produces hydroxyl radical (HO•), a strong oxidant that can react with and damage several biomolecules (Takeda, *et al*. 1995). In addition, chloroplasts have a complex system of membranes rich in polyunsaturated fatty acids, which are potential targets for peroxidation (Halliwell and Gutteridge 1999). Under normal conditions, these ROS–generating processes are slow, but they can be accelerated by xenobiotics, chemical contaminants, pollutants and other factors, such as high light or UV exposure (Rao, *et al*. 1996; Mackerness, *et al*. 1999).

3.0 MATERIALS AND METHODS

The procedures for conducting toxicity tests described in this chapter are based on established methodologies (ASTM, 1993; APHA, AWWA and WPCF, 1998; Reish and Oshida, 1986; Ward and Parrish, 1982) with some modifications. Quality Assurance and Quality Control was ensured by using standard laboratory procedures in all toxicity tests including proper documentation, proper cleaning, an avoidance of contamination and maintenance of test conditions. All test organisms (algae) were maintained in a separate area from the place where toxicity tests were conducted, test solutions prepared or equipment cleaned. The sub culturing process for algal maintenance and the initiation of the tests were carried out in the UV-sterilized chambers (ESCO, Lamina Flow Clean Device, EVC-A, ESCO Micro (M) Sdn Bhd, Malaysia) to avoid possible contamination. The algal toxicity test were carried out in rooms and in an incubation chamber and shelves with constant temperature ($25 \pm 1^{o}C$) and appropriate illumination (42 µmol photon/s/m^2 with 12h : 12h, light : dark cycle).

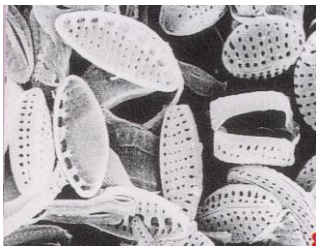
All containers used in this study were made of disposable plastic ware, glass and high density polyethylene plastic to minimize the dissolution, leaching and sorption of the metal cations. Prior to use and at the end of the test, all the glassware and plastic ware (to be used again in the toxicity tests) were cleaned with non-phosphate detergent and rinsed with running tap water. Then rinsed once with 20% HNO_3 (to remove metals) followed twice by distilled water. After that, rinsed with concentrated acetone (to remove organics) and rinsed again with distilled water for three times and finally oven dried before use. The chemical contaminants used in this study will now on be referred to as toxicants.

3.1 EFFECT OF SELECTED TOXICANTS ON GROWTH OF ALGAE AND TOXICITY STUDIES

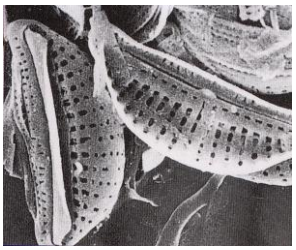
3.1.1 Preparation of Test Organisms

3.1.1.1 Source of the test organisms

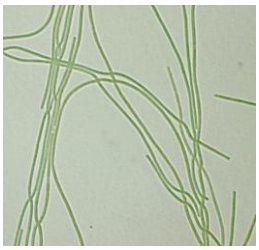
Twenty five species of microalgae and two species of green macroalgae (seaweed) namely *Boergesenia forbesii* (Harvey) Feldman and *Ventricaria ventricosa* (Agardh) Olsen & West were used in the study (Fig. 3.1). All microalgae were obtained from the University of Malaya Algal Culture Collection (UMACC) at the Institute of Graduate Studies, University of Malaya. These microalgae were isolated and purified from the known origin or collection site as shown in Table 3.1. Taxonomic identifications of the cultures were confirmed prior to study. Twenty five species of marine phytoplankton used as test organisms were *Chlorella vulgaris* Beijerinck (UMACC 103), *Chlorella vulgaris* Beijerinck (UMACC 104), *Nitzschia inconspicua* Grunow (UMACC 111); *Amphora turgida* Gregory (UMACC 113); *Oscillatoria* sp. (UMACC 115); *Oscillatoria* sp. (UMACC 118); *Oscillatoria* sp. (UMACC 119); *Oscillatoria* sp. (UMACC 123); Bacillariophyta (UMACC 125); *Oscillatoria* sp. (UMACC 126); *Tetraselmis* sp. (UMACC 129); *Chaetoceros* sp. (UMACC 133); *Oscillatoria* sp. (UMACC 136); *Chaetoceros* sp. (UMACC 140); *Isochrysis galbana* Parke (UMACC 141); *Chaetoceros* sp. (UMACC 142); *Tetraselmis tetrahele* (West) Butcher (UMACC 144); *Tetraselmis* sp. (UMACC 145); *Tetraselmis* sp. (UMACC 146); *Chaetoceros calcitrans* Takano (UMACC 147); *Chaetoceros* sp. (UMACC 148); *Nannochloropsis oculata* (Droop) Hilberg (UMACC 166); *Nannochloropsis* sp. (UMACC 167); *Porphyridium cruentum* (Agardh) Naegeli (UMACC 196) and *Chlorella vulgaris* Beijerinck (UMACC 245).



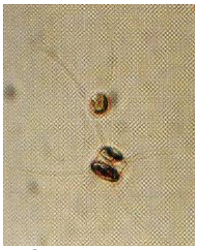
Nitzchia incospicua
UMACC 111



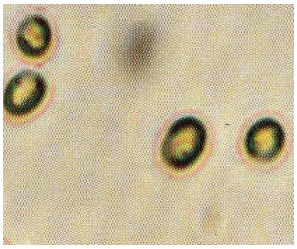
Amphora turgida
UMACC 113



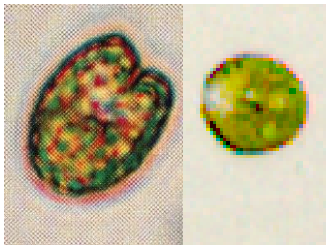
Oscillatoria sp.
UMACC 118



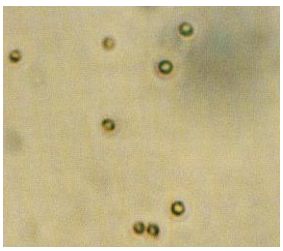
Chaetoceros sp.
UMACC 140



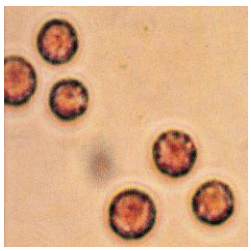
Isochrysis galbana
UMACC 141



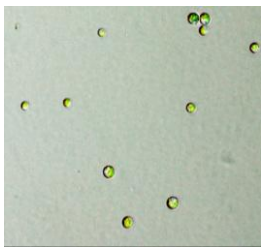
Tetraselmis tetrahele
UMACC 144



Nannochloropsis oculata
UMACC 167

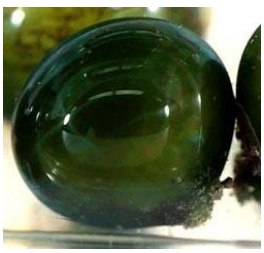


Porphyridium cruentum
UMACC 196



Chlorella vulgaris
UMACC 245

Boergesenia forbesii



Ventricaria ventricosa

Figure 3.1: Algae used in the study

Table 3.1: Microalgal Cultures

Species	Original Location of Sample
Chlorella vulgaris Beijerinck (UMACC 103)	Klang Estuary, Selangor
Chlorella vulgaris Beijerinck (UMACC 104)	Muddy waters of Sementa Mangrove, Selangor
Nitzschia inconspicua Grunow (UMACC 111)	Boat scraping, Malacca
Amphora turgida Gregory (UMACC 113)	Boat scraping, Malacca
Oscillatoria sp. (UMACC 115)	Boat scraping, Malacca
Oscillatoria sp. (UMACC 118)	Boat scraping, Malacca
Oscillatoria sp. (UMACC 119)	Boat scraping, Malacca
Oscillatoria sp. (UMACC 123)	Boat scraping, Malacca
Bacillariophyta (UMACC 125)	Boat scraping, Malacca
Oscillatoria sp. (UMACC 126)	Boat scraping, Malacca
Tetraselmis sp. (UMACC 129)	Perth, Australia (Bought from Murdoch University Algal Culture Collection- MUACC- MUR 168)
Chaetoceros sp. (UMACC 133)	Coastal waters of the National Prawn Fry Production and Research Centre (NAPFPRE) Pulau Sayak, Kedah
Oscillatoria sp. (UMACC 136)	Boat scraping, Malacca
Chaetoceros sp. (UMACC 140)	Taiwan. Gift from National Prawn Fry Production and Research Centre (NAPFPRE) Pulau Sayak, Kedah
Isochrysis galbana Parke (UMACC 141)	Coastal waters of the National Prawn Fry Production and Research Centre (NAPFPRE) Pulau Sayak, Kedah

MUACC : Murdoch University Algal Culture Collection
NAPFPRE : National Prawn Fry Production and Research Centre

Table 3.1: Microalgal Cultures (continued)

Species	Original Location of Sample
Chaetoceros sp. (UMACC 142)	Tahiti. Gift from National Prawn Fry Production and Research Centre (NAPFPRE) Pulau Sayak, Kedah
Tetraselmis tetrahele (West) Butcher (UMACC 144)	Japan. Gift from National Prawn Fry Production and Research Centre (NAPFPRE) Pulau Sayak, Kedah
Tetraselmis sp. (UMACC 145)	France. Gift from National Prawn Fry Production and Research Centre (NAPFPRE) Pulau Sayak, Kedah
Tetraselmis sp. (UMACC 146)	Japan. Gift from National Prawn Fry Production and Research Centre (NAPFPRE) Pulau Sayak, Kedah
Chaetoceros calcitrans Takano (UMACC 147)	Fisheries Research Institute, Penang
Chaetoceros sp. (UMACC 148)	Fisheries Research Institute, Penang
Nannochloropsis oculata (Droop) Hilberg (UMACC 166)	Coastal waters of the National Prawn Fry Production and Research Centre (NAPFPRE) Pulau Sayak, Kedah
Nannochloropsis sp. (UMACC 167)	Coastal waters of the National Prawn Fry Production and Research Centre (NAPFPRE) Pulau Sayak, Kedah
Porphyridium cruentum (Agardh) Naegeli (UMACC 196)	Gift from North Carolina Biological Supply Company, USA
Chlorella vulgaris Beijerinck (UMACC 245)	Coastal waters at Terengganu

MUACC : Murdoch University Algal Culture Collection
NAPFPRE : National Prawn Fry Production and Research Centre

3.1.1.2 Microalgae stock culture maintenance.

The purified microalgal cultures on agar slants (2% w/v) were maintained in sterile Provasoli 50 (Prov50) Medium (CCMP, 1986). The composition of Prov50 Medium is shown in Appendix 4. The slants were placed on illuminated shelves, incubated at 25±1 ^{o}C under 12:12 h light-dark cycle with irradiance of 40 – 60 μmol photon/s/m^2. Sub-culturing was done every two months.

3.1.1.3 Preparation of inoculum for flask cultures

A loopful of each culture was taken from the slant and dispersed into 100 mL sterile Prov50 medium in 250 mL conical flasks. The flasks were shaken at 100 rpm in an incubator shaker (B.Braun, incubations schüttelschrank BS4, Melsungen AG, Germany), under illumination of 42 μmol photon/s/m^2 with 12h:12h, light:dark cycle and temperature at 25± 1^{o}C for five days or when the optical density at 620 nm (OD_{620}) reached 0.2, as measured using a spectrophotometer (Shimadzu UV- 160A, Shimadzu Corporation, Japan). The exponential phase culture was then ready to be used as innoculum. Ten percent innoculum was used in all experiments.

3.1.1.4 Quality control for microalgae contamination

For the quality control, each batch of microalgae culture for inoculum was tested for any bacteria or *Vibrio cholerae* and other enteropathogenic vibrios contamination in the culture, using Thiosulfate Citrate Bile Sucrose (TCBS) agar. *Vibrio* spp. are able to grow in media containing increased salt concentrations, and some species are halophilic. *Vibrio* spp. are natural inhabitants of seawater (Baron *et al*., 1994). TCBS agar slant was

prepared by dissolving 88g/L of agar in distilled water and boiled for one minute. After cooling down, the agar was poured into the plates. The agar obtained was clear and green-blue in colour. 50 µl of each culture for inoculum were spread on the surface of TCBS agar plate. The plates were incubated for 24 hours on the shelves, under illumination of 42 µmol photon/s/m^2 with 12h : 12h, light : dark cycle and temperature at 25$\pm$1^{o}C. The end point measurement for this test was based on colour of agar where green-blue colour indicated no contamination in culture while yellow colour indicated contamination by *Vibrio* spp. in culture. If more than 20% of the agar plates turned to yellow as measured using 1cmx 1cm plate grid, the culture was discarded and not used as inoculum.

3.1.1.5 Macroalgae (seaweed) site collection

Boergesenia forbesii and *Ventricaria ventricosa* were collected from Pulau Besar, Malacca (02°06' 56.56" N; 102°19' 57.70" E) and Pulau Tioman, Pahang (02° 49' 51.44" N; 104° 09' 43.03" E) respectively. On the site of collection, *Boergesenia forbesii* are found at the subtidal mudflat (Figure 3.2). This macroalgae usually grows on the dead corals or attached at the stem or under the leaves of the larger seaweeds like *Rhipilia tomentosa* and *Avrainvillea* sp. *Ventricaria ventricosa* samples were collected by diving at depths down to 18m. Due to limitation of the macroalgae abbundance at the site of collection, both species used in this study were cultured continuosly and maintained in the laboratory to supply materials for the toxicity tests. The macroalgae were cultured as described in Section 3.1.1.7.

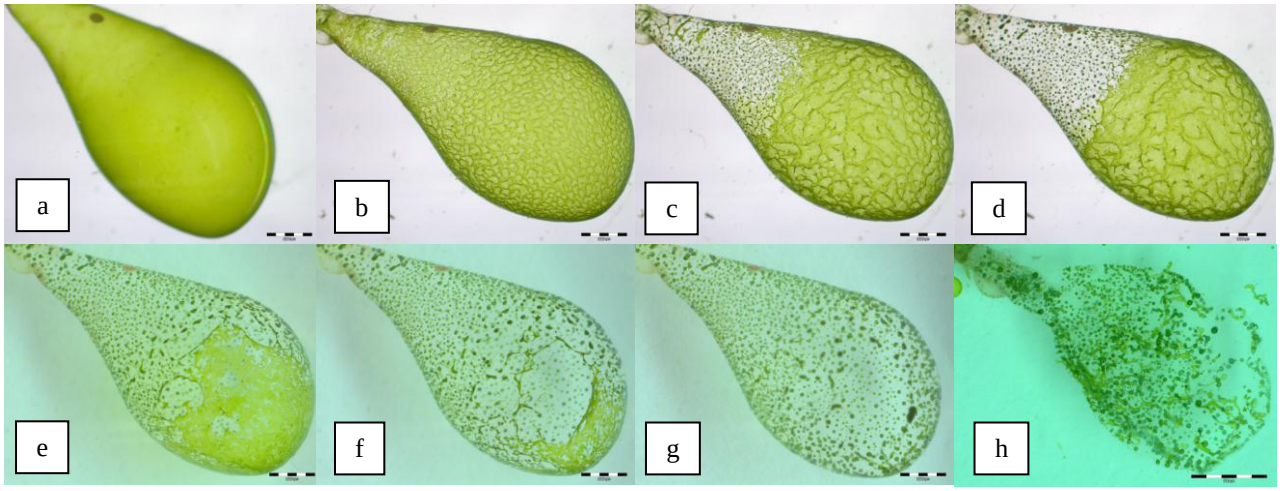
3.1.1.6 Water quality measurement at the site of collection

Water quality analyses were carried out on the site of sample collection for *Boergesenia forbesii* at Pulau Besar, Melaka. The water sample was collected for three batches and was kept in the refrigerator at $4\pm1^{o}C$. Analyses were done to determine the basic nutrient level and metal content at the site of collection. The results from these analyses were used as the nutrient and toxicant baseline parameters for the growth and toxicity studies. Physical parameters such irradiance, pH, dissolved oxygen, salinity, conductivity, air temperature, water temperature, colour and total hardness were determined. The nutrient/chemical content such as ammoniacal-nitrogen (NH_3-N), orthophosphate (PO_4^{3-}), Chemical oxygen demand (COD), nitrate (NO_3-N), Calcium (Ca), Chloride (Cl), Potassium (K), Silica (SiO_2), Sodium (Na) and Sulphate (SO_4) were also determined. For metal concentrations, the water samples were sent to a commercial laboratory (PERMULAB Sdn. Bhd, Malaysia). Twelve metals including cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) were analysed using the Atomic Absorbtion Spectrophotometer (AAS). The analytical methods for this parameter are described in Sections 3.1.4.

3.1.1.7 Macroalgae (seaweed) culturing and maintenance

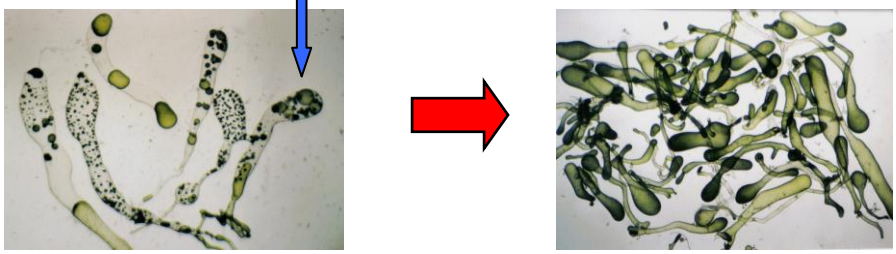
Both macroalgae used in this study were cultured in the laboratory through applanospore formation, with removal of contaminating microorganisms. Figure 3.2 summarizes the process of production of aplanospores to mature plants for *Boergesenia forbesii.*

Boergesenia forbesii (Harvey) Feldman, collected from Pulau Besar, Malacca, Peninsular Malaysia



In the laboratory, the coenocytic thalli are wounded to produce aplanosores

Aplanospores



Applanospore were grown to young plants and maintained in Prov50 Medium until the size of the plant reached 10mm x 3mm, before harvesting for the assays.

Figure 3.2: Process of obtaining aplanospores to mature plants for *Boergesenia forbesii.*

In the laboratory, the coenocytic thalli were wounded to produce aplanosores (Enomoto and Hirose, 1972; La Claire, 1982). The algae were maintained and grown in multi-well plates containing Prov50 Medium. The multi-well plates were placed on the shelves under cool-white fluorescent light, 15 µmol photon/s/m^2 irradiance with 12h:12h light:dark cycle and temperature of 25±1ºC. Every month, the culture solutions were refreshed and any microorganisms growing on the surface of the macroalgae were removed. The macroalgae were cultured for three to six months or until the size of the plant reached 10mm x 3mm (excluded the length of the rhizoid) for *Boergesenia forbesii* and 5mm x 5mm for *Ventricaria ventricosa*, before harvesting for the assays. For *Boergesenia forbesii*, only 1st generation daughter cells were used for the assay.

3.1.2 Screening of Microalgae For The Toxicity Studies

3.1.2.1 Preliminary toxicity test

All 25 microalgal species as mentioned in Section 3.1.1.1 were subjected to a preliminary toxicity test to cadmium (Cd). The test solution was prepared from the metallic salt, cadmium chloride which was dissolved in sterilized Prov50 medium. The toxicity tests were conducted using the microalgae in their exponential growth phase. Non-renewal, 96 hours Static Exposure System test was used. The end-point of the test was based on percentage mortality of the cultures as indicated by cell viability. The summary of the test condition is shown in Table 3.2. A preliminary toxicity test was carried out as a screening experiment to select the marine microalgal species with (a) high tolerance to cadmium, (b) single cell of morphology, (c) big size of cell that could be used for further detailed studies on 12 types of toxicants.

Table 3.2: Summary of test conditions for algal toxicity tests.

	Preliminary Toxicity Test	Growth and Toxicity Test	
Test Parameter	25 species of microalgae	*Chlorella vulgaris* UMACC 245 *Tetraselmis tetrahele* UMACC144	*Boergesenia forbesii* *Ventricaria ventricosa*
Test system	Static, non renewal	Non renewal	Non renewal
Temperature	25 °C± 1°C	25 °C± 1°C	25 °C± 1°C
Light source	Cool-white flouresence	Cool-white flouresence	Cool-white flouresence
Light intensity	42 μmol photon/s/m^2	42 μmol photon/s/m^2	42 μmol photon/s/m^2
Photoperiod	12:12, light: dark cycle	12:12, light: dark cycle	12:12, light: dark cycle
pH	8.0±0.5	8.0±0.5	8.0±0.5
Salinity	30±2.0 ppt	30±2.0 ppt	30±2.0 ppt
Test chamber	Multiwell plate	1L conical flask	Multiwell plate
Test Volume	10 mL	500 mL	10 mL
Replicates	3	3	3
Media	Prov 50 medium	Prov 50 medium	Prov 50 medium
Concentration	10 mg/L & 100 mg/L $CdCl_2$.	0, 0.01, 0.1,1,10,100 & 500 mg/L	0, 0.01, 0.1,1,10,100 & 500 mg/L
Test duration	96 hours	4 & 10 days	4 & 10 days
Aeration	None; Shaken once daily	Shaken at 100 rpm	Shaken at 100 rpm
Assessment endpoint	Percentage of culture mortality	Biomass, IC_{50}	Biomass, IC_{50}
Measurement endpoint	Cell viability	Chlorophyll *a* content	Chlorophyll *a* content

Each of the 25 microalgae were grown in sterile Prov50 medium containing 10 and 100 mg/L of $CdCl_2$ for 96 hours in multiwell plates with total volume of 10mL. An inoculum of 10% from exponential phase cultures standardized at OD_{620}= 0.2 (measured by Shimadzu UV- 160A Spectrophotometer, Shimadzu Corporation, Japan) was used. Cultures grown in sterile Prov50 medium without $CdCl_2$ were taken as control. All treatments were carried out in triplicate. The cultures in multiwell plates, were incubated on the shelves at 25±1°C, under cool-white fluorescence light, 42 µmol photon/s/m^2 and 12:12 light:dark cycle.

After 96 hours, the cultures were transferred into 15mL screw-cap plastic centrifuge tubes and centrifuged (using centrifuge Kubota 2100, Kubota Corporation, Japan) at 14g for 5 minutes. The pellet obtained was transferred into 1.5ml microcentrifuge tube and washed once with Prov50 medium. The pellet was then re-dissolved in 1ml Prov50 medium containing 0.4%w/v trypan blue solution for the cell viability test. The cells in the microcentrifuge tubes were then incubated on the shelves for two hours at 25±1°C under cool-white fluorescence light, 42 µmol photon/s/m^2.

Cell viability was monitored by cell count using an Improved Double-Neubauer Haemacytometer. The end point measurement for this test was based on colour of the microalgae cell where colourless or white coloured cell indicated live cells while blue coloured cell indicated dead of cells. The percentage of cell mortality was determined by the following relationship. The species with highest tolerance for the cadmium were selected for further toxicity testing.

$$\text{Percentage of cell mortality (\%)} = \frac{\text{No. of dead cells}}{\text{Total No. of cells}} \times 100\%$$

3.1.3 Toxicity Test on Selected Algae Based on Growth And Mortality

Based on the preliminary toxicity test, two microalgae, *Chlorella vulgaris* UMACC 245 and *Tetraselmis tetrahele* UMACC 144, which had high tolerance to cadmium, were single cell and had big size of cell (8-10µm to 14-16µm diameter) were selected for the detailed toxicity tests. In addition the two macroalgae *Boergesenia forbesii* and *Ventricaria ventricosa* were used.

Twelve toxicants including seven metals [Cadmium (Cd); Cobalt (Co); Chromium (Cr); Copper (Cu); Iron (Fe); Manganese (Mn) and Zinc (Zn)], three textile dyes [Supranol Br. Red 3Bur (Acidic dye); Astrazon Red FBL (Basic dye) and Lanaset Red 2GA (Metal complex dye)] and two organophosphate pesticides [Malathion and Dichlovos] in a range of concentrations were tested individually for each algal species. Non-renewal exposure system test was used with the test duration of 4 and 10 days. The summary of the test conditions shown in Table 3.2.

3.1.3.1 Preparation of metal stock solutions

Metal solutions with concentrations of 1000mg/L were prepared by dissolving $CdCl_2.H_2O$; $CoSO_2.7H_2O$; $CrCl_3.6H_2O$; $CuSO_4.7H_2O$; $FeSO_4.7H_2O$; $MnCL_2.4H_2O$ and $ZnSO_4.7H_2O$ salts individually in Prov50 medium based on the formula below:

$$\text{Salt (g)} = \frac{Y \times V \times \text{Mol. Wt} \times 10^{-4}}{\text{At. Wt} \times N \times P}$$

Where Y = Concentration of metal in mg/L
V = Volume required (m^3)
Mol. Wt = Molecular weight of salt (g)
At. Wt = Atomic weight of metal (g)
N = Normality
P = Purity

Details of parameters used in the determination of the amounts of salts for Cd, Co, Cr, Cu, Fe, Mn and Zn metal ion are shown in Table 3.3 and Table 3.4. A one liter volumetric flask was filled with approximately 700ml of Prov50 medium and a fixed amount of metal salts added and dissolved. Additional Prov50 medium was added slowly until 1.0L level was reached. Then, the solution was transferred into the 1.0L high density polyethylene bottle for storage at $4\pm1\ ^{0}C$ as stock solution.

Table 3.3: Parameters used in the determination of the amounts of salts for preparation of the stock solutions

Metal	Salt	Mol. Wt (g)	At. Wt (g)	Normality	Purity
Cd	$CdCl_2.H_2O$	201.32	112.410	1	98.00
Co	$CoSO_2.7H_2O$	281.10	58.933	1	99.00
Cr	$CrCl_3.6H_2O$	266.45	51.996	1	93.00
Cu	$CuSO_4.7H_2O$	249.68	63.546	1	99.75
Fe	$FeSO_4.7H_2O$	295.65	55.847	1	99.00
Mn	$MnCL_2.4H_2O$	197.91	54.938	1	100.00
Zn	$ZnSO_4.7H_2O$	287.55	65.390	1	99.50

Table 3.4: Amount of salt needed for preparing 1000 mg of metal ions in 1 litre volumes

Metal	Source	Concentration (mg/L)	Amount (g)
Cd	$CdCl_2.H_2O$	1000	1.827
Co	$CoSO_2.7H_2O$	1000	4.818
Cr	$CrCl_3.6H_2O$	1000	5.510
Cu	$CuSO_4.7H_2O$	1000	3.938
Fe	$FeSO_4.7H_2O$	1000	5.347
Mn	$MnCL_2.4H_2O$	1000	3.602
Zn	$ZnSO_4.7H_2O$	1000	4.419

3.1.3.2 Preparation of textile dye stock solutions

Three textile dyes (Supranol Br. Red 3Bur, Astrazon Red FBL and Lanaset Red 2GA) used in this study were obtained from Textile Kim Fashion Knitware (M) Sdn

Bhd, Senawang, Negeri Sembilan. Textile dye stock solutions with concentrations of 1000mg/L were prepared by dissolving 1g of Supranol Br. Red 3Bur, Astrazon Red FBL and Lanaset Red 2GA powder in a one liter volumetric flask containing 1.0 L Prov50 medium individually. The solutions were then transferred into the 1.0L polyethylene bottle for storage at 4±1°C as stock solution.

3.1.3.3 Preparation of organophosphate pesticide stock solutions

Two organophosphate pesticides (Malathion and Dichlovos) used in this study were purchased from the garden-store (Sin Seng Huat Seeds Sdn Bhd, Selangor). The concentration of 1000mg/L organophosphate pesticides solutions in a one liter volumetric flask were prepared by mixing 1.1904ml Malathion (84%w/w) and 2.2522ml Dichlovos (44.4% w/w) in 998.8096ml and 997.7478 ml Prov50 medium respectively. These stock solutions were transferred into 1.0L polyethylene bottle for storage at room temperature ($25 \pm 1\ ^{0}C$).

3.1.3.4 Preparation of test solutions

A series of concentrations (0.01, 0.1, 1, 10, 100 and 500 mg/L) of Cd, Co, Cr, Cu, Fe, Mn, Zn, Supranol Br. Red 3Bur, Astrazon Red FBL, Lanaset Red 2GA, Malathion and Dichlovos including the control were prepared by serial dilution technique . The pH of the test solution was adjusted to 8.0 and autoclaved (using TOMY Autoclave SS-325, Tomy Kogyo Co. Ltd, Japan).

3.1.3.5 Quality control for test solution

For quality control in determining the actual concentrations of metal in the test solutions used, a sample of each metal test solution prepared, was sent to the commercial laboratory (PERMULAB Sdn Bhd, Malaysia) for the metal content analysis. Seven metals including Cd, Co, Cr, Cu, Fe, Mn and Zn with different metal concentrations (0.01, 0.1, 1, 10, 100 and 500 mg/L) were determined using the Atomic Absorbtion Spectrophotometer (AAS).

For the textile dye test solutions, the colour content in textile dyes (Supranol Br. Red 3Bur, Astrazon Red FBL and Lanaset Red 2GA) at different concentrations (0.01, 0.1, 1, 10, 100 and 500 mg/L) was measured in PtCo unit, at the wavelength 465nm using Hach spectrophotometer (Hach Odyssey DR/2500, Hach Company, USA) on the initial day and on the last day of experiment.

3.1.3.6 Exposure of microalgae to selected toxicants

Two marine green microalgae, *Chlorella vulgaris* UMACC 245 and *Tetraselmis tetrahele* UMACC 144 were used in the study. The total volume of 500ml cultures in 1000ml conical flasks with 10% inoculum from exponential phase cultures standardized at an optical density 620nm (OD_{620}) of 0.2 (measured by Shimadzu UV- 160A Spectrophotometer, Shimadzu Corporation, Japan) were grown in Prov50 medium containing various concentrations of individual selected toxicant (0.01, 0.1, 1.0, 10, 100 and 500 mg/L). The high concentrations of toxicants, although less environmentally realistic, had to be used in order to observe a complete dose response from 0 to 100% inhibition. Initial pH of the growth medium was adjusted to 8.0 prior to inoculation.

Prov50 medium without toxicant was taken as positive control and conical flask containing individual test solution at different concentrations (0.01, 0.1, 1.0, 10, 100 and 500 mg/L) without microalgae cultures was taken as negative control. All treatments were carried out in triplicate. The cultures were incubated in an incubator shaker (B.Braun, incubations schüttelschrank BS4, Melsungen AG, Germany), shaken at 100 rpm under illumination of 42 µmol photon/s/m^2 irradiance with 12h:12h, light:dark cycle and temperature at 25$\pm$1^oC for 4 and 10 days. The summary of the test conditions is shown in Table 3.2.

3.1.3.7 Exposure of macroalgae (seaweed) to selected toxicants

Boergesenia forbesii and *Ventricaria ventricosa* were also used for the growth study and toxicity test. The total volume of 10 ml cultures in disposable sterile six-welled Falcon^{TM} multi-well plates with 5.0g and 1.0g FW of *Boergesenia forbesii* and *Ventricaria ventricosa* respectively were grown in sterilized Prov50 medium, containing different concentrations of individual selected toxicant (0.01,0.1,1,10, 100 & 500 mg/L). Initial pH of the growth medium was adjusted to 8.0 prior to inoculation. Prov50 medium without toxicant was taken as positive control and multi-well plates containing individual test solution at different concentrations (0.01, 0.1, 1.0, 10, 100 and 500 mg/L) without macroalgae cultures was taken as negative control. All treatments were carried out in triplicate. The cultures were incubated for 4 and 10 days on the shelves at 15 µmol photon/s/m^2 irradiance with 12h:12, light:dark cycle and temperature of 25$\pm$1ºC. The summary of the test conditions is shown in Table 3.2.

3.1.3.8 Growth parameter measurement

Growth for both microalgae and macroalgae was assessed on day 0, 4 and 10 by chlorophyll *a* (Chl *a*) analysis. The growth rate (GR), based on chlorophyll *a* (Chl *a*) content was determined using the following relationship (Guillard, 1973).

$$\text{Growth rate, GR } (\text{day}^{-1}) = \frac{\ln N_2 - \ln N_1}{t_2 - t_1}$$

where N_2 = Chlorophyll *a* content at times t_2

N_1 = Chlorophyll *a* content at times t_1

t_1 = the beginning of the selected time interval

t_2 = the end of the selected time interval

Carotenoid content and algal DW of harvested macroalgae were also measured on day 0, day 4 and day 10. The inhibition concentration (IC_{50}) value was calculated based on the chlorophyll *a* content results on day 4 and day 10. The IC_{50} value are concentrations of the test material resulting in 50% inhibitory effect, are the standard end-points. These values provide a specific point estimate of the effect being measured. It was determined using the ICPIN software programme (Norberg-King, 1993). The ICPIN software programme, which was developed by the U.S. EPA and uses a bootstrap method of calculation, to calculate IC_{50} value for sublethal test data such as growth and reproduction end-points.

3.1.4 Analytical Methods

3.1.4.1 Determination of pH, temperature, dissolved oxygen, salinity, conductivity and irradiance

These parameters were obtained via instrumental measurement. Waterproof Cyberscan PCD650 (Eutech Instruments Pte Ltd, Singapore) was used to measure all the environmental parameters such as pH, dissolved oxygen, salinity, conductivity, air and water temperature which were taken on-site during sample collection. In the laboratory, pH of the water samples was measured using a pH meter (Delta 320 Mettler Toledo, Mettler-Toledo Group, Shanghai China). Temperature (°C) was measured using a thermometer. Irradiance (k Lux) was determined by a light meter (Lux/FC 840020, Cole-Parmer Instrument Co, Taiwan). The unit for irradiance was converted to μmol photon/s/m^2 through the following equation (Thimijan, *et al.*, 1982):

10 000 lux = 134.5 μmol photon/s/m^2

The colour content of the water sample and test solution was measured in PtCo unit, at the wavelength 465nm using Hach spectrophotometer (Hach Odyssey DR/2500, Hach Company, USA).

3.1.4.2 Determination of Ammoniacal-nitrogen (NH_3-N), Orthophosphate (PO_4^3), Nitrate (NO_3-N) and Chemical oxygen demand (COD) content

The nutrients such as ammoniacal-nitrogen (NH_3-N), orthophosphate (PO_4^{3-}), nitrate (NO_3-N) and Chemical oxygen demand (COD) content in seawater samples were analysed using the Method 8155, Method 8048, Method 8171 and Method 8000

respectively as shown in the Hach HandBook Odyssey DR/2500 Spectrophotometer Procedure Manual (Hach Company, USA, 2001).

The NH_3–N content of the seawater sample was determined by the salicylate method [Method 8155] (APHA, 1998), A green colour solution will develop if the ammoniacal-nitrogen is present and the absorbance of the colour was read at 665nm using Hach spectrophotometer (Hach Odyssey DR/2500, Hach Company, USA).

Orthophosphate is referred to phosphate that responds to colorimetric tests without prior hydrolysis or oxidative digestion of the sample (APHA, 1998). The assay employed was the ascorbic acid method [Method 8048], based on the molybdenum blue colour development from phosphomolybdic acid and the absorbance of the colour was read at 880nm using Hach spectrophotometer (Hach Odyssey DR/2500, Hach Company, USA).

The nitrate content of the seawater sample was determined by the cadmium reduction method [Method 8171] (APHA, 1998), A amber colour solution will develop if the nitrate is present and the absorbance of the colour was read at 507nm using Hach spectrophotometer (Hach Odyssey DR/2500, Hach Company, USA).

The COD content of the seawater sample was determined by the 8000 method (APHA, 1998), An amber colour solution will develop if the COD is present and the absorbance of the colour was read at 620nm using Hach spectrophotometer (Hach Odyssey DR/2500, Hach Company, USA). Based on COD value, the carbon (C) content can be calculated using the formula given by Edwards *et al*., (1980):

$$\text{Carbon (C) in mg/L} = \text{COD (mg/L)} \times \frac{12}{32}$$

3.1.4.3 Determination of nutrient/chemical and metal content

For the measurement of the chemical/nutrient content [total hardness ($CaCO_3$), calcium (Ca), chloride (Cl), potassium (K), silica (SiO_2), sodium (Na), sulphate (SO_4)] and metal concentrations [cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), zinc (Zn)] in water sample, three batches of the water samples were sent to a commercial laboratory (PERMULAB Sdn. Bhd, Malaysia). All these nutrient/chemicals and metal content were analyses using the Atomic Absorption Spectrophotometer (AAS).

3.1.4.4 Algal cell count

The microalgal cells were counted using an Improved Double-Neubauer Haemacytometer (Germany). Appropriate dilution and homogenization of the algal cultures was performed prior to counting, to limit the cell number to below 100 cells per field counted.

3.1.4.5 Determination of chlorophyll *a* (Chl. *a*) content for microalgae

The Chl *a* content was determined using the spectrophotometric method (APHA, 1998). 10 ml of algal culture collected on a filter paper (glass fiber cellulose, 0.45µm, GF/C-47mm) was mashed and mixed homogenously with 10 ml of 100% acetone in a screw-cap centrifuge tube. The tubes were kept overnight at 4 ± 1 °C in the dark before centrifugation (using Kubota 2100 centrifuge, Kubota Corporation,

Japan) at 14g, for 10 minutes. The optical density of the supernatant at 630, 645 and 665nm was measured using Spectrophotometer (Shimadzu UV-160A Spectrophotometer, Shimadzu Corporation, Japan). Acetone was used as the blank. The Chl *a* content was calculated through the following equation (Parsons & Strickland, 1963);

$$\text{Chl } a \ (\text{mgm}^{-3}) = \frac{\text{Ca X acetone volume (ml)}}{\text{Algae culture volume (L)}}$$

Where $Ca = 11.6 (OD_{665}) - 1.31(OD_{645}) - 0.14 (OD_{630})$

3.1.4.6 Determination of chlorophyll *a* (Chl *a*) and carotenoid content for macroalgae (seaweed)

The Chl *a* and carotenoid content was determined using the spectrophotometric method (APHA, 1998). A known weight of macroalgae was washed once in sterilled Prov50 medium followed by distilled water to eliminate the metal residue and salts on the seaweed, before being mashed and mixed homogenously with 5 ml of 100% acetone in a screw-cap centrifuge tube. The tubes were kept overnight at 4±1°C in the dark and then centrifuged at 14g, for 10 minutes using Kubota 2100 centrifuge (Kubota Corporation, Japan) . The optical density of the supernatant at 630, 645 and 665nm (for Chl *a*) and 452nm (for carotenoid) was measured (using Shimadzu UV-160A Spectrophotometer, Shimadzu Corporation, Japan). Acetone was used as the blank.

The chl *a* content was calculated through the following equation (Parsons & Strickland, 1963):

$$\text{Chl. a } (\text{mgm}^{-3}) = \frac{\text{Ca X acetone volume (ml)}}{\text{Weight of macroalgae (g)}}$$

Where Ca = 11.6 (OD_{665}) - 1.31(OD_{645}) - 0.14 (OD_{630})

The carotenoid content was calculated using the following equation:

$$\text{Total carotenoid } (\mu g/g) = OD\ 452 \times 3.86 \times \frac{\text{Volume of acetone (ml)}}{\text{Weight of macroalgae (g)}}$$

3.1.4.7 Determination of microalgae dry weight (DW)

A known volume of microalgal culture was filtered through a preweighed dried 0.45 µm glass fiber cellulose (GF/C-47mm) filter paper. The pre-weight filters should be pre-combusted prior to use to remove any organic contaminants. The filters containing algae were dried at $100\pm1^{o}C$ in an oven (ULM-600 Memmert Oven, Schwabach, W.Germany) for 24 hours, cooled in a desicator filled with silica gel and weighed. The algal dry weight is determined through the following equation:

$$DW\ (mg/L) = \frac{[\text{weight of filters with dried algal biomass, (mg)}] - [\text{weight of blank filters, (mg)}]}{\text{Volume of algal culture (L)}}$$

3.1.4.9 Determination of macroalgae (seaweed) dry weight (DW)

A known weight of seaweed was put into a pre-weighed dried crucible. The pre-weight crucible should be pre-combusted prior to use to remove any organic contaminants. The crucible containing seaweed were dried at $100\pm1^{o}C$ in an oven (ULM-600 Memmert Oven, Schwabach, W.Germany) for 24 hours, cooled in a desicator filled with silica gel and weighed. The drying process was repeated until the constant weight

measurement is obtained. The seaweed dry weight is determined through the following equation:

$$DW\ (mg/g) = \frac{[\text{weight of crucible with dried algal biomass, (mg)}]-[\text{weight of blank crucible(mg)}]}{\text{Weight of algal culture (g)}}$$

3.1.5 Statistical Analyses

The data were analysed using One-way Analyses of Variance (ANOVA). Post Hoc test like Newman-Keuls test was used to determine the significant difference between different types of concentrations group means in an analysis. All statistical analyses were performed using the statistical software Statistica Version 5.5.

3.1 EFFECT OF SELECTED TOXICANTS ON BIOCHEMICAL COMPOSITION AND TOXICITY STUDIES OF ALGAE

Effect of toxicants on the biochemical composition in two green microalgae namely, *Chlorella vulgaris* UMACC 245 and *Tetraselmis tetrahele* UMACC 144, and two macroalgae *Boergesenia forbesii* and *Ventricaria ventricosa* was investigated. Twelve toxicants including seven metals [cadmium (Cd); cobalt (Co); chromium (Cr); copper (Cu); iron (Fe); manganese (Mn) and zinc (Zn)], three textile dyes [Supranol Br. Red 3Bur (Acidic dye); Astrazon Red FBL (Basic dye) and Lanaset Red 2GA (Metal complex dye)] and two organophosphate pesticides [Malathion and Dichlovos] in different range of concentrations were exposed individually to each algal species. After

exposure for 4 and 10 days to the selected toxicant, the biochemical composition (carbohydrate, protein and lipid content) in harvested algae was determined.

3.2.1 Procedure

The procedures used for the toxicity test was describe in Section 3.1.3.6 and Section 3.1.3.7, for the exposure of selected toxicants to microalgae and macroalgae (seaweed) respectively.

3.2.2 Determination of Total Carbohydrate Content in Microalgae

The total carbohydrate content in microalgae was determined using Kochert (1978); Dubois (1956); Granum and Myklestad (2002) method, based on colourmetric measurement. A known volume of filtered algae (filtered on 0.45 µm glass fiber cellulose, GF/C-47mm filter paper) was mashed in 5ml 2M hydrochloric acid (HCL) using glass hand-homoginiser and transferred into a plastic screw-cap centrifuge tube. The tube was incubated in the water-bath (BS-11 Lab Companion, Jeio Tech, Korea) for one hour at 80$\pm$1°C and mixed regularly. The sample was then centrifuged at 14g for 10 minutes using Kubota 2100 centrifuge (Kubota Corporation, Japan). The residue was re-extracted again in 5ml 2M HCl as followed in above procedures. The supernatant obtained was pooled to make up to 10mL. A volume of 500µL of the supernatant was transferred into a wide-mouth glass tube and mixed with 1.5 mL distilled water. 100 µL of phenol reagent (100%) was added slowly into the glass tube followed by 5mL of concentrated sulphuric acid (H_2SO_4) and mixed homogenously using Vortex mixer (S0200-230-UK, Labnet International Inc, USA). The sample was incubated for 30

minutes at 25±1°C and the absorbance at 485nm was read using Shimadzu UV-160 spectrophotometer (Shimadzu Corporation, Japan). A standard curve was prepared by using a series of glucose solution at concentrations of 0- 50 µg as reference.

3.2.3 Determination of Total Carbohydrate Content in Macroalgae (Seaweed)

The total carbohydrate content in macroalgae (seaweed) was determined using Kochert (1978); Dubois (1956); Granum and Myklestad (2002) method with some modification, based on colourmetric measurement. A known weight of macroalgae (seaweed) was ground in 10ml of 2M hydrochloric acid (HCL) using mortar and pestle before being transferred into a plastic screw-cap centrifuge tube. The tube was incubated in the water-bath (BS-11 Lab Companion, Jeio Tech, Korea) for one hour at 80±1°C and mixed regularly. The sample was then centrifuged at 14g for 10 minutes using Kubota 2100 centrifuge (Kubota Corporation, Japan). The residue was re-extracted again in 10ml 2M HCl as followed in above procedures. The supernatant obtained was pooled to make up to 20mL. A volume of 500µL of the supernatant was transferred into a wide-mouth glass tube and mixed with 1.5 mL distilled water. 100 µL of phenol reagent (100%) was added slowly into the glass tube followed by 5mL of concentrated sulphuric acid (H_2SO_4) and mixed homogenously using Vortex mixer (S0200-230-UK, Labnet International Inc, USA). The sample was incubated for 30 minutes at 25±1°C and the absorbance at 485nm was read using Shimadzu UV-160 spectrophotometer (Shimadzu Corporation, Japan). A standard curve was prepared by using a series of glucose solution at concentrations of 0- 50 µg as reference.

3.2.4 Determination of Total Protein Content in Microalgae

The total protein content in microalgae was determined using dye-binding method (Bradford, 1976). A known volume of filtered algae (filtered on 0.45 µm glass fiber cellulose, GF/C-47mm filter paper) was mashed in 5ml 0.5M Sodium hydroxide (NaOH) using glass hand-homoginiser and transferred into a plastic screw-cap centrifuge tube. The tube was incubated in the water-bath (BS-11 Lab Companion, Jeio Tech, Korea) for 20 minutes at 80±1°C and mixed regularly. The sample was then centrifuged at 14g for 10 minutes using Kubota 2100 centrifuge (Kubota Corporation, Japan). The residue was re-extracted again in 5ml 0.5M NaOH as followed in above procedures. The supernatant obtained was pooled to make up to 10mL. A volume of 100µL of the supernatant was transferred into a glass test tube and mixed with 3mL protein reagent. The sample was incubated for 30 minutes at 25±1°C and the absorbance at 595nm was read using Shimadzu UV-160 spectrophotometer (Shimadzu Corporation, Japan). A standard curve was prepared by using a series of Bovine Serum Albumin (BSA) solution at concentrations of 0-100 µg/mL as reference. Protein reagent was prepared by (1) mixing 20mL Bio-Rad solution with 80mL distilled water or (2) dissolving 100mg of Coomasie Brilliant Blue G-25 (Sigma-Aldrich) in 50mL ethanol. This solution was then filtered on the filter paper and the supernatant obtained was mixed with 100 mL of 85%v/v phosphoric acid. Additional distilled water was added slowly until 1.0L level was reached. The solution was mixed homogenously before transferring into the 1.0L dark glass bottle for storage at 4 ± 1^{0}C.

3.2.5 Determination of Total Protein Content in Macroalgae (Seaweed)

The total protein content in macroalgae (seaweed) was determined using dye-binding method (Bradford, 1976) with some modification. A known weight of macroalgae (seaweed) was ground in 10ml 0.5M Sodium hydroxide (NaOH) using mortar and pestle before been transferred into a plastic screw-cap centrifuge tube. The tube was incubated in the water-bath (BS-11 Lab Companion, Jeio Tech, Korea) for 20 minutes at 80±1°C and mixed regularly. The sample was then centrifuged at 14g for 10 minutes using Kubota 2100 centrifuge (Kubota Corporation, Japan). The residue was re-extracted again in 10ml 0.5M NaOH as followed in above procedures. The supernatant obtained was pooled to make up to 20mL. A volume of 100µL of the supernatant was transferred into a glass test tube and mixed with 3mL protein reagent. The sample was incubated for 30 minutes at 25±1°C and the absorbance at 595nm was read using Shimadzu UV-160 spectrophotometer (Shimadzu Corporation, Japan). A standard curve was prepared by using a series of Bovine Serum Albumin (BSA) solution at concentrations of 0-100 µg/mL as reference. Protein reagent was prepared by (1) mixing 20mL Bio-Rad solution with 80mL distilled water or (2) dissolving 100mg of Coomasie Brilliant Blue G-25 (Sigma-Aldrich) in 50mL ethanol. This solution was then filtered on the filter paper and the supernatant obtained was mixed with 100 mL of 85%v/v phosphoric acid. Additional distilled water was added slowly until 1.0L level was reached. The solution was mixed homogenously before transferring into the 1.0L dark glass bottle for storage at 4 ± 1^{0}C.

3.2.6 Determination of Total Lipid Content in Microalgae

The total lipid content in microalgae was determined using Bligh and Dyer (1959) method, based on gravimetric measurement. A known volume of filtered algae (filtered on 0.45 µm glass fiber cellulose, GF/C-47mm filter paper) was mashed in 5ml Methanol-Chloroform (2:1 v/v) solution using glass hand-homoginiser and transferred into a plastic screw-cap centrifuge tube. The sample was then centrifuged (using Kubota 2100 centrifuge, Kubota Corporation, Japan) at 14g for 10 minutes. The residue was re-extracted again in 5ml Methanol-Chloroform (2:1 v/v) solution as followed in above procedures. The supernatant obtained was pooled in new centrifuge tube and 2mL of chloroform was added followed by 2mL of distilled water. The tube were shaken thoroughly using the vortex (S0200-230-UK Vortex mixer, Labnet International Inc, USA) until the mixture turned milky green colour. The sample was then centrifuged at 14g for 10 minutes (using Kubota 2100 centrifuge, Kubota Corporation, Japan). The lower layer (green colour) was removed using a special drawn out Pasteur pipette (*The Pasteur pipette is heated under the flame of a spirit lamp, drawn out using a forcep, break the end to obtain a sharp tip*) and transferred into a new screw-cap glass tube. The sample was then blowed dry with gentle stream of nitrogen gas (Purified Nitrogen 98-M, MOX-Linde Gases Sdn Bhd, Malaysia). After dried, the sample extract was re-dissolved in 1mL of chloroform and transferred into a pre-weight 3.5 mL borosilicate glass vial. The extract was blowed dry again with gentle stream of nitrogen gas (Purified Nitrogen 98-M, MOX-Linde Gases Sdn Bhd, Malaysia) and the dry extract was kept in a desiccator containing silica gel for 24 hours before weighing and kept for the fatty acid transesterification. The lipid content in percentage of lipid per dry weight unit was calculated using the following equation:

$$\text{Lipid content} = \frac{[\text{Weight of lipid + vial (}\textit{final weight}\text{)}] - [\text{Weight of empty vial }]}{X \text{ (g) biomass (DW microalgae)}} \times 100\%$$

3.2.7 Determination of Total Lipid Content in Macroalgae (Seaweed)

The total lipid content in macroalgae (seaweed) was determined using Bligh and Dyer (1959) method with some modification, based on gravimetric measurement. A known weight of macroalgae (seaweed) was ground in 10ml Methanol-Chloroform (2:1 v/v) solution using mortar and pastel and then was transferred into a plastic screw-cap centrifuge tube. The sample was then centrifuged (using Kubota 2100 centrifuge, Kubota Corporation, Japan) at 14g for 10 minutes. The residue was re-extracted again in 10ml Methanol-Chloroform (2:1 v/v) solution as followed in above procedures. The supernatant obtained was pooled in new centrifuge tube and 4mL of chloroform was added followed by 4mL of distilled water. The tube were shaken thoroughly using the vortex (S0200-230-UK Vortex mixer, Labnet International Inc, USA) until the mixture turned milky green colour. The sample was then centrifuged at 14g for 10 minutes (using Kubota 2100 centrifuge, Kubota Corporation, Japan). The lower layer (green colour) was removed using a special drawn out Pasteur pipette (*The Pasteur pipette is heated under the flame of a spirit lamp, drawn out using a forcep, break the end to obtain a sharp tip*) and transferred into a new screw-cap glass tube. The sample was then blowed dry with gentle stream of nitrogen gas (Purified Nitrogen 98-M, MOX-Linde Gases Sdn Bhd, Malaysia). After dried, the sample extract was re-dissolved in 1mL of chloroform and transferred into a pre-weight 3.5 mL borosilicate glass vial. The extract was blowed dry again with gentle stream of nitrogen gas (Purified Nitrogen 98-M, MOX-Linde Gases Sdn Bhd, Malaysia) and the dry extract was kept in a desiccator containing silica gel for

24 hours before weighing and kept for the fatty acid transesterification. The lipid content in percentage of lipid per dry weight unit was calculated using the following equation:

$$\text{Lipid content} = \frac{[\text{Weight of lipid + vial (}\textit{final weight}\text{)}] - [\text{Weight of empty vial }]}{X \text{ (g) biomass (DW seaweed)}} \times 100\%$$

3.2.8 Statistical Analysis

The data were analysed using One-way Analyses of Variance (ANOVA). Post Hoc test like Newman-Keuls test was used to determine the significant difference between different types of concentrations group means in an analysis. All statistical analyses were performed using the statistical software Statistica Version 5.5.

3.3 EFFECT OF SELECTED TOXICANTS ON DNA DAMAGE AND TOXICITY STUDIES (RANDOM AMPLIFIED POLYMORPHIC DNA)

Random Amplified Polymorphic DNA (RAPD) analysis was carried out in *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa* as an assessment of response to toxicants.

One hundred and ninety primers were screened to select eight suitable primers to be used for further studies. Optimization of PCR conditions of the selected primers were carried out to determine the suitable annealing temperature for each primer.

Three metals including cadmium (as reference metal) and two other metals that represent the most toxic and the least toxic metal to each type of algae, three textile dyes [Supranol Br. Red 3Bur (Acidic dye); Astrazon Red FBL (Basic dye) and Lanaset Red 2GA (Metal complex dye)] and two organophosphate pesticides [Malathion and Dichlovos] in a range of concentrations were exposed individually to each algal species. After exposure for 4 and 10 days to the selected toxicant, the harvested algae were used for the RAPD analysis.

3.3.1 Genomic DNA Extraction

Genomic DNA extraction was carried out using modified CTAB-chloroform protocol by Raz and Ecker (Birren, *et al*.,1997). For the microalgae culture, 500mL of microalgae culture from individual sample were centrifuged at 14g for 10 min (using Eppendorf centrifuge 5810R, Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany) and the supernatant were discarded. The pellet obtained was washed once with Prov50 medium followed by steriled distilled water to eliminate the salts and other toxicant. The pellet then was frozen in liquid nitrogen (-185°C), ground with a mortar and pestle until become powdered.

For the macroalgae (seaweed), a known fresh weight of macroalgae (seaweed) from individual sample was rinse once with Prov50 medium followed by steriled distilled water to eliminate the salts and other toxicant. The macroalgae (seaweed) then were in ground liquid nitrogen (-185°C) until become powdered using a mortar and pestle.

Then 5ml of lysis buffer (100mM Tris-HCl pH8, 1.4 M NaCl, 20mM EDTA pH8, 3% CTAB, 1% mercaptoethanol) was added into the mortar and mixed thoroughly to homogenize the powdered algae in the buffer before been transferred into a 50ml Nelgene centrifuge tube. The tubes were incubated in water-bath (BS-11 Lab Companion, Jeio Tech, Korea) at 65±1°C for one hour. The composition of lysis buffer is shown in Table 3.5. Then Proteinase K (Fermentas Life Science, USA) was added in a tube with a final concentration of 200µg/mL and were incubated in water-bath (BS-11 Lab Companion, Jeio Tech, Korea) at 37±1°C for one hour. The DNA was extracted twice where 5ml of chloroform:isoamyl alcohol (24:1) solution was added into the tubes and mixed homogenously using Vortex mixer (S0200-230-UK, Labnet International Inc, USA) for 1 minutes followed by centrifugation at 1988g for 15 minutes (using MR1822 Jouan Centrifuge, France). The aqueous phase (upper layer) of the solution was taken out and transferred into a new 50mL Nelgene centrifuge tube. The residue was re-extracted again in 5ml of chloroform:isoamyl alcohol (24:1) solution as followed in above procedures. Then Ribonuclease A (Fermentas Life Science, USA) with a final concentration of 25µg/mL was added in a tubes and were incubated in water-bath (BS-11 Lab Companion, Jeio Tech, Korea) at 37±1°C for one hour. The DNA was re-extracted again in 5ml of chloroform:isoamyl alcohol (24:1) solution and was mixed by inverting the tube a few times followed by centrifugation at 1988g for 15 minutes (using MR1822 Jouan Centrifuge, France). The aqueous phase (upper layer) of the solution was taken out and transferred into a new 50mL Nelgene centrifuge tube. Then 5ml of ice-cold isopropanol was added into the tube and was mixed by inverting the tube a few times. The tube was kept at 4±1°C in the refrigerator for overnight to precipitate the DNA from the aqueous phase.

The following day, precipitated DNA was harvested by centrifugation at 1988g for 15 minutes (using MR1822 Jouan Centrifuge, France). The DNA pellet obtained was transferred into 1.5ml microcentrifuge tube and re-centrifuged again at 1988g for 15 minutes (using Eppendorf microcentrifuge 5415R, Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany). The DNA pellet was then washed with 500µl of 70% ethanol followed by centrifugation using Eppendorf microcentrifuge 5415R (Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany) at 1988g for 10 minutes. The supernatant was discarded and the DNA pellet was air dried in the polypropylene-polycarbonate desiccator (Kartell Labware Division, Italy).The final pellet was dissolved in 200µl TE buffer (10mM Tris-HCl pH8, 1mM EDTA pH8). The DNA concentration was measured and the final concentration was adjusted to 100µg/mL with TE buffer (10mM Tris-HCl pH8, 1mM EDTA pH8). The amount and quality of DNA was estimated using spectrophotometric and agarose gel electrophoresis method. The genomic DNA was stored at -20$\pm$1°C in a Chiller (ACF200/200L Acson Chiller, QYL Industries Bhd, Malaysia). All chemicals used throughout the described procedure were obtained from Sigma-Aldrich (Poole, UK).

Table 3.5: Composition of DNA Extraction Buffer (Birren, *et al.*,1997)

Component and final comcentration	Amount to add per 0.5 Liter
1.4 M NaCl	140ml of 5 M
20 mM EDTA	20ml of 0.5 M (pH 8)
100 mM Tris-Cl	50ml of 1M (pH 8 at 25^0C)
3% (w/v) CTAB	15g
Distilled water (H_2O)	to make 500 mL
1% (w/v) ß-mercaptoethanol	5ml of 14.3M

* Combine all of the components except the ß-maercaptoethanol. Add ß-mercaptoethanol just before use.

3.3.1.1 Quantification of DNA

There are two methods to measure DNA concentration:

(i) Agarose-Gel electrophoresis

Electrophoresis of genomic DNA products was performed in 1.0 % (w/v) agarose. The agarose powder was dissolved in a Tris-Borate-EDTA buffer (1 x TBE = 90mM Tris-base, 90mM Boric acid and 2mM EDTA) by heated on a hot plate (Hot plate 84532, Snijders Scientific B.V, Nederlands) and shaken regularly or heated in microwave oven for 1 minute. The dissolved agarose was cooled at room temperature and was poured into a horizontal electrophoresis gel container (Major Science, Taipei Hsein, Taiwan) and a 12 well-comb was immersed into the agarose solution. The agarose solution will be solidified at room temperature (25±1°C) and the comb was taken out slowly after the gel solidification.

The solidified agarose gel was then placed in a horizontal electrophoresis gel system (Major Science, Taipei Hsein, Taiwan) and 1x TBE buffer was poured into the electrophoresis container until all the surface of the gel is covered with the TBE buffer. 10µl of genomic DNA sample was mixed with 2µl of gel loading buffer (analytical grade water containing 40% sucrose, 0.25% bromophenol blue) equivalent to ratio 1:5 of gel loading buffer: genomic DNA and loaded into the well of agarose gel. A DNA molecular weight marker [M = 1kb marker, Fermentas Life Science (CA, USA)] was run for each agarose gel. Band visualized from top to bottom; 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500 and 1000bp. One of the well on agarose gel which was loaded with gel loading buffer without genomic DNA was taken as negative control. Genomic DNA samples were subjected to electrophoresis at 80V, 500 mA for two hours and subsequently the genomic DNA will move from the negative to positive pole. The

movement of the DNA was monitored and stopped when the bands reached 1cm from the bottom. Then the agarose gel was stained in a 1 x TBE buffer containing ethidium bromide (at final concentration of 0.5µg/mL) for one hour. Then the gel was de-stained in 1 x TBE buffer for one hour. Gels were photographed under UV illumination using a digital camera (Alpha Innotech, USA). Genomic DNA band pattern were analysed using Alpha ImagerTM 2200 Image Analysis Software (Alpha Innotech, USA). The size of the genomic DNA sample was estimated based on DNA molecular size marker.

(ii) UV absorbance- Spectrophotometric method

Eppendorf 04612 Biophotometer (Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany) was used to measure the genomic DNA concentrations and purity based on the optical density (OD) at 260nm and 280nm. TE buffer was used as blank. The purity of the nucleic acid sample can be estimated by calculating the ratio between the optical density at 260nm and 280nm (OD_{260nm}/OD_{280nm}). High purity DNA should give a ratio between 1.8 to 2.0. An OD of 1.0 at 260nm is equivalent to 50µg/mL of double-stranded DNA (dsDNA) or 36µg/mL of single-stranded DNA (ssDNA). The DNA concentrations are calculated as follows:

$$\text{ds-DNA concentration in µg/mL} = \text{measured } OD_{260} \times \frac{50\mu g/mL}{1OD_{260}} \times \text{dilution factor}$$

$$\text{ss-DNA concentration in µg/mL} = \text{measured } OD_{260} \times \frac{36\mu g/mL}{1OD_{260}} \times \text{dilution factor}$$

3.3.2 RAPD Reaction

3.3.2.1 Polymerase Chain Reaction (PCR)

PCR amplifications were performed in 25µl volumes containing approximately 80 – 100ng of genomic DNA, 10pmol primer, 200µM deoxy-trinucleotide phosphate (dNTP Mix), 2.5mM $MgCl_2$, 2U of *Thermus aquaticus* (*Taq*) DNA polymerase, 1 x Taq buffer with KCl and ionized distilled water was added to make a total volume of 25µl . PCR chemicals were obtained from Fermentas Life Science (CA, USA). For every batch of PCR amplification, a negative control consisted of all the PCR mixture except the DNA was done. The PCR amplification was carried out using a Eppendorf Mastercycler Gradient (Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany). Thermal cycling parameters consisted of 5 min denaturation (94ºC) followed by 45 cycles of 1 min denaturation (94 ºC), 1 min annealing (44ºC) & 2 min extension (72ºC); with the final extension period adjusted to 2 min. Reaction mixtures were stored in freezer (MDF-U281 Sanyo UltraLow, Sanyo Corporation, Japan) at -80$\pm$1ºC prior to use.

3.3.2.2 Agarose gel electrophoresis

Electrophoresis of RAPD products was performed in 1.2% (w/v) agarose using a Tris-Borate-EDTA buffer (1 x TBE = 90mM Tris-base, 90mM Boric acid and 2mM EDTA) buffer system. A known weight of agarose was dissolved in 1 X TBE buffer by heated on a hot plate (Hot plate 84532, Snijders Scientific B.V, Nederlands) and shaken regularly or heated in microwave oven for 1 minute. The dissolved agarose was cooled at room temperature and was poured into a Multi-sub horizontal agarose gel electrophoresis container (Cleaver Scientific Ltd, United Kingdom) and a 24 well-comb was immersed

into the agarose solution. The agarose solution will be solidified at room temperature (25°C) and, the comb was taken out slowly after the gel solidification.

The agarose gel was then placed in a Multi-sub horizontal agarose gel electrophoresis system (Cleaver Scientific Ltd, United Kingdom) and 1x TBE buffer was poured into the electrophoresis container until all the surface of the gel is covered with the TBE buffer. Amplified DNA was mixed with $1/5^{th}$ volume of gel loading buffer (analytical grade water containing 40% sucrose, 0.25% bromophenol blue) with 20µl of this solution loaded onto the agarose gel. One of the well on agarose gel which was loaded with gel loading buffer without RAPD reaction product was taken as negative control. A DNA molecular weight marker [M = 1kb marker & 100bp marker, Fermentas Life Science (CA, USA)] was run for each agarose gel. Band visualized from top to bottom; 10000, 8000, 6000, 5000, 4000, 3500, 3000, 2500, 2000, 1500, 1000, 900, 800, 750, 700, 600, 500, 400, 300, 250, 200 and 100bp. PCR amplification products were subjected to electrophoresis at 80V, 500 mA for seven hours. The movement of the DNA was monitored and stopped when the bands reached 1cm from the bottom. Then the agarose gel was stained in a 1 x TBE buffer containing ethidium bromide (at final concentration of 0.5 µg/mL) for one hour. Then the gel was de-stained in 1 x TBE buffer for one hour. Gels were photographed under UV illumination using a digital camera (Alpha Innotech, USA).

3.3.2.3 Analysis of DNA profiles

DNA band pattern were analysed using Alpha ImagerTM 2200 Image Analysis Software (Alpha Innotech, USA). The size of each band were measured based on the

DNA molecular weight marker and each changes observed in the RAPD profiles (disappearance and appearance of bands) in comparison with their control treatment was given the arbitrary score of +1. The average was then calculated for each experimental group.

3.3.2.4 Statistical analysis

The data were analysed using One-way Analyses of Variance (ANOVA). Post Hoc test like Newman-Keuls test was used to determine the significant difference between different types of concentrations group means in an analysis. All statistical analyses were performed using the statistical software Statistica Version 5.5.

3.3.3 Screening of The Suitable Primers For The RAPD Analysis

For this study 190 primers (OPA1-20, OPK 1-20, OPN 1-20 & S1-130), obtained from Operon Technologies (UK) were screened in each type of algae and the primer which can amplify the most number of clear band will be used for further studies. Table 3.6 shows the list of the 190 primers used in the study.

The procedures in Section 3.3.1, 3.3.2, 3.3.2.1, 3.3.2.2, 3.3.2.3 and 3.3.2.4 were used for DNA extraction, RAPD reaction, PCR, agarose gel electrophoresis, analysis of DNA profiles and statistical analysis respectively.

Table 3.6: List of primers used in RAPD analysis

No	Primer	Sequence (5' to 3')	No	Primer	Sequence (5' to 3')	No	Primer	Sequence (5' to 3')
1	OPA1	CAGGCCCTTC	34	OPK14	CCCGCTACAC	67	S7	GGTGACGCCAG
2	OPA2	TGCCGAGCTG	35	OPK15	CTCCTGCCAA	68	S8	GTCCACACGG
3	OPA3	AGTCAGCCAC	36	OPK16	GAGCGTCGAA	69	S9	TGGGGGACTC
4	OPA4	AATCGGGCTG	37	OPK17	CCCAGCTGTG	70	S10	CTGCTGGGAC
5	OPA5	AGGGGTCTTG	38	OPK18	CCTAGTCGAG	71	S11	GTAGACCCGT
6	OPA6	GGTCCCTGAC	39	OPK19	CACAGGCGGA	72	S12	CCTTGACGCA
7	OPA7	GAAACGGGTG	40	OPK20	GTGTCGCGAG	73	S13	TTCCCCCGCT
8	OPA8	GTGACGTAGG	41	OPN1	CTCACGTTGG	74	S14	TCCGCTCTGG
9	OPA9	GGGTAACGCC	42	OPN2	ACCAGGGGCA	75	S15	GGAGGGTGTT
10	OPA10	GTGATCGCAG	43	OPN3	GGTACTCCCC	76	S16	TTTGCCCGGA
11	OPA11	CAATCGCCGT	44	OPN4	GACCGACCCA	77	S17	AGGGAACGAG
12	OPA12	TCGGCGATAG	45	OPN5	ACTGAACGCC	78	S18	CCACAGCAGT
13	OPA13	CAGCACCCAC	46	OPN6	GAGACGCACA	79	S19	ACCCCCGAAG
14	OPA14	TCTGTGCTGG	47	OPN7	CAGCCCAGAG	80	S20	GGACCCTTAC
15	OPA15	TTCCGAACCC	48	OPN8	ACCTCAGCTC	81	S21	CAGGCCCTTC
16	OPA16	AGCCAGCGAA	49	OPN9	TGCCGGCTTG	82	S22	TGCCGAGCTG
17	OPA17	GACCGCTTGT	50	OPN10	ACAACTGGGG	83	S23	AGTCAGCCAC
18	OPA18	AGGTGACCGT	51	OPN11	TCGCCGCAAA	84	S24	AATCGGGCTG
19	OPA19	CAAACGTCGG	52	OPN12	CACAGACACC	85	S25	AGGGGTCTTG
20	OPA20	GTTGCGATCC	53	OPN13	AGCGTCACTC	86	S26	GGTCCCTGAC
21	OPK1	CATTCGAGCC	54	OPN14	TCGTGCGGGT	87	S27	GAAACGGGTG
22	OPK2	GTCTCCGCAA	55	OPN15	CAGCGACTGT	88	S28	GTGACGTAGG
23	OPK3	CCAGCTTAGG	56	OPN16	AAGCGACCTG	89	S29	GGGTAACGCC
24	OPK4	CCGCCCAAAC	57	OPN17	CATTGGGGAG	90	S30	GTGATCGCAG
25	OPK5	TCTGTCGAGG	58	OPN18	GGTGAGGTCA	91	S31	CAATCGCCGT
26	OPK6	CACCTTTCCC	59	OPN19	GTCCGTACTG	92	S32	TCGGCGATAG
27	OPK7	AGCGAGCAAG	60	OPN20	GGTGCTCCGT	93	S33	CAGCACCCAC
28	OPK8	GAACACTGGG	61	S1	GTTTCGCTCC	94	S34	TCTGTGCTGG
29	OPK9	CCCTACCGAC	62	S2	TGATCCCTGG	95	S35	TTCCGAACCC
30	OPK10	GTGCAACGTG	62	S3	CATCCCCCTG	96	S36	AGCCAGCGAA
31	OPK11	AATGCCCCAG	64	S4	GGACTGGAG	97	S37	GACCGCTTGT
32	OPK12	TGGCCCTCAC	65	S5	TGCGCCCTTC	98	S38	AGGTGACCGT
33	OPK13	GGTTGTACCC	66	S6	TGCTCTGCCC	99	S39	CAAACGTCGG

Table 3.6: List of primers used in RAPD analysis (continued)

No	Primer	Sequence (5' to 3')	No	Primer	Sequence (5' to 3')	No	Primer	Sequence (5' to 3')
100	S40	GTTGCGATCC	133	S73	AAGCCTCGTC	166	S106	ACGCATCGCA
101	S41	ACCGCGAAGC	134	S74	TGCGTGCTTG	167	S107	CTGCATCGTG
102	S42	GGACCCAACC	135	S75	GACGGATCAG	168	S108	GAAACACCCC
103	S43	GTCGCCGTCA	136	S76	CACACTCCAG	169	S109	TGTAGCTGGG
104	S44	TCTGGTGAGG	137	S77	TTCCCCCCAG	170	S110	CCTACGTCAG
105	S45	TGAGCGGACA	138	S78	TGAGTGGGTG	171	S111	CTTCCGCAGT
106	S46	ACCTGAACGG	139	S79	GTTGCCAGCC	172	S112	ACGCATCGCA
107	S47	TTGGCACGGG	140	S80	ACTTCGCCAC	173	S113	GACGCCACAC
108	S48	GTGTGCCCCA	141	S81	CTACGGAGGA	174	S114	ACCAGGTTGG
109	S49	CTCTCCAGAC	142	S82	GGCACTGAGG	175	S115	AATGGCGCAG
110	S50	GGTCTACACC	143	S83	GAGCCCTCCA	176	S116	TCTCAGCTGG
111	S51	AGCGCCATTG	144	S84	AGCGTGTCTG	177	S117	CACTCTCCTC
112	S52	CACCGTATCC	145	S85	CTGAGACGGA	178	S118	GAATCGGCCA
113	S53	GGGGTGACGA	146	S86	GTGCCTAACC	179	S119	CTGACCAGCC
114	S54	CTTCCCCAAG	147	S87	GAACCTGCGG	180	S120	GGGAGACATC
115	S55	CATCCGTGCT	148	S88	TCACGTCCAC	181	S121	ACGGATCCT
116	S56	AGGGCGTAAG	149	S89	CTGACGTCAC	182	S122	GAGGATCCCT
117	S57	TTTCCCACGG	150	S90	AGGGCCGTCT	183	S123	CCTGATCACC
118	S58	GAGAGCCAAC	151	S91	TGCCCGTCGT	184	S124	GGTGATCAGG
119	S59	CTGGGGACTT	152	S92	CAGCTCACGA	185	S125	CCGAATTCCC
120	S60	ACCCGGTCAC	153	S93	CTCTCCGCCA	186	S126	GGGAATTCGG
121	S61	TTCGAGCCAG	154	S94	CGATGAGACC	187	S127	CCCATATCCC
122	S62	GTGAGGCGTC	155	S95	ACTGGGACTC	188	S128	GGGATATCGG
123	S63	GGGGGTCTTT	156	S96	AGCGTCCTCC	189	S129	CCAAGCTTCC
124	S64	CCGCATCTAC	157	S97	ACGACCGACA	190	S130	GGAAGCTTGG
125	S65	GATGACCGCC	158	S98	GGCTCATGTG			
126	S66	GAACGGACTC	159	S99	GTCAGCGCAC			
127	S67	GTCCCGACGA	160	S100	TCTCCCTCAG			
128	S68	TGGACCGGTG	161	S101	GGTCGGAGAA			
129	S69	CTCACCGTCC	162	S102	TCGGACGTGA			
130	S70	TGTCTGGGTG	163	S103	AGACGTCCAC			
131	S71	AAAGCTGCGG	164	S104	GGAAGTCGCC			
132	S72	TGTCATCCCC	165	S105	AGTCGTCCCC			

3.3.4 Optimization of Primer Annealing Temperature For RAPD

Based on the primer screening experiment, eight primers for each alga species used in the study (Table 3.7). Optimization of the PCR conditions for selected primers was carried out to determine the suitable annealing temperature for each primer in each algae. Six different annealing temperatures (34, 36, 38, 40, 42 and 44°C) were tested and the best annealing temperature for each primer will be used for further RAPD analysis in algae exposed to selected toxicant .

The procedures in Section 3.3.1, 3.3.2, 3.3.2.1, 3.3.2.2, 3.3.2.3 and 3.3.2.4 were used for DNA extraction, RAPD reaction, PCR, agarose gel electrophoresis, analysis of DNA profiles and statistical analysis respectively.

Table 3.7: Primers selected for use for the four species of algae

Algae	*Chlorella vulgaris* UMACC 245	*Tetraselmis tetrahele* UMACC 144	*Boergesenia forbesii*	*Ventricaria ventricosa*
Primer	a) OPA13 b) OPN13 c) S17 d) S67 e) S112 f) S118 g) S124 h) S125	a) OPA13 b) OPN13 c) OPN16 d) S17 e) S67 f) S68 g) S87 h) S118	a) OPA13 b) OPK14 c) OPN6 d) OPN13 e) S17 f) S67 g) S105 h) S124	a) OPA13 b) OPK14 c) OPN12 d) OPN13 e) S17 f) S20 g) S67 h) S86

3.3.5 RAPD Analysis in Four Algae Exposed to Selected Toxicants

Random Amplified Polymorphic DNA (RAPD) analysis was carried out in four algae, *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa*. After exposure to 4 and 10 days to the selected toxicant, the harvested algae were used for the RAPD analysis. Table 3.8 shows the list of toxicants that were used for each type of algal species in RAPD analysis.

The procedures used for the toxicity test was describe in Section 3.1.3.6 and Section 3.1.3.7 for the exposure of selected toxicants to microalgae and macroalgae (seaweed) respectively. The procedures in Section 3.3.1, 3.3.2, 3.3.2.1 and 3.3.2.2 were used for DNA extraction, RAPD reaction, PCR and agarose gel electrophoresis respectively.

3.36 Analysis of DNA Profiles

The DNA band pattern were analysed using Alpha ImagerTM 2200 Image Analysis Software (Alpha Innotech, USA).

3.3.6.1 DNA band size

The DNA molecular weight (base-pair size) of each band obtained in the RAPD profile was measured using the Molecular Weight Calculation programme, as followed the procedures shown in Alpha ImagerTM 2200 Image Analysis Manual Book (2000). In this programme, the value of known molecular weights markers [1kbp marker & 100bp marker, Fermentas Life Science (CA, USA)] was entered and the molecular weight of unknown band on the image was determined.

Table 3.8: List of toxicants that were used for each algal species in RAPD analysis

Algae species	Selected toxicant used for RAPD analysis		
	Metal	Textile Dye	Organophosphate pesticide
Chlorella vulgaris UMACC 245	a) Iron (Fe) b) Manganese (Mn) c) Cadmium (Cd)	a) Supranol Br. Red 3Bur (Acidic dye) b) Astrazon Red FBL (Basic dye) c) Lanaset Red 2GA (Metal complex dye)	a) Malathion b) Dichlovos
Tetraselmis tetrahele UMACC 144	a) Copper (Cu) b) Chromium (Cr) c) Cadmium (Cd)	a) Supranol Br. Red 3Bur (Acidic dye) b) Astrazon Red FBL (Basic dye) c) Lanaset Red 2GA (Metal complex dye)	a) Malathion b) Dichlovos
Boergesenia forbesii	a) Copper (Cu) b) Zinc (Zn) c) Cadmium (Cd)	a) Supranol Br. Red 3Bur (Acidic dye) b) Astrazon Red FBL (Basic dye) c) Lanaset Red 2GA (Metal complex dye)	a) Malathion b) Dichlovos
Ventricaria ventricosa	a) Copper (Cu) b) Zinc (Zn) c) Cadmium (Cd)	a) Supranol Br. Red 3Bur (Acidic dye) b) Astrazon Red FBL (Basic dye) c) Lanaset Red 2GA (Metal complex dye)	a) Malathion b) Dichlovos

3.3.6.2 Appearance of new bands, disappearance of bands and similarity of the band

Change observed in the RAPD profiles (appearance of new bands, disappearance of bands and similarity of the band) in comparison to control group in the RAPD profiles was measured by given arbitrary score of +1 or -1.

(i) Appearance of new band : +1

(ii) Disappearance of band : -1

The average was then calculated for each experimental group. All the data were recorded and transformed to percentage using the following formula:

Percentage of new band appearance (%)

$$= \frac{\text{Number of new bands appeared in test culture}}{\text{Number of bands in control culture}} \times 100$$

Percentage of band disappearance (%)

$$= \frac{\text{Number of bands disappeared in test culture}}{\text{Number of bands in control culture}} \times 100$$

Percentage of band similarity (%)

$$= \frac{\text{Number of bands in test culture similar to control}}{\text{Number of bands in control culture}} \times 100$$

3.3.6.3 Intensity of the band

Band intensity in the RAPD profiles was measured using the 1D-Multi (Lane desitometry) programme as followed the procedures shown in Alpha ImagerTM 2200 Image Analysis Manual Book (2000). In this programme, the density of each band (per unit square area) can be scan and quantified. The density values for 1D-Multi graph of

each band was used as parameter to measure intensity of the band. The average value of the each band intensity was then calculated for each experimental group. All the data were recorded and transformed to percentage using the following formula:

$$\text{Percentage of band intensity (\%)} = \frac{\text{Intensity of bands in test culture}}{\text{Intensity of bands in control culture}} \times 100$$

3.3.6.4 Estimation of genomic template stability

The genomic template stability is related to the level of DNA damage, is a qualitative measurement reflecting changes in RAPD pattern. The genomic template stability (%) was calculated using formula below:

Genomic template stability (%) = 100 – (100a/n)

where a = The average number of changes in DNA profiles

n = The number of bands selected in control DNA profiles.

Example of Genomic template stability calculation:

Number of new bands appeared = 3

Number of bands disappeared = 5

Number of bands in control = 10

Genomic template stability (%) = 100 – (100a/n)

Genomic template stability (%)

= 100 – (100 [No. of band appeared + No. of band disappeared] / No. of band in control culture)

= 100 – (100 [3 + 5]/10)

= 100 – 80

= 20

Therefore Genomic template stability = 20%

3.3.6.5 Statistical analysis

The data were analysed using One-way Analyses of Variance (ANOVA). Post Hoc test like Newman-Keuls test was used to determine the significant difference between different types of concentrations group means in an analysis. All statistical analyses were performed using the statistical software Statistica Version 5.5.

3.4 EFFECT OF SELECTED TOXICANTS ON DNA DAMAGE AND TOXICITY STUDIES (AP-SITE COUNTING)

The AP-Site (Abasic-site) counting was determined using a DNA Damage Quantification Kit- AP-Site counting (Dojindo Molecular Technologies Inc., Japan) according to the manufacturer's protocol (Dojindo, 2009).

Oxidative attack by hydroxyl radicals on the deoxyribose moiety will lead to the release of free bases from DNA, generating strand breaks with various sugar modifications and simple abasic-sites (AP-site). Aldehyde Reactive Probe (ARP) reagent (N'-amino oxymethyl carbonyl hydrazon-D-biotin reacts specifically with an aldehyde group which is the open ring form of the AP-site. This reaction will make it possible to detect DNA modifications that result in the formation of an aldehyde group. After treating DNA containing AP-sites with ARP reagent, AP-sites are tagged with biotin residues. By using an excess amount of ARP, all AP-sites can be converted to biotin-tagged AP-sites. Therefore, AP-sites can be quantified using avidin-biotin assay followed by colourimetric detection of peroxidase or alkaline phosphatase conjugated to

the avidin (Fig.3.3). DNA Damage Quantification Kit contains all the necessary solutions, enabling the determination of 1 to 40 AP-sites per $1x10^5$ base-pair.

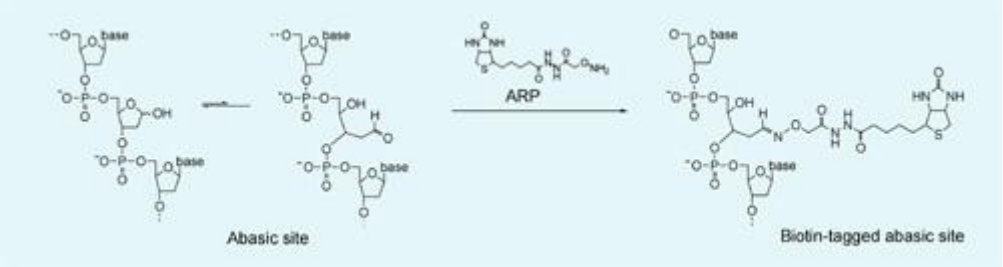


Figure 3.3: Mechanism of ARP tagging at an abasic site (AP-site)

3.4.1 Description of the AP-site counting kit

This kit was used to measure the AP-sites (Abasic-site) in DNA samples, which corresponded to colourimetric 96-well microplate assay. ARP Standard DNA solutions in this kit were prepared by heat/acid depurination (Lindahl and Nyberg, 1972; Kubo, *et al.,* 1992) of calf thymus DNA to control the number of abasic-sites from 0 to 40 per 1 x 10^5 bp. These DNA solutions are treated with ARP and purified. The Standard 0 ARP-DNA was prepared using methoxyamine-treated calf thymus DNA without heat/acid depurination.

Standard ARP DNA and purified ARP-treated sample DNA were fixed on the 96 well plate with DNA Binding Solution. Then, the number of AP sites in the sample DNA can be determined by the biotin-avidin-peroxidase assay. Since the standard ARP DNA are double stranded DNA, this kit is not applicable to abasic-sites detection of single stranded DNA. The O.D. value of a single stranded DNA sample will be nearly twice as high as that of a double stranded DNA sample under the same assay condition (Dojindo, 2009).

3.4.2 AP-Site Counting Analysis in Four Algae Exposed to Selected Toxicants

AP-Site counting analysis was carried out in four algae namely, *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa*. Each alga was exposed to two metals that represent the most toxic and the least toxic metal to each algal, in a range of concentrations. After exposure to 4 and 10 days to the selected toxicant, the harvested algae were used for the AP-Site counting analysis. Table 3.9 shows the list of toxicants that were used for each algal species in AP-Site counting analysis.

Table 3.9: List of toxicants that were used for each type of algal species in AP-site counting analysis

Algae species	Selected toxicant used for AP-site counting analysis
Chlorella vulgaris UMACC 245	a) Iron (Fe) b) Manganese (Mn)
Tetraselmis tetrahele UMACC 144	a) Copper (Cu) b) Chromium (Cr)
Boergesenia forbesii	a) Copper (Cu) b) Zinc (Zn)
Ventricaria ventricosa	a) Copper (Cu) b) Zinc (Zn)

The procedures used for the toxicity test was describe in Section 3.1.3.6 and Section 3.1.3.7 for the exposure of selected toxicants to microalgae and macroalgae (seaweed) respectively.

3.4.2.1 DNA extraction

DNA in each algal was extracted as followed by procedure mentioned in Section 3.31. It is important for an accurate assay that the DNA concentration is adjusted exactly to 100µg/mL. The absorbance of DNA at $1OD_{260nm}$ = 50 µg/mL. The ratio of OD_{260nm}/OD_{280nm} of highly purified DNA solution is 1.8 or higher is compulsory in this assay. Protein contamination in the sample solution may cause a positive error.

3.4.2.2 ARP reaction (Preparation of ARP-labeled DNA)

10µL of purified genomic DNA solution (100 µg/mL) was mixed with 10 µL of ARP Solution in a 0.5mL microcentrifuge tube and incubated in water-bath (BS-11 Lab Companion, Jeio Tech, Korea) at 37$\pm$1°C for one hour. Then, the inside of the Filteration Tube cup was washed twice with 100 µL TE buffer. 380 µL of TE buffer was added to the ARP-labeled DNA solution (20 µL) and the mixed solution was transferred to the Filtration Tube. The Filtration Tube was centrifuged at 2500g for 15 min (using Eppendorf microcentrifuge 5415R, Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany) and the filtrate solution is discarded.

400 µL of TE buffer is added to the Filtration Tube and the DNA on the filter was re-suspend in the TE buffer using a pipette. The Filtration Tube was then centrifuged at 2500g for 15 min (using Eppendorf microcentrifuge 5415R, Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany). After that the DNA on the filter was re-suspend in 200 µL of TE buffer using a pipette and the DNA solution was transferred to the 1.5mL microcentrifuge tube. The process was repeated again (where remaining DNA on the filter was re-suspend again in 200 µL of TE and transferred into the 1.5mL tube) to

make sure all ARP-labeled DNA on the filter completely transferred into the 1.5mL microcentrifuge tube. The recovery rate of DNA using the filtration tube is 90%, therefore the concentration of the ARP-labeled DNA is 2.25 µg/mL. For more accurate determination of the number of AP-sites in the sample DNA, it is recommended to measure the DNA concentration using a Biophotometer (Eppendorf 04612 Biophotometer, Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany). The purified ARP-labeled genomic DNA solution (in total volume of 400 µL with DNA concentration of 2.25 µg/mL) was stored at 0-5°C in a Chiller (ACF200/200L Acson Chiller, QYL Industries Bhd, Malaysia).

3.4.2.3 Determination of the number of AP-site (abasic-sites) in extracted DNA

(i) Day 1

90 µL of the ARP-labeled genomic DNA was diluted with 310 µL of TE buffer. Then, 60 µL of the diluted ARP-labeled genomic DNA solution was added per well into a 96-well Microplate U bottom. For each sample three replicates were carried out (three wells per one sample).

To prepare a Standard calibration curve, Standard ARP-DNA Solution were used. 60 µL of each Standard ARP-DNA Solution (0, 2.5, 5,10, 20 and 40 ARP-DNA Standard Solution) was added per well. Three wells were used per standard solution (three replicate). Then, 100 µL of the DNA Binding Solution was added to each well for both ARP-labeled genomic DNA solution and Standard ARP-DNA Solution. These solutions were incubated in room temperature (25°C) for overnight.

(ii) Day 2

The DNA Binding Solution in the wells were discarded and each well was washed with 250 µL Washing Buffer for five times. [*The Washing buffer was prepared by dissolved the contents of the Washing Buffer packet in 1 Liter of deionized or distilled water and stored at room temperature*]. After discarding the solution, the microplate was inverted and taped it on a paper towel several times to remove the solution completely. 150 µL of diluted HRP-Strepavidin solution was added to each well and the plate was incubated at 37±1°C for one hour in water-bath (BS-11 Lab Companion, Jeio Tech, Korea). [*Diluted HRP-Streptavidin solution was prepared by added 10 µL of HRP-Streptavidin solution into 40 mL of Washing Buffer solution (1/4000 diluted working solution) and mixed well. This working solution is not stable, always use freshly prepared solution*].

After one hour incubation, the solution in the well was discarded and the well was washed with 250 µL Washing Buffer for 5 times. After discarding the solution, the microplate was inverted and taped it on a paper towel several times to remove the solution completely.100 µL of Substrate Solution was added to each well and the microplate was incubated in water-bath (BS-11 Lab Companion, Jeio Tech, Korea) at 37±1°C for one hour. The OD at 650nm was measured within 1 hour after the incubation is finished. If the 650nm filter is not available for the measurement of OD after colour development, 50 µL of the solution was transferred in each well to a well of a new microplate. Then, 50 µL of 1M sulfuric acid was added and the OD at 450nm was measured using Dynex MRX Elisa Reader (Dynex Technologies, MTX Lab Systems Inc, USA). A calibration curve was prepared using the data obtained with standard ARP-

DNA solutions. The number of AP-sites in the genomic DNA was determined using the calibration curve.

3.4.3 Statistical Analysis

The data were analysed using One-way Analyses of Variance (ANOVA). Post Hoc test like Newman-Keuls test was used to determine the significant difference between different types of concentrations group means in an analysis. All statistical analyses were performed using the statistical software Statistica Version 5.5.

3.5 EFFECT OF SELECTED TOXICANTS ON SUPEROXIDE DISMUTASE (SOD) ACTIVITY AND TOXICITY STUDIES

The Superoxide dismutase (SOD) analysis was determined using a a SOD Assay Kit-WST (Dojindo Molecular Technologies Inc., Japan) according to the manufacturer's protocol (Dojindo, 2006).

Superoxide dismutase (SOD), which catalyzes the dismutation of the superoxide anion (O_2^-) into hydrogen peroxide and molecular oxygen, is one of the important antioxidative enzymes. In order to determine the SOD activity, several direct and indirect methods have been developed. Among these methods, an indirect method using nitroblue tetrazolium (NBT) is commonly used due to its convenience and ease of used. However, there are several disadvantages to the NBT method, such as poor water solubility of the formazan dye and the interaction with the reduced form of xanthine oxidase.

SOD Assay Kit-WST allows very convenient SOD assaying by utilizing Dojindo's highly water soluble tetrazolium salt, WST-1 1 [2-(4-Iodophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt] that produces a water-soluble formazan dye upon reduction with a superoxide anion. The rate of the reduction with $O_2^{\cdot -}$ are linearly related to xanthine oxidase (XO) activity and is inhibited by SOD as shown in Figure 3.4. Therefore, the IC_{50} (50% inhibition activity of SOD or SOS-like materials) can be determined by colorimetric method.

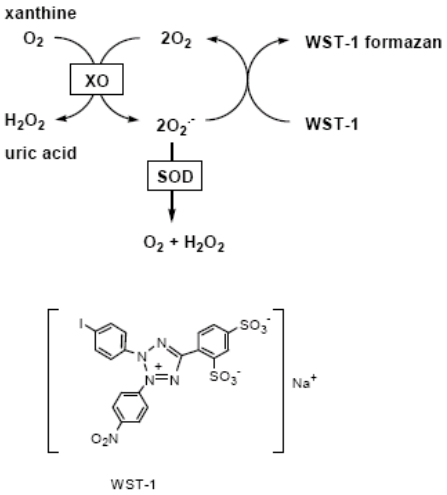


Figure 3.4: Principle of the SOD Assay Kit (Dojindo Molecular Technologies Inc., 2006)

3.5.1 SOD Analysis in Four Algae Exposed to Selected Toxicants

Superoxide dismutase (SOD) analysis was carried out in four algae namely, *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa*.

Three metals including cadmium (as reference metal) and two other metals that represent the most toxic and the least toxic metal to each algae, three textile dyes [Supranol Br. Red 3Bur (Acidic dye); Astrazon Red FBL (Basic dye) and Lanaset Red

2GA (Metal complex dye)] and two organophosphate pesticides [Malathion and Dichlovos] in a range of concentrations were exposed individually to each algal species (Table 3.10).

The procedures used for the toxicity test was describe in Section 3.1.3.6 and Section 3.1.3.7 for the exposure of selected toxicants to microalgae and macroalgae (seaweed) respectively. After exposure of 4 and 10 days to the selected toxicant, the harvested algae were used for the SOD analysis.

3.5.1.1 Preparation of the SOD enzyme from the algae

For the microalgae culture, 100mL of microalgae culture from individual samples were centrifuged at 14g for 10 min (using Eppendorf centrifuge 5810R, Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany) and the supernatant discarded. The pellet obtained was washed once with Prov50 medium followed by sterile distilled water to eliminate the salts and other toxicant. The pellet then was frozen in liquid nitrogen (-185^{0}C), ground with a mortar and pestle until become powdered and re-suspended in 100µl SOD Assay Kit-WST dilution buffer.

This was then centrifuged at 14g for 5 min (using Eppendorf microcentrifuge 5415R, Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany). The supernatant obtained was used for the assay.

Table 3.10: List of toxicants that were used in Superoxide dismutase (SOD) analysis

Algae species	Selected toxicant used for SOD analysis		
	Metal	Textile Dye	Organophosphate pesticide
Chlorella vulgaris UMACC 245	a) Iron (Fe) b) Manganese (Mn) c) Cadmium (Cd)	a) Supranol Br. Red 3Bur (Acidic dye) b) Astrazon Red FBL (Basic dye) c) Lanaset Red 2GA (Metal complex dye)	a) Malathion b) Dichlovos
Tetraselmis tetrahele UMACC 144	a) Copper (Cu) b) Chromium (Cr) c) Cadmium (Cd)	a) Supranol Br. Red 3Bur (Acidic dye) b) Astrazon Red FBL (Basic dye) c) Lanaset Red 2GA (Metal complex dye)	a) Malathion b) Dichlovos
Boergesenia forbesii	a) Copper (Cu) b) Zinc (Zn) c) Cadmium (Cd)	a) Supranol Br. Red 3Bur (Acidic dye) b) Astrazon Red FBL (Basic dye) c) Lanaset Red 2GA (Metal complex dye)	a) Malathion b) Dichlovos
Ventricaria ventricosa	a) Copper (Cu) b) Zinc (Zn) c) Cadmium (Cd)	a) Supranol Br. Red 3Bur (Acidic dye) b) Astrazon Red FBL (Basic dye) c) Lanaset Red 2GA (Metal complex dye)	a) Malathion b) Dichlovos

For the macroalgae (seaweed), a known fresh weight of macroalgae (seaweed) from individual samples was rinsed once with Prov50 medium followed by sterile distilled water to eliminate the salts and other toxicant. The macroalgae (seaweed) were ground in liquid nitrogen (-185^{0}C) until powdered using a mortar and pestle and re-suspended in 100µl SOD Assay Kit-WST dilution buffer. This was then centrifuged at 14g for 5 min (using Eppendorf microcentrifuge 5415R, Eppendorf Asia Pacific Sdn Bhd, Eppendorf AG, Germany). The supernatant obtained were used for the assay.

3.5.1.2 Preparation of solutions (for one 96-well plate)

i) *WST working solution:*

Prepared by diluting 1 mL of WST Solution in 19 mL of Buffer Solution

ii) *Enzyme working solution:*

Prepared by centrifuged the enzyme Solution tube for 5 sec, mixed by pipetting and diluted 15 µL of Enzyme Solution with 2.5 mL of Dilution Buffer.

iii) *Standard solutions of Fe-SOD$_{Ec}$ (Sigma-Aldrich Inc,USA)*

Prepared at concentrations of 200,100,50,20,10,5,1,0.1,0.05,0.01 and 0.001 U/mL by serial dilution where the Fe-SOD$_{Ec}$ Stock Solution (3000 units, 1.7 mg solid, 2629 unit/mg solid obtained from S5389- Fe-SOD$_{Ec,}$ Sigma-Aldrich Inc,USA) was diluted in the SOD Assay Kit-WST dilution buffer.

3.5.1.3 SOD assay kit-WST protocol

In U-bottom microplate, 20µl of each algae sample supernatant were added into a sample solution well and the Blank 2 well. 20µl of double distilled water (ddH_2O) were added into a Blank 1 well and Blank 3 well. All the algae sample supernatant and 3 controls (Blank 1, Blank 2 and Blank 3) were carried out in triplicate. 200 µl of WST working solution was added in all wells and 20 µL enzyme working solution was added into a sample solution well and Blank 1 well. In the Blank 2 well and Blank 3 well, 20 µl dilution buffer was added. The reactions were mixed thoroughly using a pipette and incubated at 37±1°C for 20 min in water-bath (BS-11 Lab Companion, Jeio Tech, Korea).

The absorbance was read at 450 nm using a microplate reader (Dynex MRX Elisa Reader, Dynex Technologies, MTX Lab Systems Inc, USA). Table 3.11 summarized the amount of each solution for sample, Blank 1, 2 and 3. To prepare a Standard SOD calibration curve, the SOD Standard Solution ($Fe\text{-}SOD_{EC}$, Sigma-Aldrich Inc, USA) with concentrations of 200, 100, 50, 20, 10, 5, 1.0, 0.5, 0.1, 0.05, 0.01 and 0.001 U/mL were used and the same procedure were applied as the algae sample solution.

Table 3.11: Amount of each solution for sample, Blank 1, 2 and 3

	Sample	Blank 1	Blank 2	Blank 3
Sample Solution	20 µL	-	20 µL	-
Double distilled water (ddH_2O)	-	20 µL	-	20 µL
WST Working Solution	20 µL	20 µL	20 µL	20 µL
Enzyme Working Solution	20 µL	20 µL	-	-
Dilution Buffer	-	-	20 µL	20 µL

The SOD activity (inhibition rate %) was calculated using the following equation and plotted onto an inhibition curve.

$$\text{SOD activity (inhibition rate \%)} = \frac{(A_{\text{Blank1}} - A_{\text{Blank3}}) - (A_{\text{Sample}} - A_{\text{Blank2}})}{(A_{\text{Blank1}} - A_{\text{Blank3}})} \times 100$$

The amount of SOD activity (inhibition rate %) in the tested algae sample was determined from the curve in U/mL. The protein content in the tested algae samples were determined by spectrophotometry (Bradford, 1976) as mention in Section 3.2.4 and 3.2.5. The SOD enzyme activity in mg protein unit was calculated.

3.5.2 Statistical Analysis

The data were analysed using One-way Analyses of Variance (ANOVA). Post Hoc test like Newman-Keuls test was used to determine the significant difference between different types of concentrations group means in an analysis. All statistical analyses were performed using the statistical software Statistica Version 5.5.

4.0 RESULTS

4.1 EFFECT OF SELECTED TOXICANTS ON GROWTH OF ALGAE AND TOXICITY STUDIES

4.1.1 Water Quality At The Sample Collection Sites

During the study period, the seawater samples were collected during noon (12pm-2pm). Average water characteristics was determined as: pH 7.84±0.01, air temperature (29.0±0.1 °C), water temperature (32.0±0.1 °C), dissolved oxygen (7.33±0.12 mg/L) and irradiance at 1345.0±10 µmol photon/s/m^2 (Table 4.1). The COD content (818.33 mg/L) was high compared to NH_3-N (0.04 mg/L), PO_4^{3-} (0.03 mg/L) and NO_3-N (0.25 mg/L) content. The average C:N:P ratio in water samples was 8767.85: 8.28: 1.00, showing that the carbon content was in excess but still suitable for algal growth. The other nutrients like Ca (202 mg/L), Cl (20706 mg/L) and Na (9393 mg/L) were high while K (2.80 mg/L), SiO2 (1.25 mg/L) were low and SO_4 was not detectable in this study. The concentrations of metals such as Cu (0.14 mg/L), Fe (0.04 mg/L) and Zn (0.04 mg/L) were low while Cd, Cr, Co and Mn were undetectable, that was less than the minimum detection limit reported.

4.1.2 Screening of Microalgae For The Studies

4.1.2.1 Preliminary toxicity test

The results for 25 microalgal species and two macroalgae (seaweed) which were subjected to a preliminary toxicity test are shown in Table 4.2.

Table 4.1: Water Quality Analysis at Pulau Besar, Melaka (the site of sample collection for *Boergesenia forbesii*)

Water Quality Analysis Parameter	Result
Environmental Parameter	
pH	pH 7.84 ± 0.01
Air temperature (°C)	29.0 ± 0.1 °C
Water temperature (°C)	32.0 ± 0.1 °C
Irradiance (µmol photon/s/m^2)	1345.0 ± 10.0 µmol photon/s/m^2
Dissolved oxygen (mg/L)	7.33 ± 0.12 mg/L
Salinity (ppt)	30.0 ± 0.1 ppt
Conductivity (mS)	40.47 ± 0.15 mS
Colour (PtCo)	34.33 ± 0.58 PtCo
Total Hardness ($CaCO_3$)	1120 ± 1.0 mg/L
Nutrient content	
Chemical oxygen demand (COD)	818.33 ± 8.82 mg/L
Ammoniacal-nitrogen (NH_3-N)	0.040 ± 0.010 mg/L
Orthophosphate (PO_4^{3-})	0.035 ± 0.006 mg/L
Nitrate (NO_3-N)	0.250 ± 0.055 mg/L
Calcium (Ca)	202 ± 1.0 mg/L
Chloride (Cl)	20706 ± 1.0 mg/L
Potassium (K)	2.80 ± 0.1 mg/L
Silica (SiO2)	1.25 ± 0.1 mg/L
Sodium (Na)	9393 ± 1.0 mg/L
Sulphate (SO4)	ND (<0.5)
Metal concentrations	
Cadmium (Cd)	ND (<0.01)
Chromium (Cr)	ND (<0.03)
Cobalt (Co)	ND (<0.01)
Copper (Cu)	0.14 ± 0.01 mg/L
Iron (Fe)	0.04 ± 0.01 mg/L
Manganese (Mn)	ND (<0.01)
Zinc (Zn)	0.04 ± 0.01 mg/L

Note: < : Less than the minimum detection limit reported; ND : Not Detected

: Triplicate samples analysed

Table 4.2: The percentage cell viability in the algae culture exposed to 10 mg/L and 100 mg/L $CdCl_2$ for four days

No	Algae	Percentage of cell viability	
		10 mg/L	100 mg/L
1	*Boergesenia forbesii* (Harvey) Feldman	95%	95%
2	*Ventricaria ventricosa* (J.Agardh) J.L Olsen & J.A West	95%	95%
3	*Chlorella vulgaris* Beijerinck UMACC 103	90%	40%
4	*Chlorella vulgaris* Beijerinck UMACC 104	80%	40%
5	*Nitzschia inconspicua* Grunow UMACC 111	90%	40%
6	*Amphora turgida Gregory* UMACC 113	80%	40%
7	*Oscillatoria* sp.UMACC 115	80%	30%
8	*Oscillatoria* sp UMACC 118.	90%	2%
9	*Oscillatoria* sp.UMACC 119	90%	20%
10	*Oscillatoria* sp UMACC 123.	90%	1%
11	*Bacillariophyta* UMACC 125	90%	50%
12	*Oscillatoria* sp.UMACC 126	90%	60%
13	*Tetraselmis* sp.UMACC 129	90%	90%
14	*Chaetoceros* sp.UMACC 133	80%	20%
15	*Oscillatoria* sp.UMACC 136	80%	2%
16	*Chaetoceros* sp.UMACC 140	80%	20%
17	*Isochrysis galbana* Parke UMACC 141	90%	20%
18	*Chaetoceros* sp.UMACC 142	90%	30%
19	*Tetraselmis tetrahele* (West) Butcher UMACC 144	95%	90%
20	*Tetraselmis* sp.UMACC 145	90%	80%
21	*Tetraselmis* sp.UMACC 146	95%	80%
22	*Chaetoceros calcitrans* Takano UMACC 147	80%	1%
23	*Chaetoceros* sp. UMACC 148	80%	1%
24	*Nannochloropsis oculata* (Droop) Hilberg UMACC 166	90%	40%
25	*Nannochloropsis oculata* (Droop) Hilberg UMACC 167	80%	40%
26	*Porphyridium cruentum* (Agardh) Naegeli UMACC 196	95%	80%
27	*Chlorella vulgaris* Beijerinck UMACC 245	95%	90%

After exposure for four days to $CdCl_2$, all the cultures were able to grow at 10 mg/L $CdCl_2$ (80-90% of cells were viable). However at 100 mg/L, only *Boergesenia forbesii, Ventricaria ventricosa, Tetraselmis* sp. and *Chlorella* sp. were still viable (90% viability). For other microalgae, only 40-60% of cells were able to survive and *Oscillatoria* sp. and *Chaetocerous* sp. (cell viability at 1-20%) did not grow in 100 mg/L $CdCl_2$.

4.1.3 Growth of Algae Exposed To 12 Toxicants

To analyse the effects of 12 toxicants [Cd, Co, Cr, Cu, Fe, Mn, Zn, Supranol Br. Red 3Bur (Acidic dye), Astrazon Red FBL (Basic dye), Lanaset Red 2GA (Metal complex dye), Malathion and Dichlovos] on growth *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa*, different concentrations (0, 0.01, 0.1, 1, 10 and 100 mg/L) of toxicants were exposed individually to the algae for four and ten days. Growth parameter such as chlorophyll *a* (Chl a) and carotenoid content were determined. The growth rate and the inhibition concentration (IC_{50}) value based on the Chlorophyll *a* (Chl a) content were also determined.

4.1.3.1 *Chlorella vulgaris* UMACC 245: Effects of 12 toxicants on growth

Figure 4.1 to Figure 4.2 show the growth rate (based on Chl *a*) and IC_{50} value (Fig. 4.3 to Fig. 4.4) of *Chlorella vulgaris* UMACC 245 after exposure to 12 toxicants

for four and ten days. Appendix 5 gives the detailed growth rate of *Chlorella vulgaris* UMACC 245 after exposure to 12 toxicants for four and ten days.

The results show, growth rate of the *Chlorella vulgaris* UMACC grown in the Prov50 Medium without toxicant (control culture) for four and ten days were 1.254$\pm$ 0.090 day^{-1} and 0.673 $\pm$ 0.106 day^{-1} respectively.

In general, the result showed, the *Chlorella* culture exposed to Cd, Cu, Fe, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion for four days and also in the *Chlorella* exposed to Cd, Cu, Mn, Acidic dye, Metal complex dye and Malathion for ten days, the growth rate of the algae decreased with increasing concentrations of toxicants. Statistical analysis using ANOVA showed there was significant difference ($p<0.05$) for all parameter in each concentration of toxicant tested compared with the control culture (Appendix 25). At the concentrations of 500 mg/L of the toxicant, there were significantly no growth or the growth parameter value were too low in the culture ($p<0.05$).

The IC_{50} values (Fig. 4.3 to Fig. 4.4) show that after 4 days exposure to toxicants, the least toxic chemicals were Supranol Br. Red 3Bur (Acidic dye) and Malathion. After ten days, the least toxic were Cu and Zn.

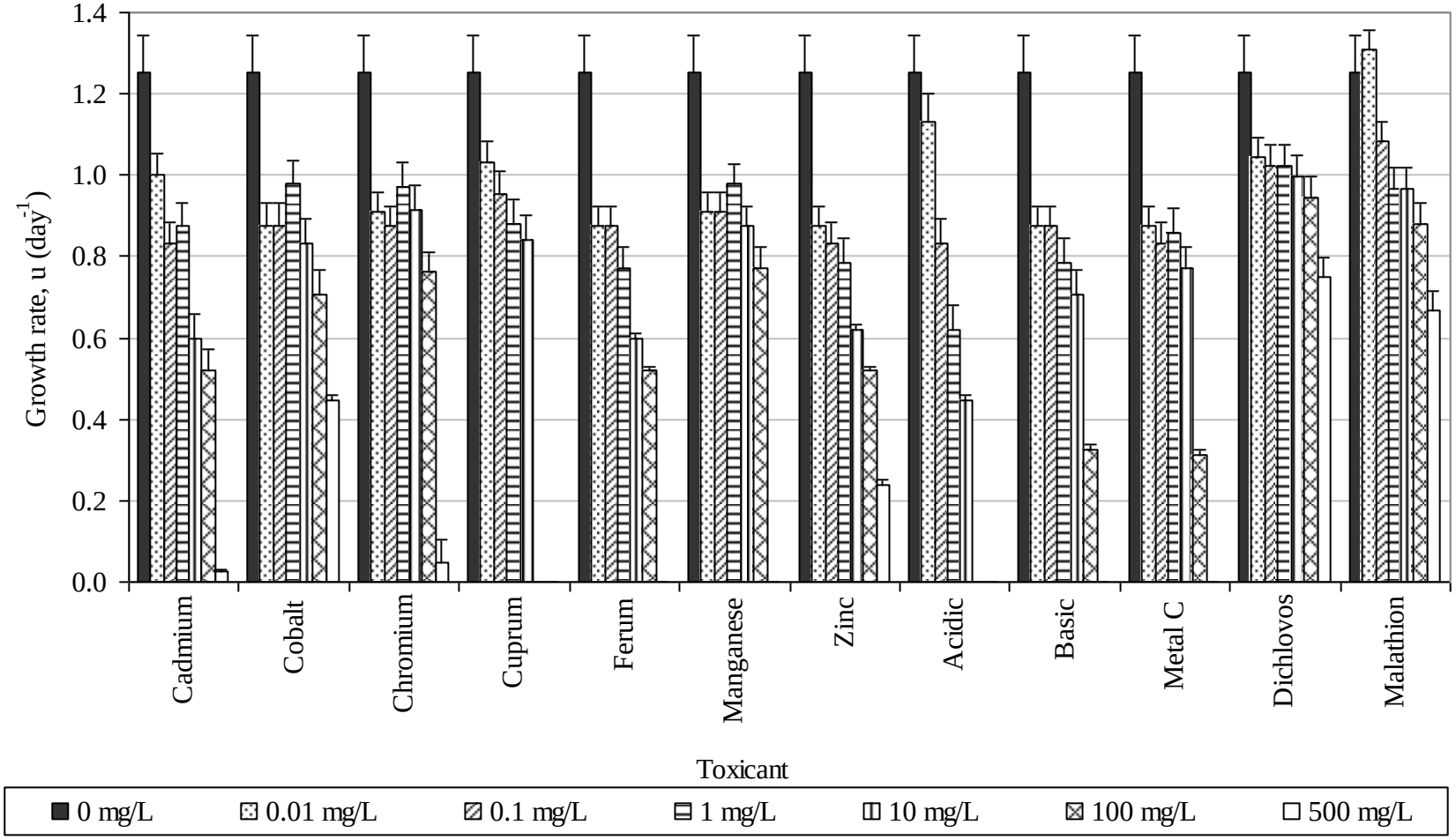


Figure 4.1: Growth rate (based on Chl *a*) of *Chlorella vulgaris* UMACC 245 after exposure to selected toxicants for four days

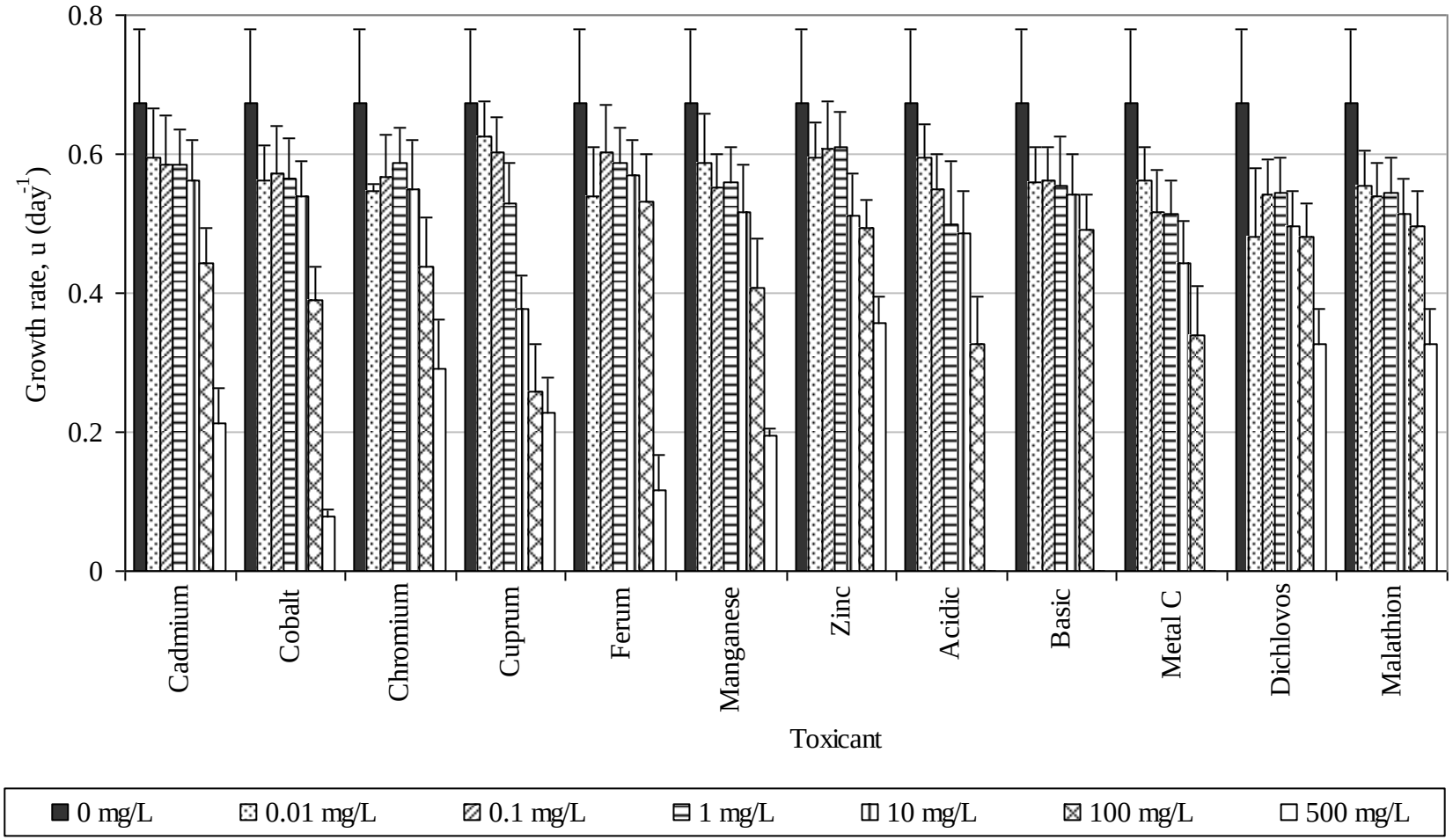


Figure 4.2: Growth rate (based on Chl *a*) of *Chlorella vulgaris* UMACC 245 after exposure to selected toxicants for ten days

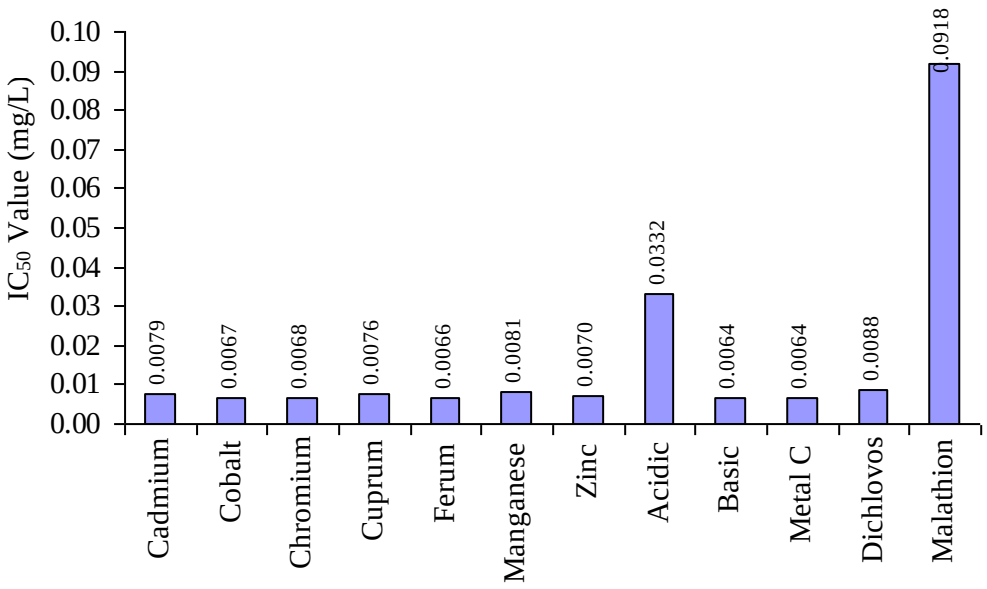


Figure 4.3: IC_{50} value (based on Chl *a*) of *Chlorella vulgaris* UMACC 245 after exposure to selected toxicants for four days

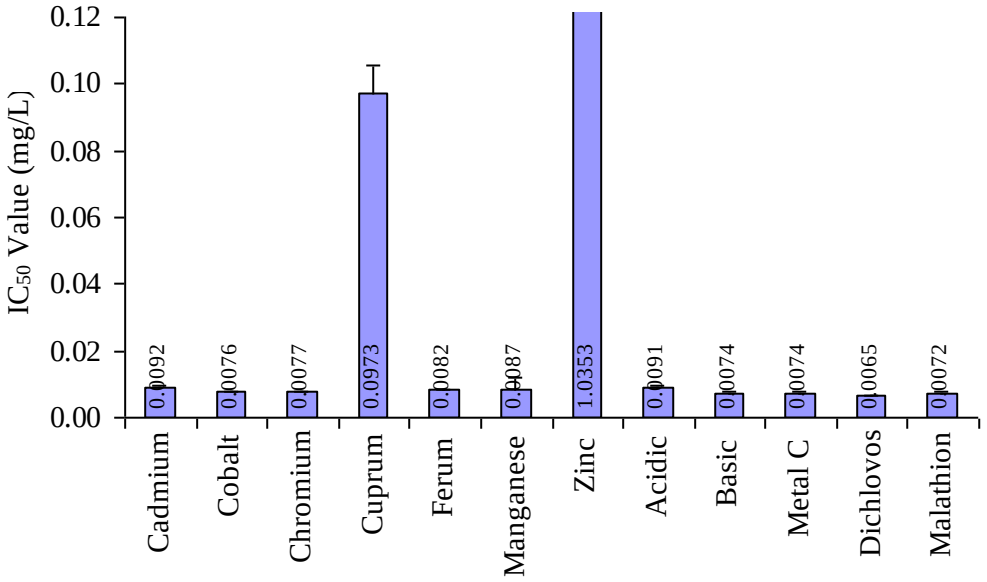


Figure 4.4: IC_{50} value (based on Chl *a*) of *Chlorella vulgaris* UMACC 245 after exposure to selected toxicants for ten days

4.1.3.2 *Tetraselmis tetrahele* UMACC 144: Effects of 12 toxicants on growth

Figure 4.5 to Figure 4.6 show the growth rate (based on Chl *a*) and IC_{50} value (Fig. 4.7 to Fig. 4.8) of *Tetraselmis tetrahele* UMACC 144 after exposure to 12 toxicants for four and ten days. Appendix 10 gives the detailed growth rate of *Tetraselmis tetrahele* UMACC 144 after exposure to 12 toxicants for four and ten days.

The results show, growth rate of the *Tetraselmis tetrahele* UMACC 144 grown in the Prov50 Medium without toxicant (control culture) for four and ten days were 1.614 ± 0.029 day^{-1} and 0.759 ± 0.046 day^{-1} respectively.

After being exposed for four days to toxicants, the results shows the growth rate of *Tetraselmis* exposed to Mn, Metal complex dye, Dichlovos and Malathion decreased with increasing concentrations of toxicant. However in the *Tetraselmis* exposed to Cd, Co, Cr, Cu, Fe, Zn, Acidic dye and Basic dye for four days and also in the *Tetraselmis* exposed to Cr, Acidic dye, Basic dye, Dichlovos and Malathion for ten days, the growth rate increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. Statistical analysis using ANOVA showed there was significant difference ($p<0.05$) for all parameter in each concentration of toxicant tested compared with the control culture (Appendix 30). The IC_{50} values (Fig. 4.7 to Fig. 4.8) show that after 4 days, the least toxic chemicals were Cr and after ten days, the least toxic were Fe.

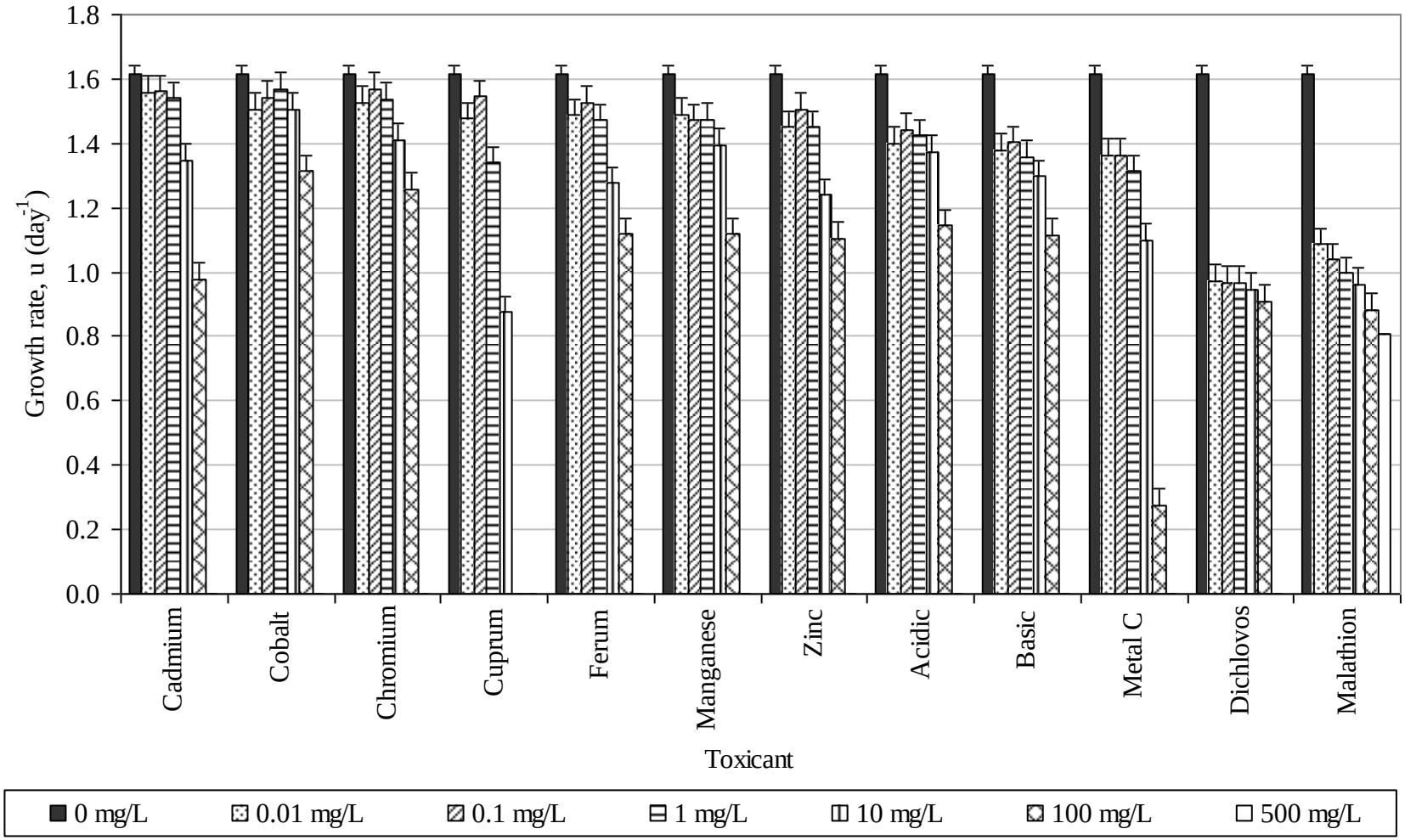


Figure 4.5: Growth rate of *Tetraselmis tetrahele* UMACC 144 after exposure to selected toxicants for four days

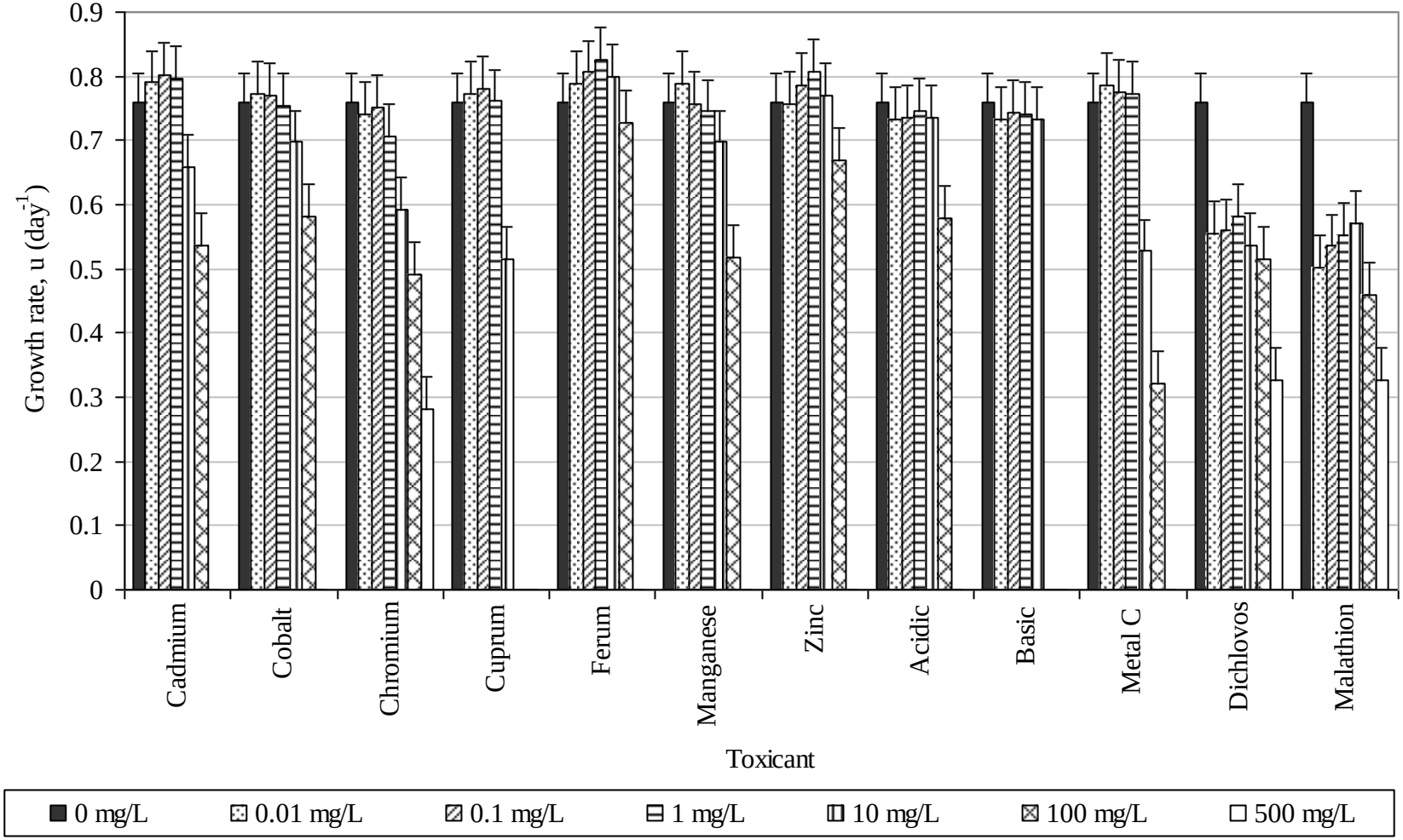


Figure 4.6: Growth rate of *Tetraselmis tetrahele* UMACC 144 after exposure to selected toxicants for ten days

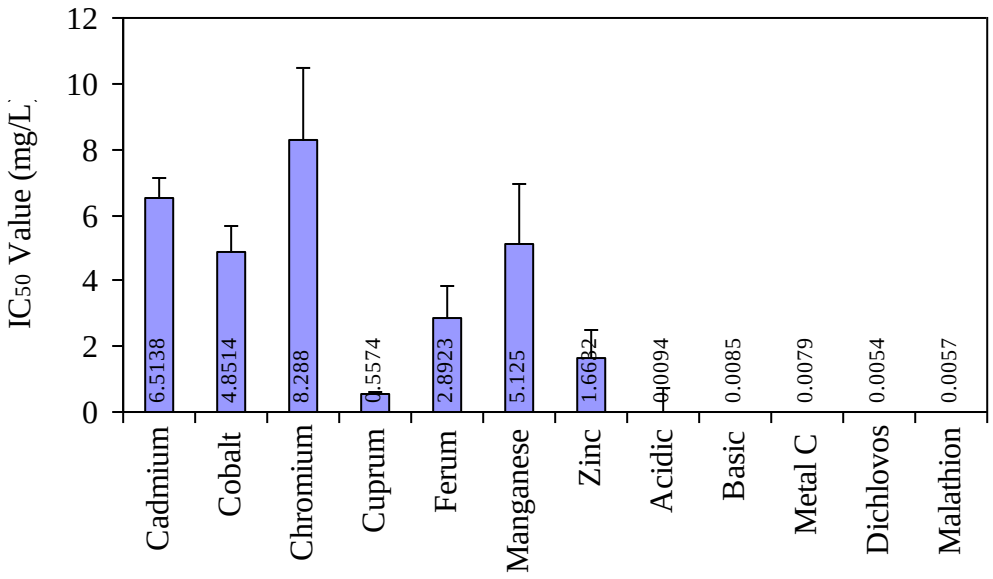


Figure 4.7: IC_{50} value (based on Chl *a*) of *Tetraselmis tetrahele* UMACC 144 after exposure to different concentrations of 12 toxicants for four days

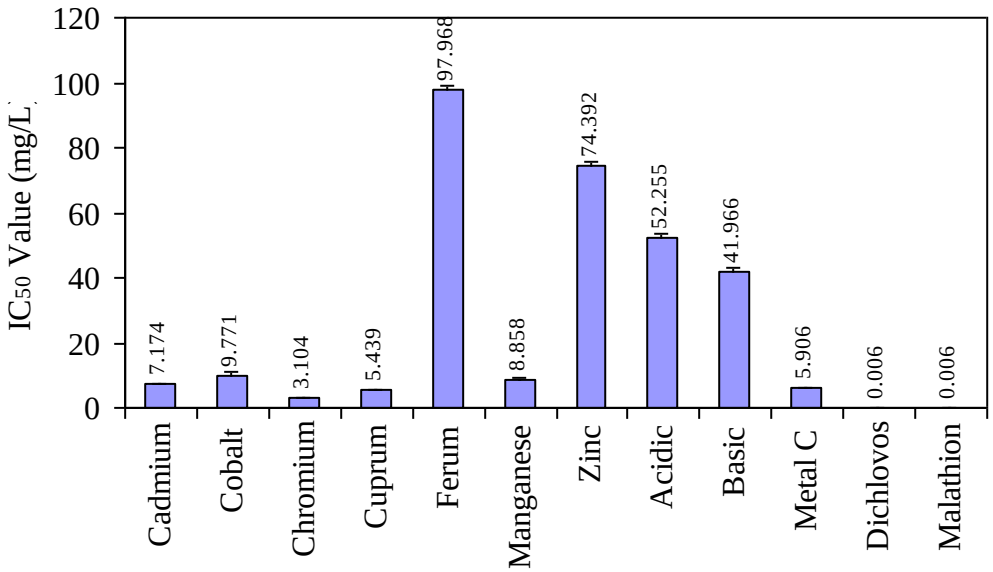


Figure 4.8: IC_{50} value (based on Chl *a*) of *Tetraselmis tetrahele* UMACC 144 after exposure to different concentrations of 12 toxicants for ten days

4.1.3.3 *Boergesenia forbesii* : Effects of 12 toxicants on growth

Figure 4.9 to Figure 4.10 show the growth rate (based on Chl *a*), carotenoid content (Fig. 4.11 to Fig. 4.12) and IC_{50} value (Fig. 4.13 to Fig. 4.14) of *Boergesenia forbesii* after exposure to 12 toxicants for four and ten days. Appendix 15 gives the detailed growth rate and carotenoid content of *Boergesenia forbesii* after exposure to 12 toxicants for four and ten days.

The growth rate and carotenoid content of algae grown in the Prov50 medium without toxicant (control culture) was 1.126 ± 0.026 day^{-1} and 0.039 ± 0.002 µg/g carotenoid respectively in four days culture and in ten days culture, the growth rate and carotenoid content were 0.463 ± 0.010 day^{-1} and 0.043 ± 0.001 µg/g carotenoid respectively.

In general, the growth of *Boergesenia* exposed to Cd, Co, Cu, Fe, Basic dye and Malathion for four days and also in Boergesenia exposed to Fe, Basic dye, Dichlovos and Malathion for ten days, the growth rate and carotenoid content decreased with increasing concentrations of toxicant. Statistical analysis using ANOVA showed there were significant difference ($p<0.05$) for all growth parameter in each concentration of toxicant tested compared with the control culture (Appendix 35).

The IC_{50} values (Fig. 4.13 to Fig. 4.14) show that after 4 days, the least toxic chemicals were Zn and after ten days, the least toxic were Acidic dye.

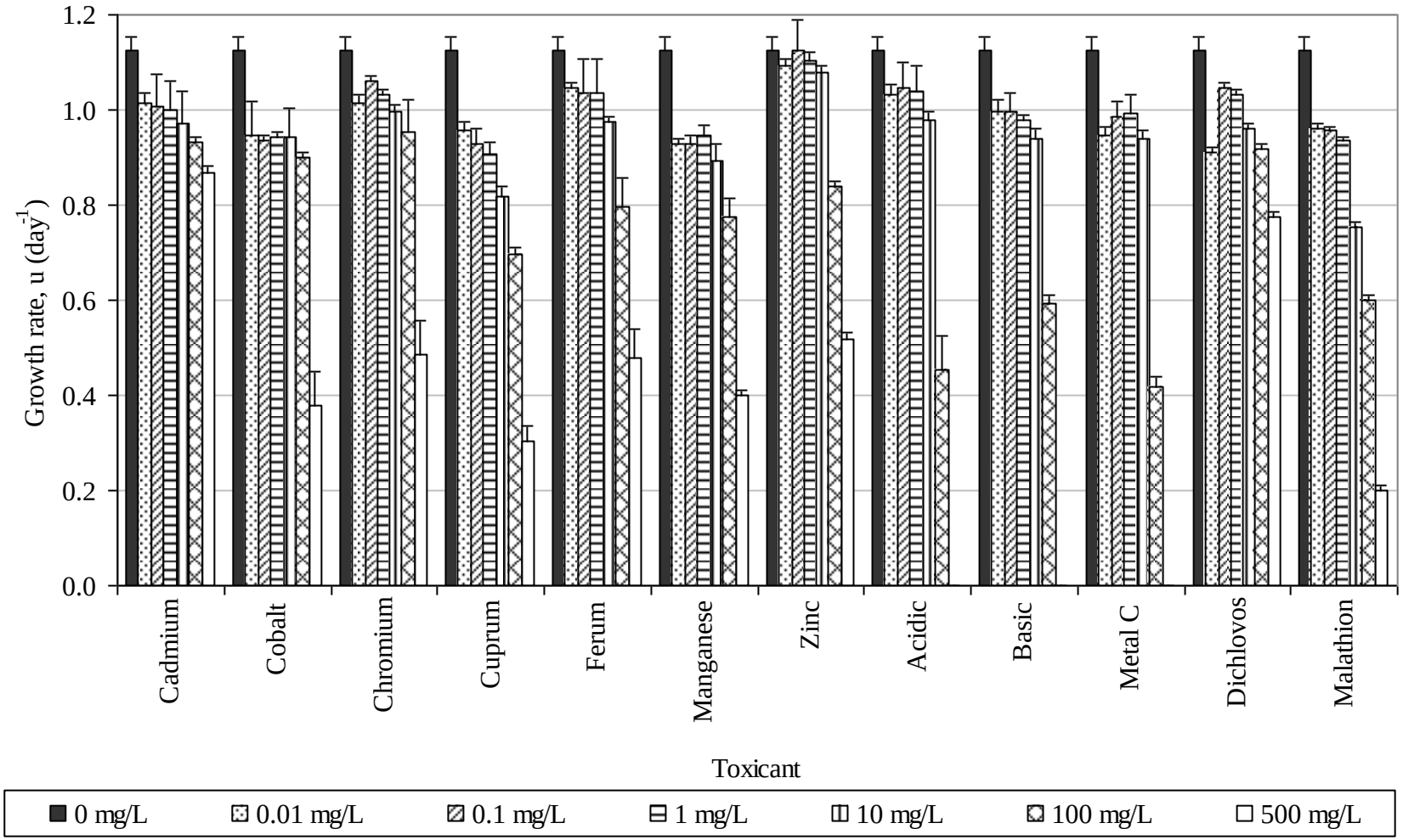


Figure 4.9: Growth rate of *Boergesenia forbesii* after exposure to selected toxicants for four days

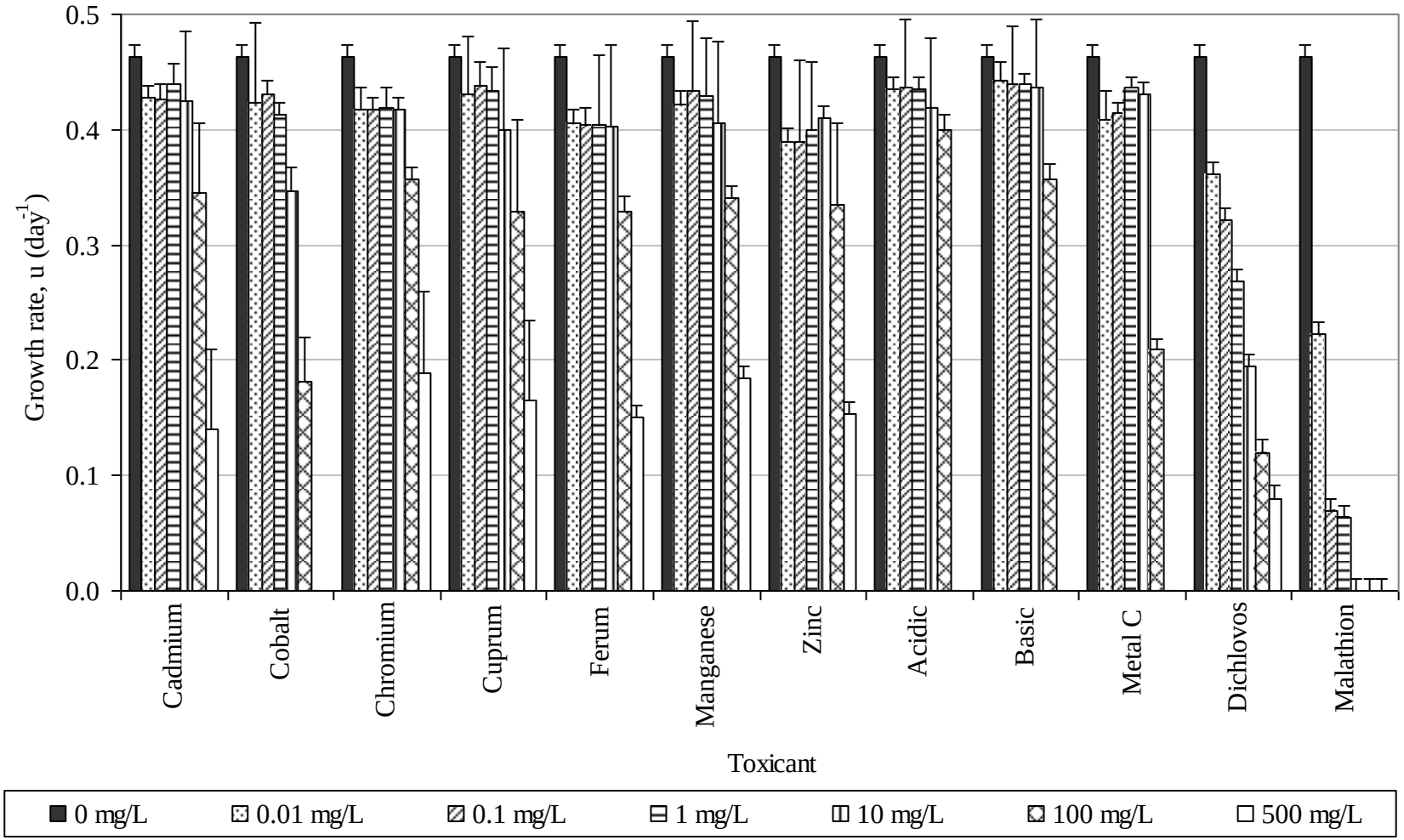


Figure 4.10: Growth rate of *Boergesenia forbesii* after exposure to selected toxicants for ten days

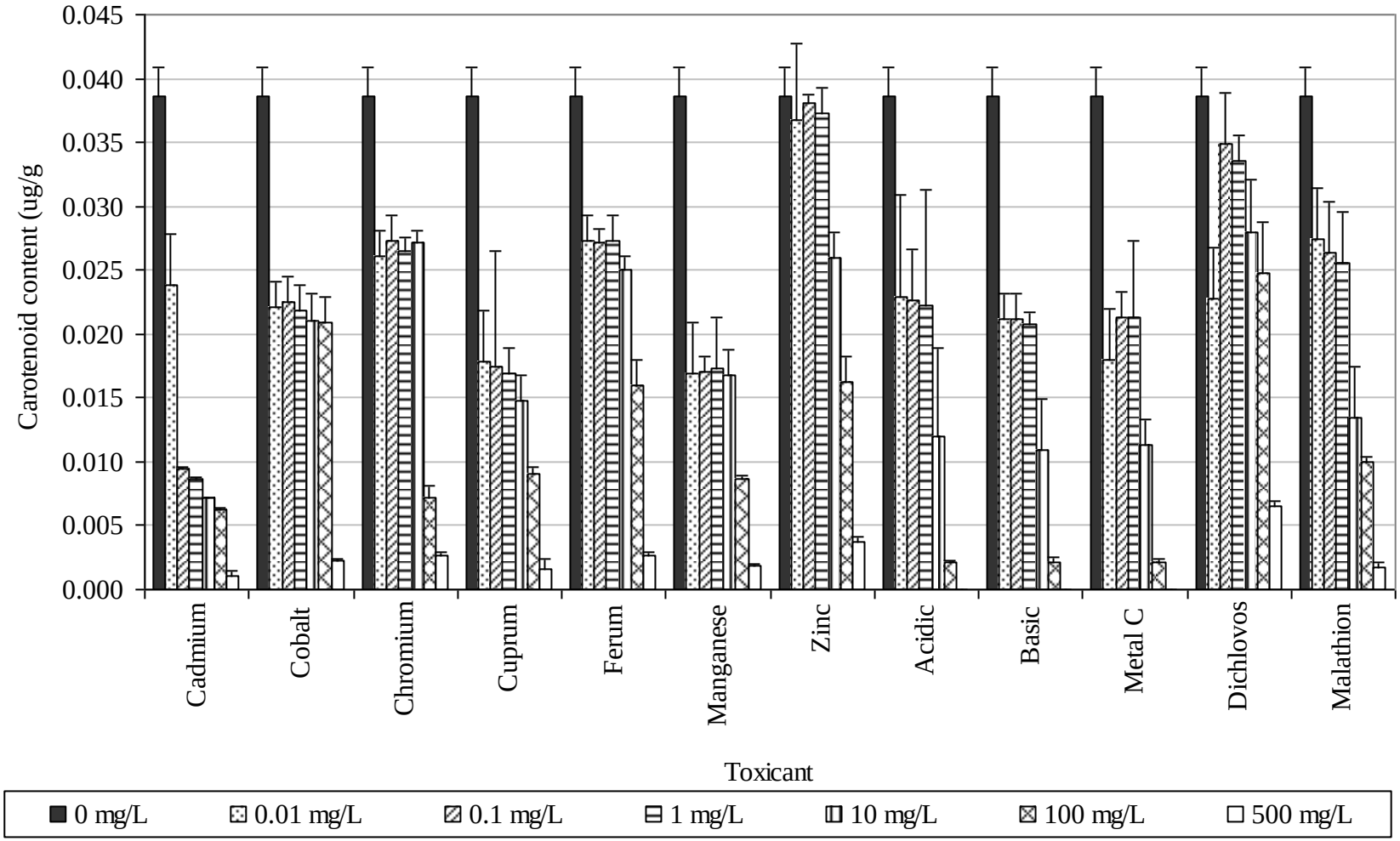


Figure 4.11: Carotenoid content of *Boergesenia forbesii* after exposure to selected toxicants for four days

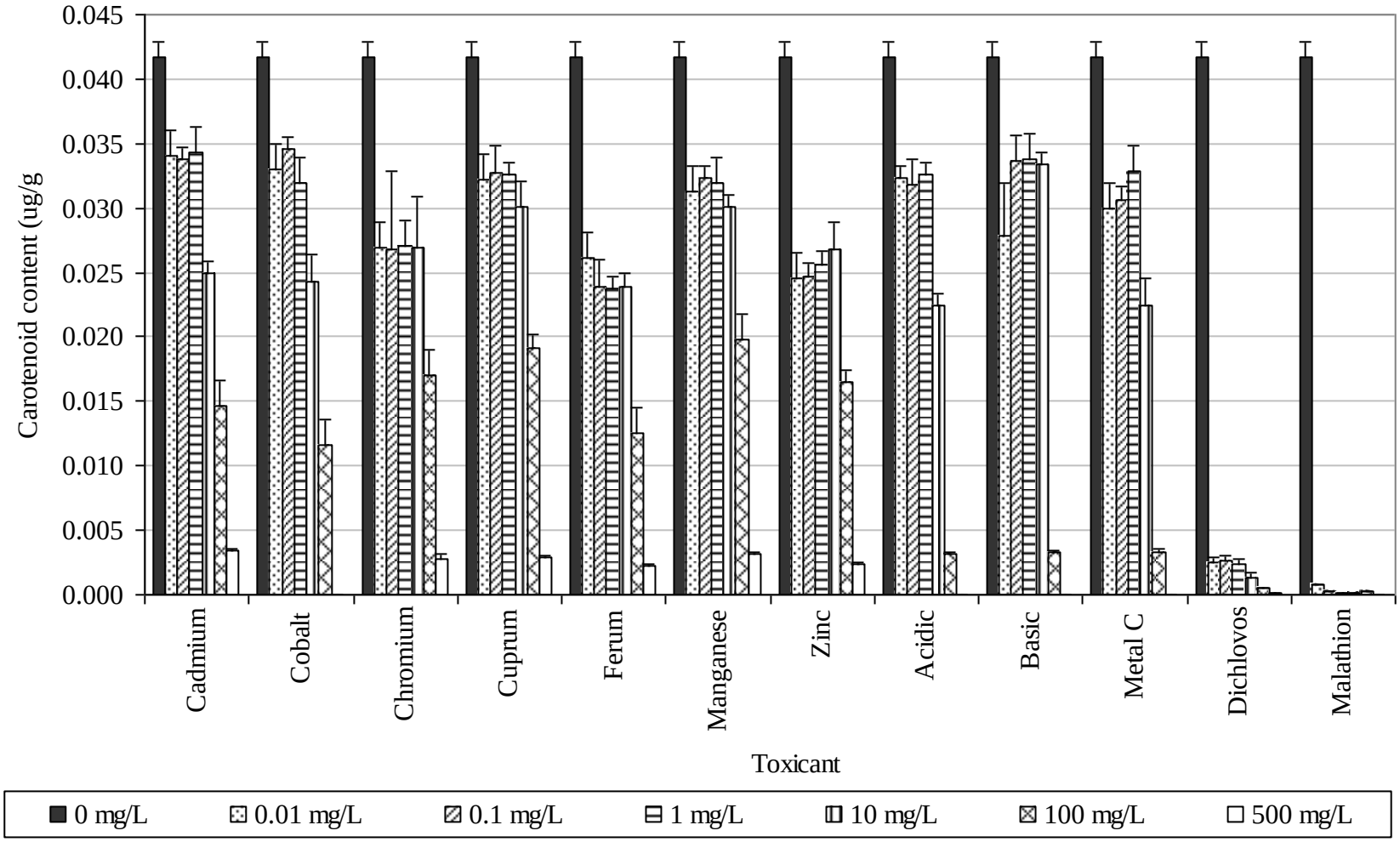


Figure 4.12: Carotenoid content of *Boergesenia forbesii* after exposure to selected toxicants for ten days

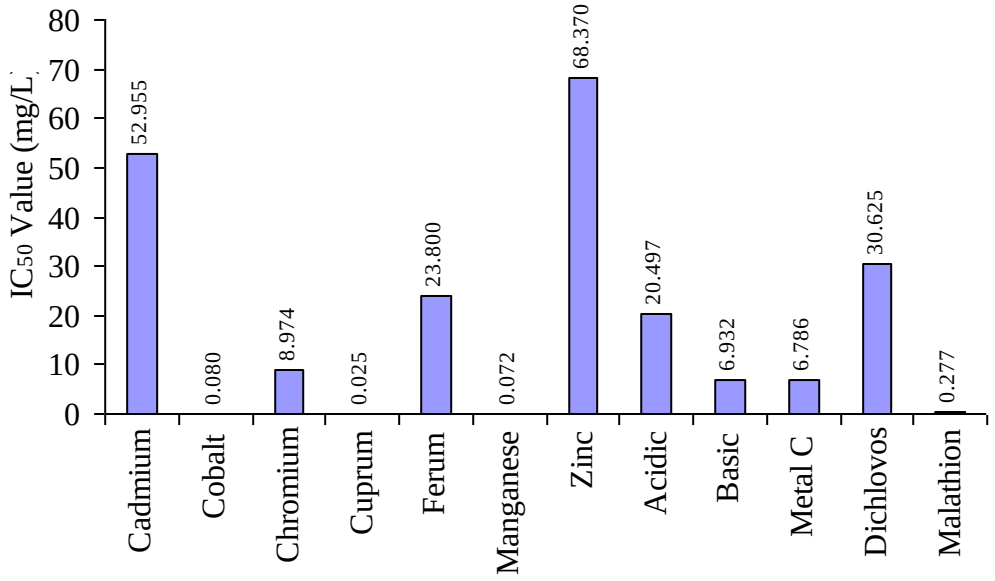


Figure 4.13: IC_{50} value (based on Chl *a*) of *Boergesenia forbesii* after exposure to different concentrations of 12 toxicants for four days

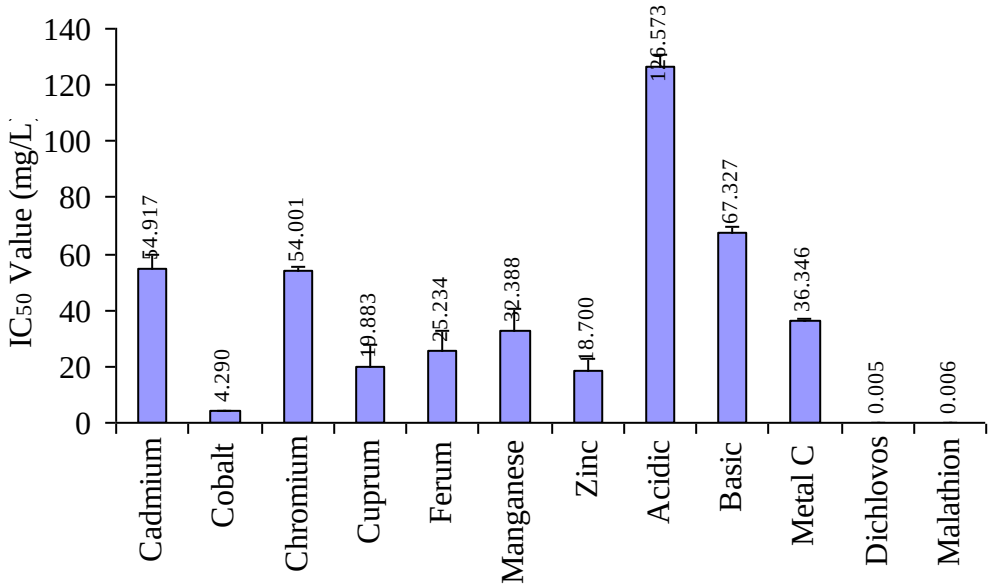


Figure 4.14: IC_{50} value (based on Chl *a*) of *Boergesenia forbesii* after exposure to different concentrations of 12 toxicants for ten days

4.1.3.4 *Ventricaria ventricosa* : Effects of 12 toxicants on growth

Figure 4.15 to Figure 4.16 show the growth rate (based on Chl *a*), carotenoid content (Fig. 4.17 to Fig. 4.18) and IC_{50} value (Fig. 4.19 to Fig. 4.20) of *Ventricaria ventricosa* after exposure to 12 toxicants for four and ten days. Appendix 20 give the detailed growth rate and carotenoid content of *Ventricaria ventricosa* after exposure to 12 toxicants for four and ten days.

The growth rate and carotenoid content of algae grown in the Prov50 medium without toxicant (control culture) was 0.912$\pm$ 0.010 day^{-1} and 0.015$\pm$0.001 µg/g carotenoid respectively in four days culture and in ten days culture, the growth rate and carotenoid content were 0.371$\pm$0.020 day^{-1} and 0.017$\pm$0.004 µg/g carotenoid respectively.

In general, the growth rate and carotenoid content between concentrations 0.01 mg/L up to 10 mg/L toxicant were markedly higher than control cultures ($p<0.05$) except in the cultures exposed to Basic dyes for four days. Statistical analysis using ANOVA showed there were significant difference ($p<0.05$) for all growth parameter in each concentration of toxicant tested compared with the control culture (Appendix 40).

The IC_{50} values (Fig. 4.19 to Fig. 4.20) show that after 4 days and ten day, the least toxic chemicals were Malathion.

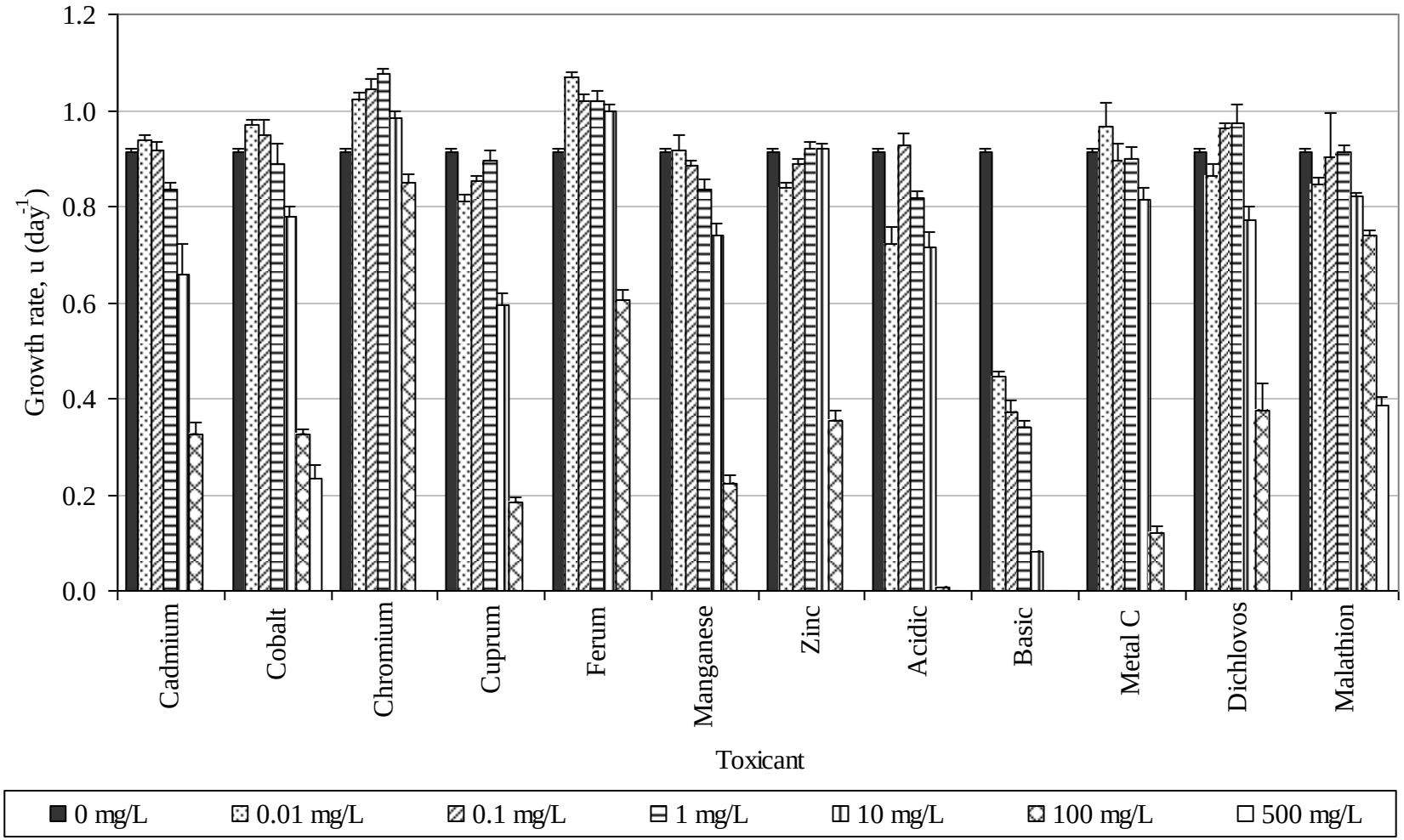


Figure 4.15: Growth rate of *Ventricaria ventricosa* after exposure to selected toxicants for four days

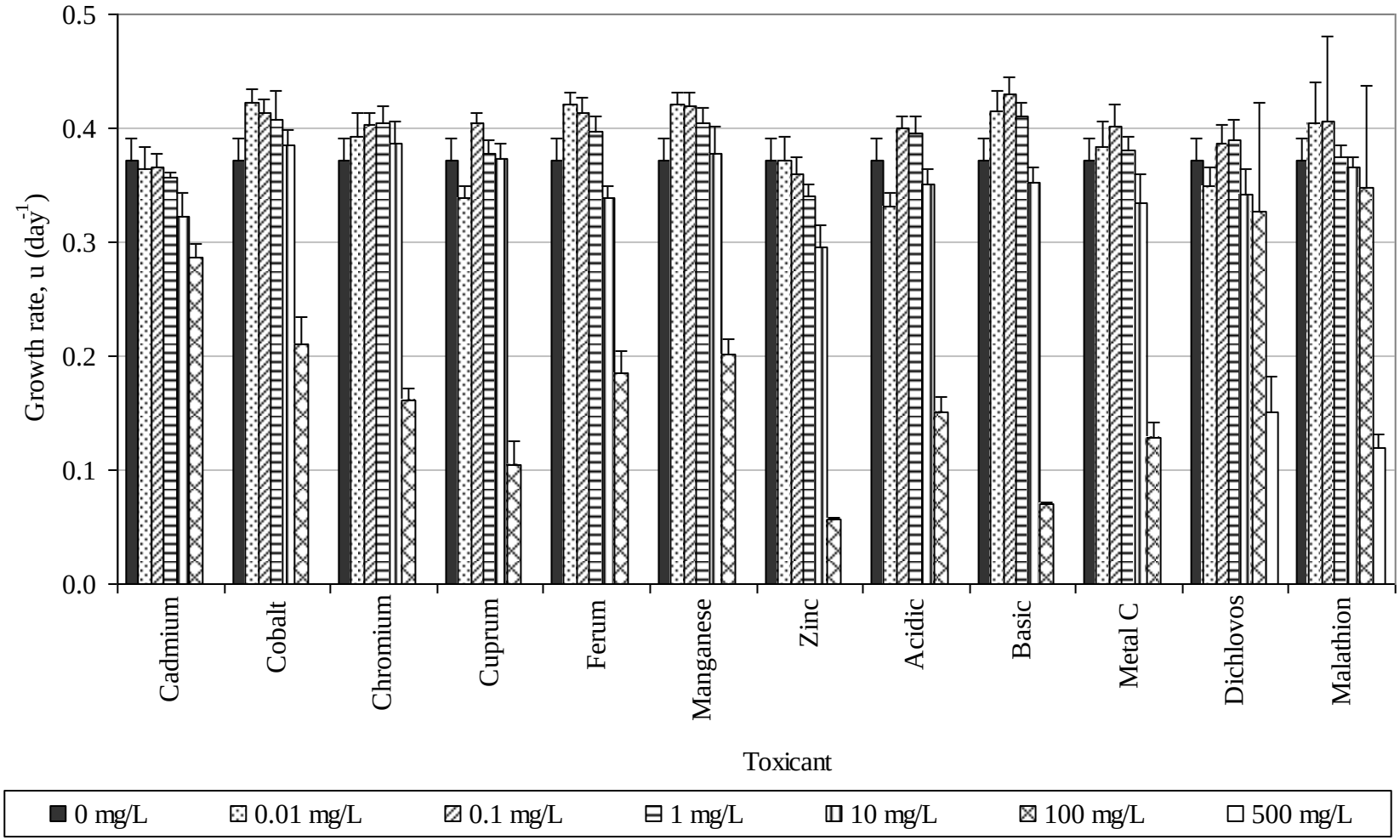


Figure 4.16: Growth rate of *Ventricaria ventricosa* after exposure to selected toxicants for ten days

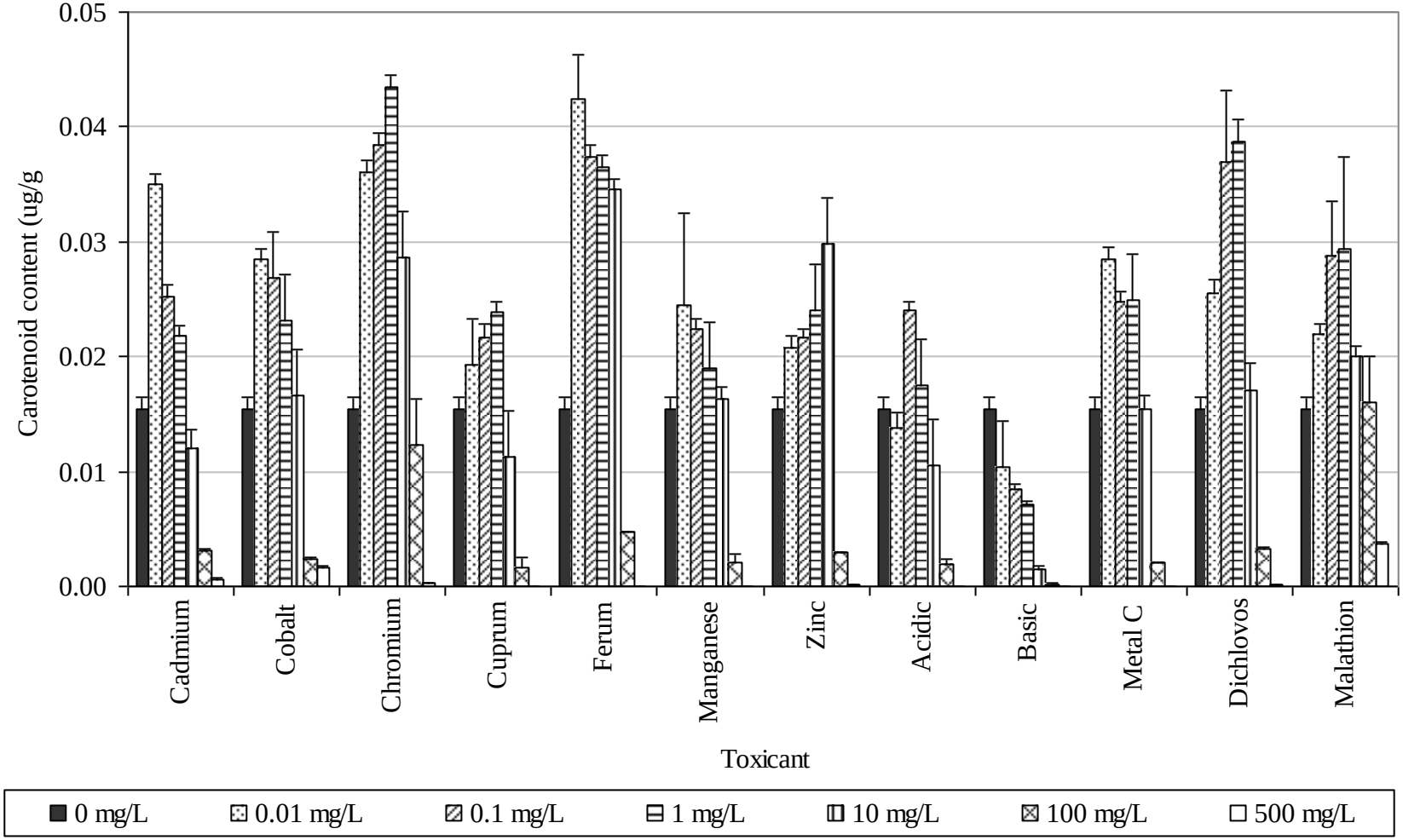


Figure 4.17: Carotenoid content of *Ventricaria ventricosa* after exposure to selected toxicants for four days

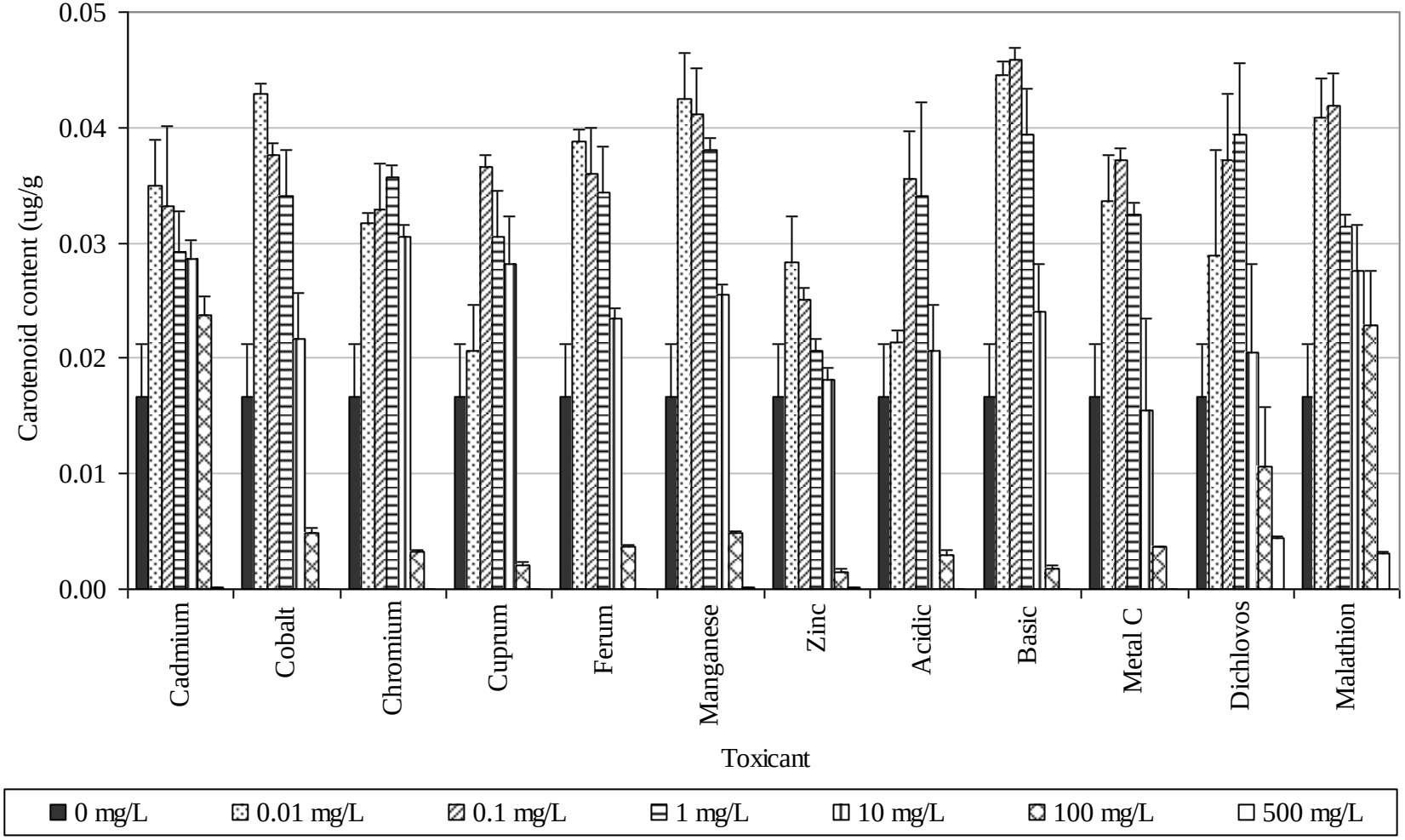


Figure 4.18: Carotenoid content of *Ventricaria ventricosa* after exposure to selected toxicants for ten days

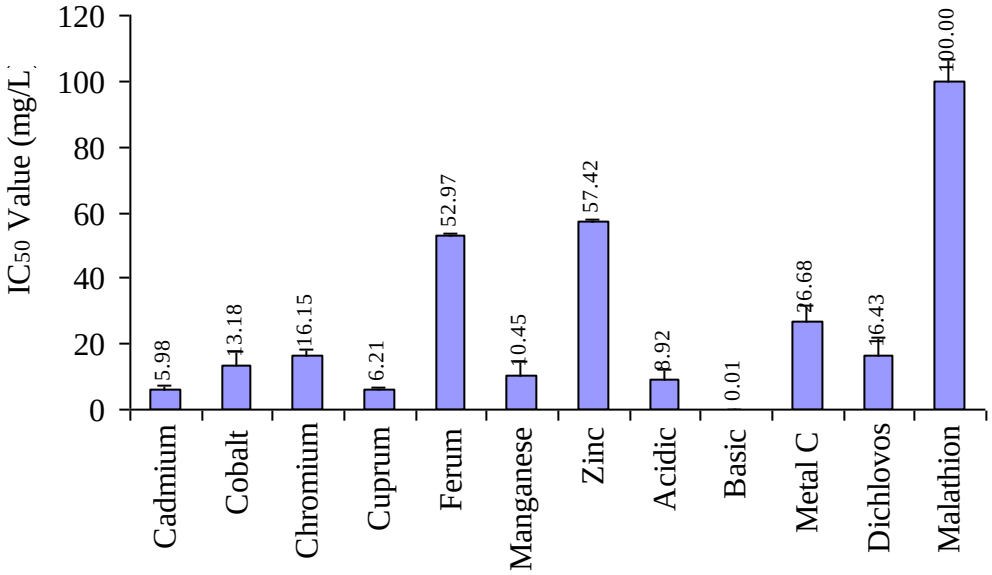


Figure 4.19: IC_{50} value (based on Chl *a*) of *Ventricaria ventricosa* after exposure to different concentrations of 12 toxicants for four days

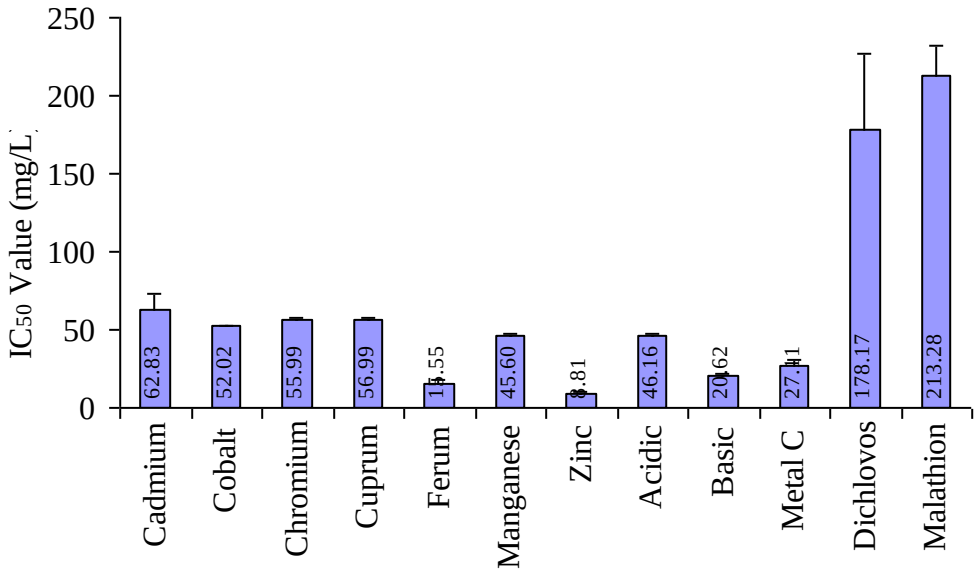


Figure 4.20: IC_{50} value (based on Chl *a*) of *Ventricaria ventricosa* after exposure to different concentrations of 12 toxicants for ten days

4.2 EFFECT OF SELECTED TOXICANTS ON BIOCHEMICAL COMPOSITION AND TOXICITY STUDIES OF ALGAE

To investigate the effects of 12 chemical contaminants [Cd, Co, Cr, Cu, Fe, Mn, Zn, Supranol Br. Red 3Bur (Acidic dye), Astrazon Red FBL (Basic dye), Lanaset Red 2GA (Metal complex dye), Dichlovos and Malathion on biochemical compositions of *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa*, each types of toxicant at different concentrations (0, 0.01, 0.1, 1, 10, 100 and 500 mg/L) were exposed individually to the algae for four and ten days. Biochemical composition in the algae including carbohydrate, protein and lipid content were determined.

4.2.1 *Chlorella vulgaris* UMACC 245: Effects of 12 Toxicants on Biochemical Composition

Figure 4.21 to Figure 4.26 summarizes the carbohydrate (Fig. 4.21 to Fig. 4.22), protein (Fig. 4.23 to Fig. 4.24) and lipid (Fig. 4.25 to Fig. 4.26) in *Chlorella vulgaris* UMACC 245 after exposure to 12 toxicants for four and ten days. Appendix 6 shows the detailed results of the biochemical compositions in *Chlorella vulgaris* UMACC 245.

The carbohydrate, protein and lipid content of the algae grown in Prov50 medium without toxicant (control cultures) for four day were 7.045$\pm$1.742% carbohydrate; 31.006$\pm$3.310% protein and 4.242$\pm$0.606% lipid respectively. In the ten days culture, the carbohydrate, protein and lipid content of the algae were 22.406$\pm$1.474% carbohydrate; 63.068$\pm$8.700% protein and 12.583$\pm$1.179% lipid respectively.

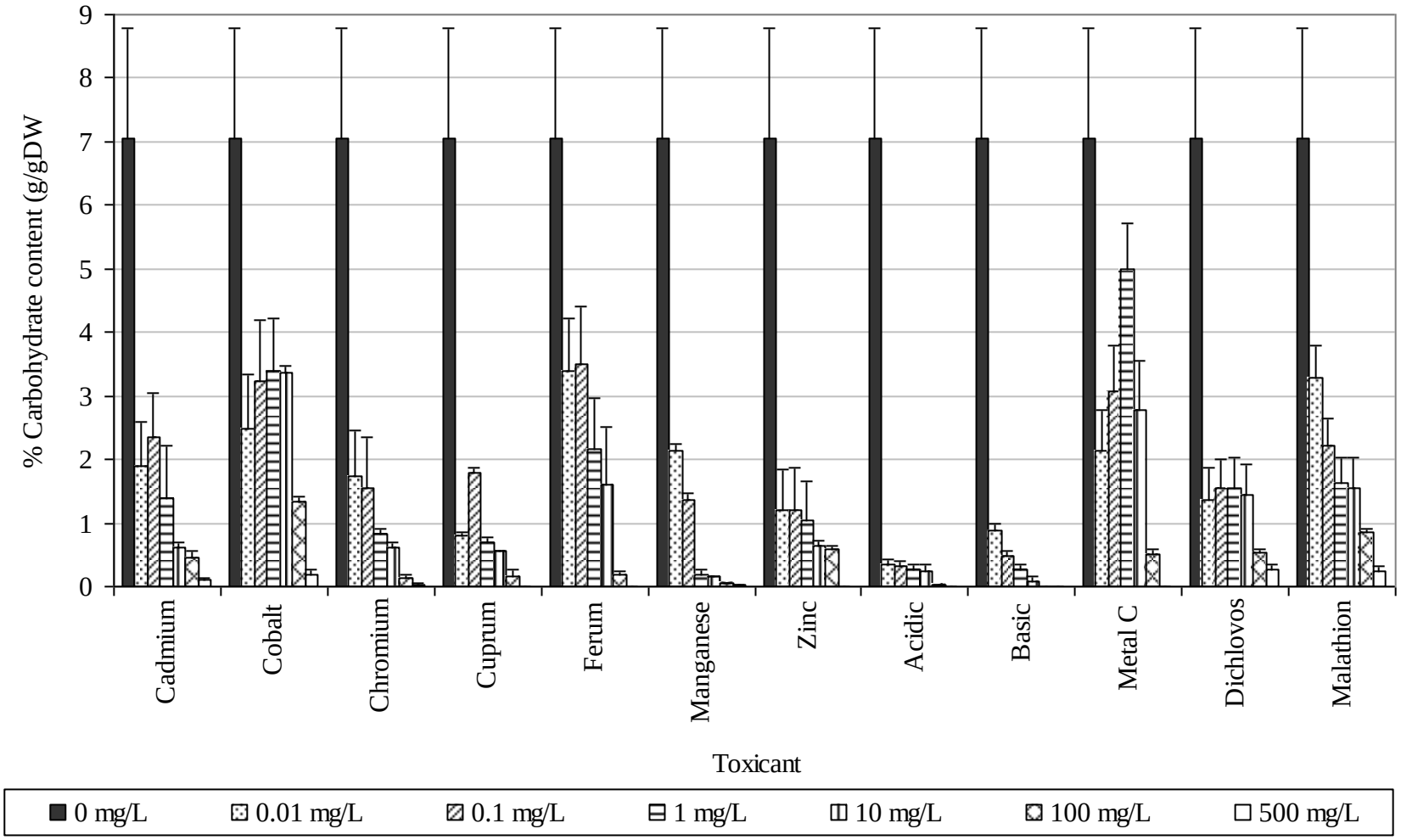


Figure 4.21: Carbohydrate content in *Chlorella vulgaris* UMACC 245 after exposure to selected toxicant for four days

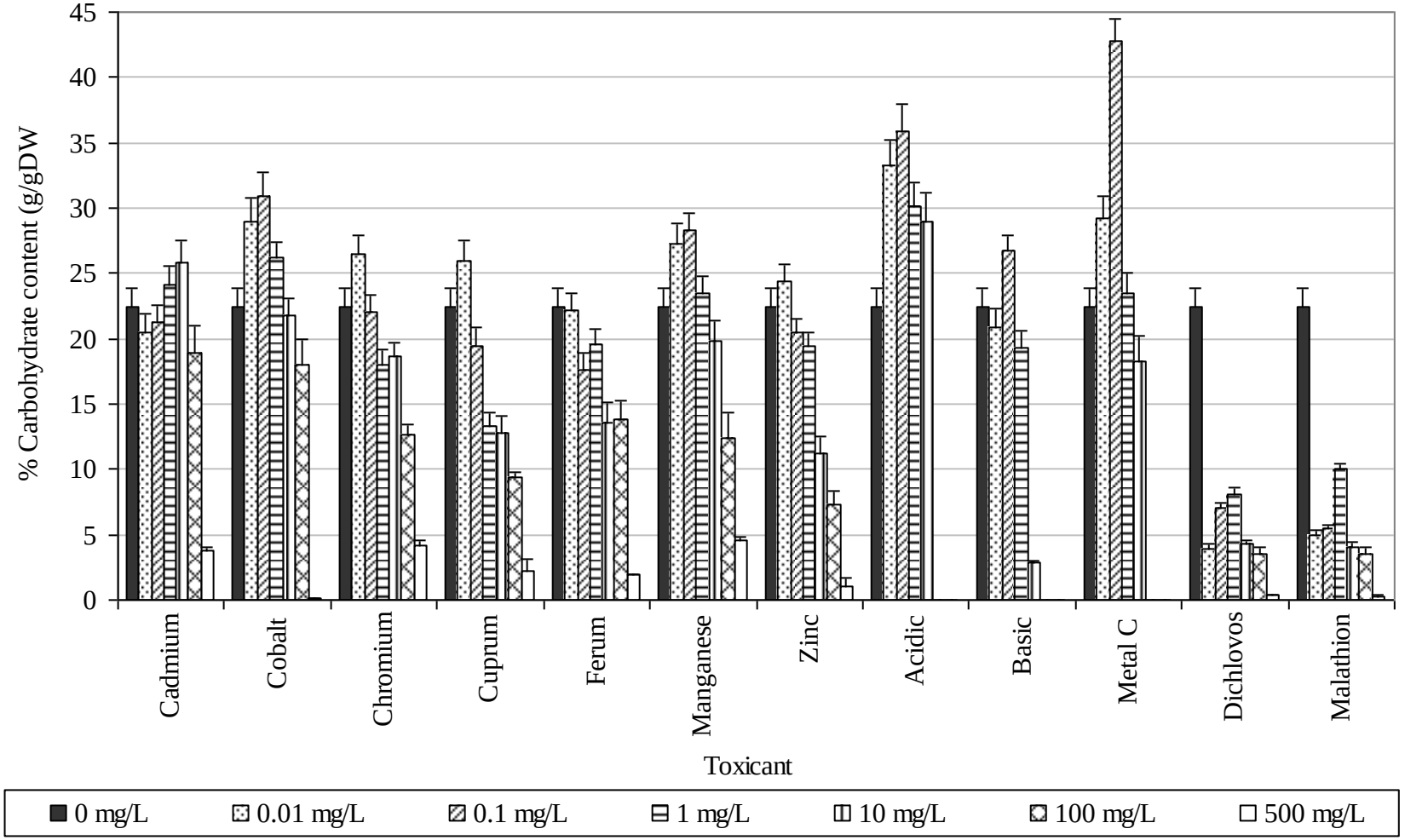


Figure 4.22: Carbohydrate content in *Chlorella vulgaris* UMACC 245 after exposure to selected toxicant for ten days

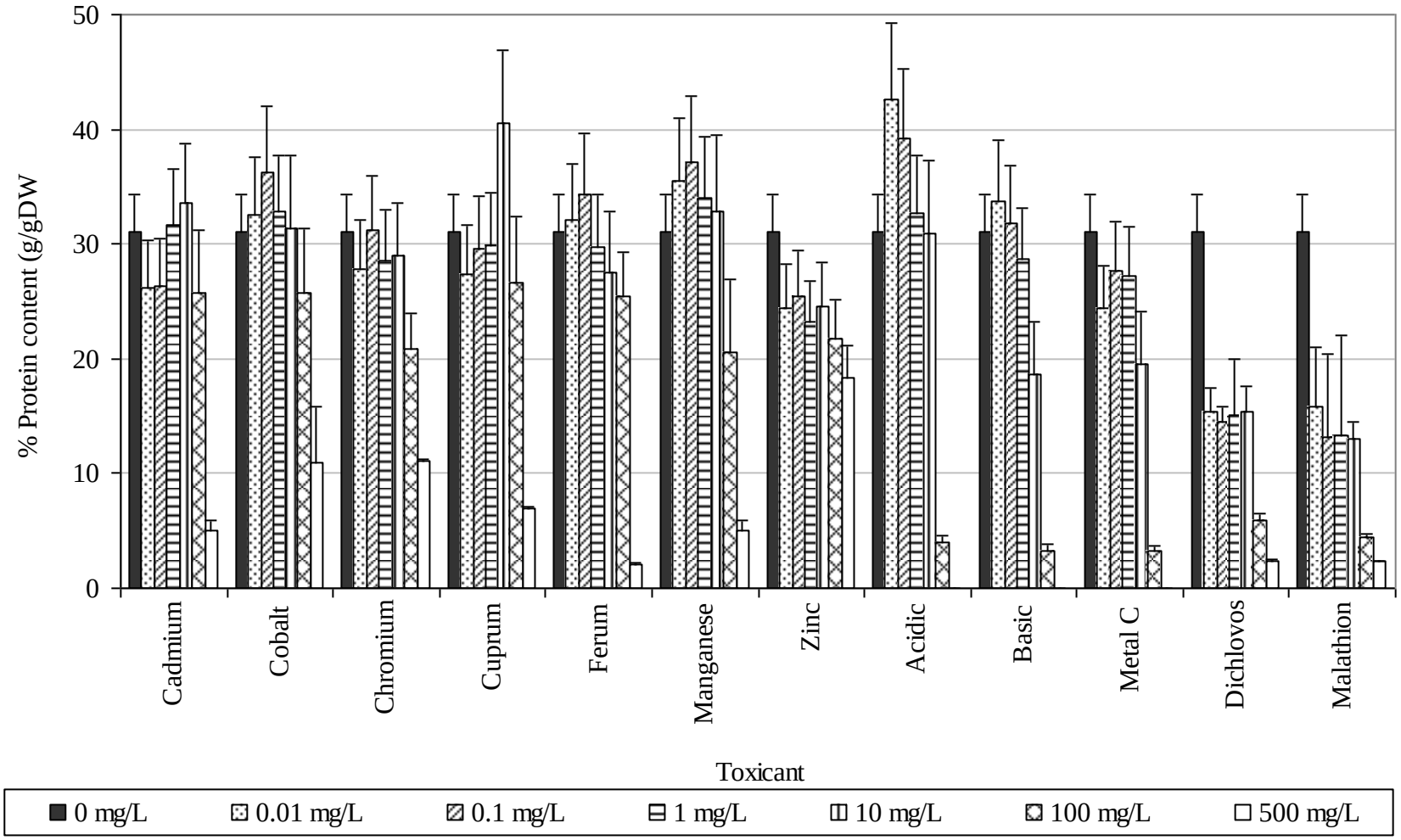


Figure 4.23: Protein content in *Chlorella vulgaris* UMACC 245 after exposure to selected toxicant for four days

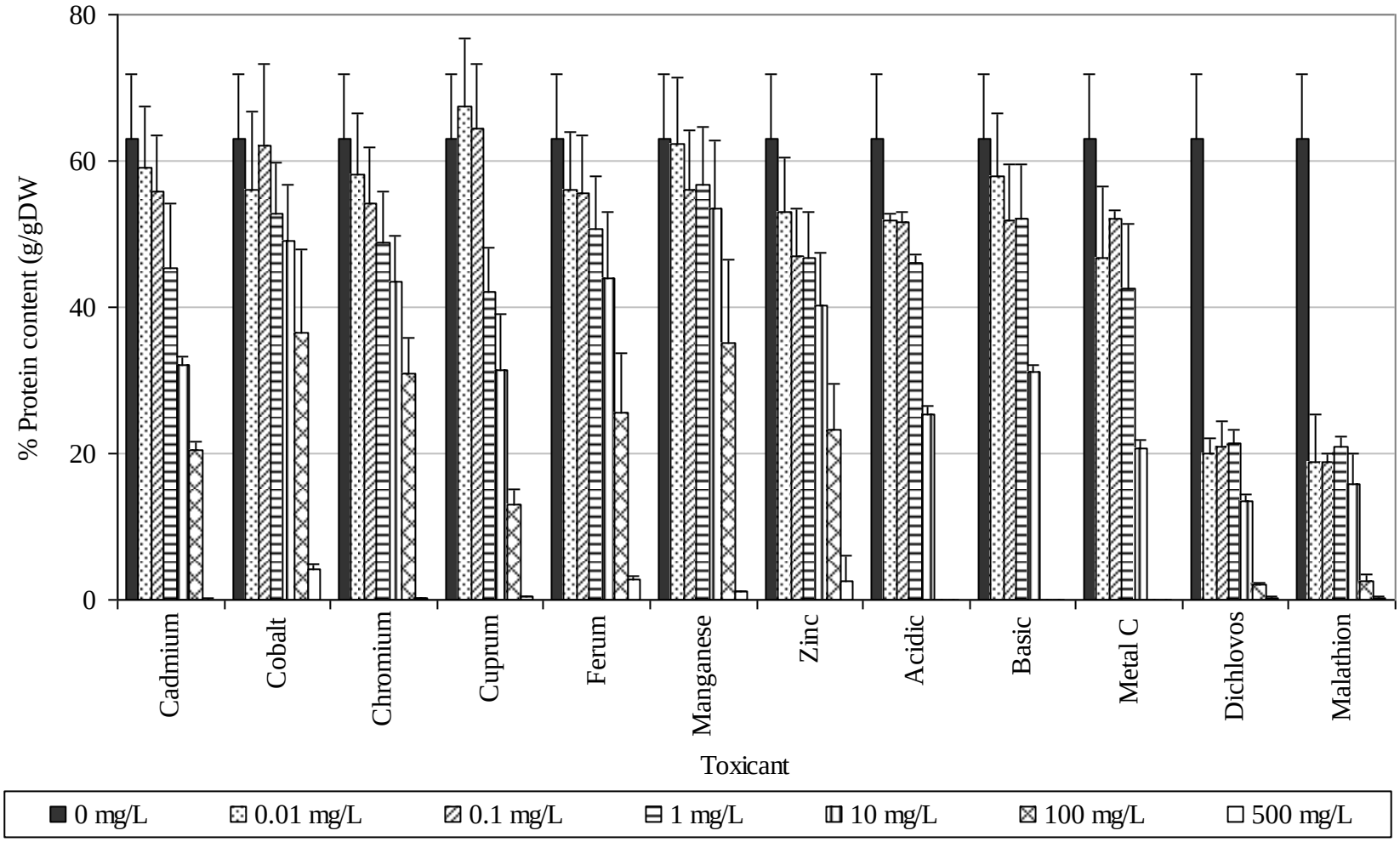


Figure 4.24: Protein content in *Chlorella vulgaris* UMACC 245 after exposure to selected toxicant for ten days

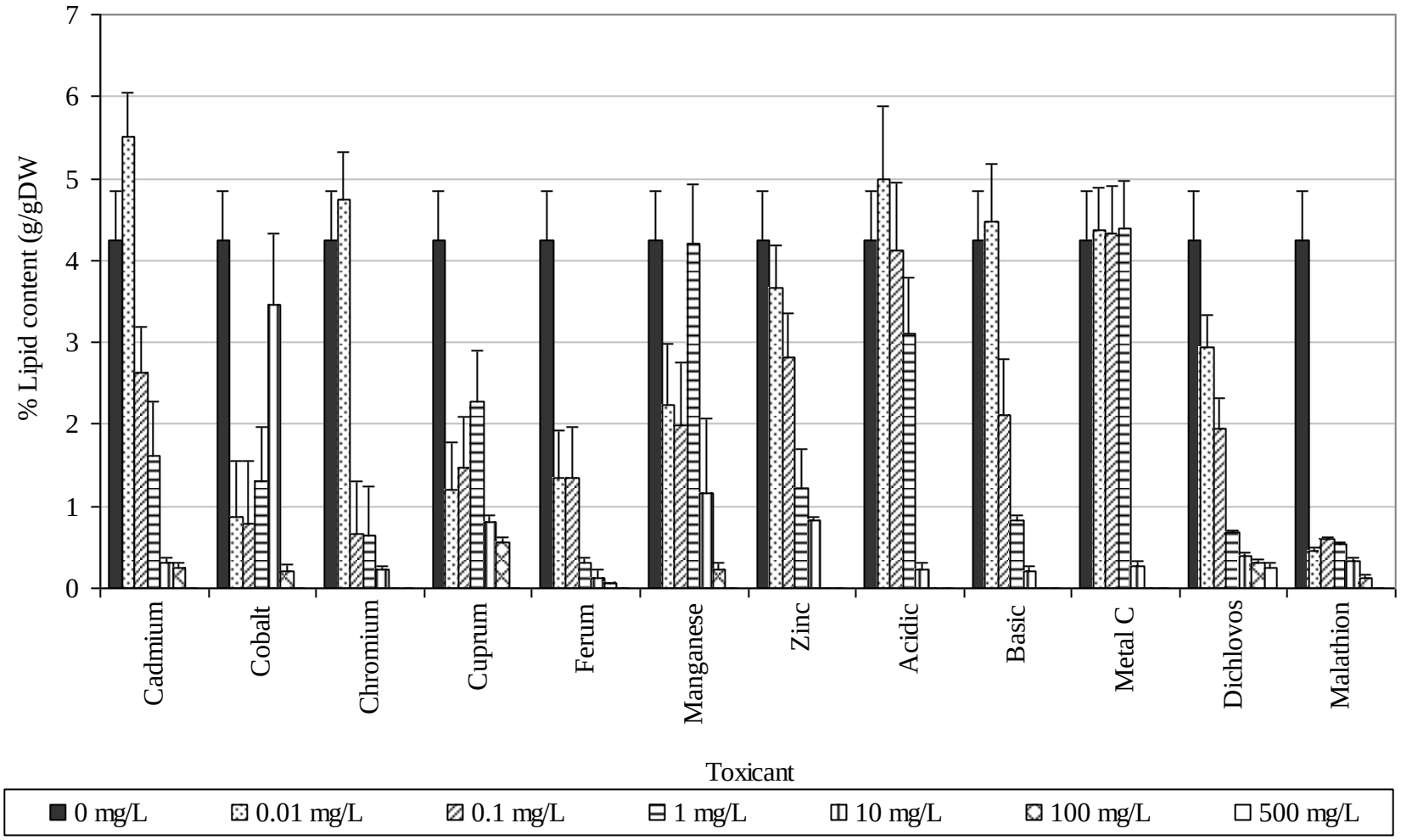


Figure 4.25: Lipid content in *Chlorella vulgaris* UMACC 245 after exposure to selected toxicant for four days

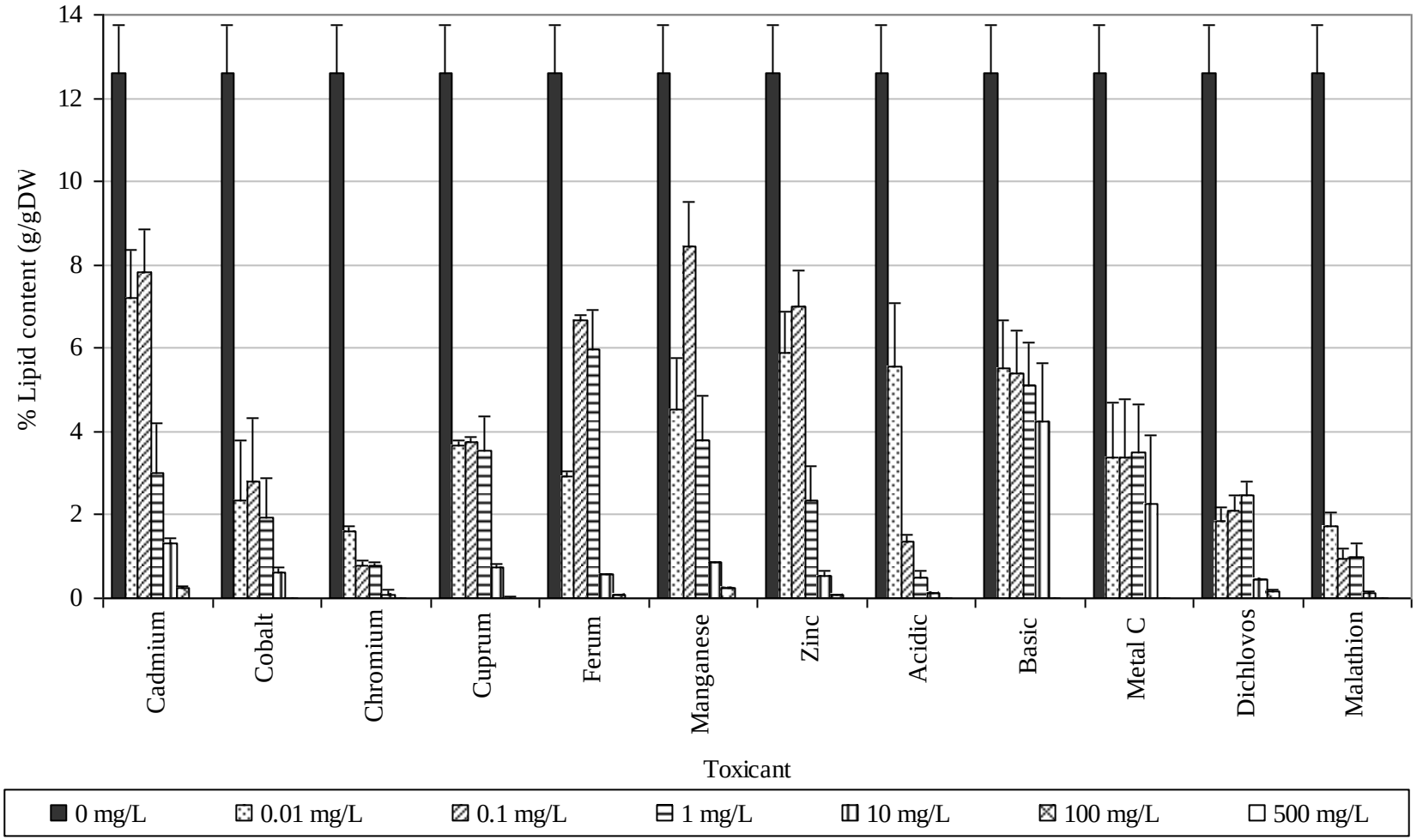


Figure 4.26: Lipid content in *Chlorella vulgaris* UMACC 245 after exposure to selected toxicant for ten days

In general, the carbohydrate content in the cultures exposed to toxicant for four days were lower than control cultures. In contrast, in the cultures exposed to toxicant for ten days, the carbohydrate content were higher ($p<0.05$) than control cultures between concentrations 0.01 mg/L to 10 mg/L toxicant except for carbohydrate content in the cultures exposed to Dichlovos and Malathion.

The results showed, in the *Chlorella* exposed to Cr, Mn, Acidic dye, Basic dye and Malathion for four days and in the cultures exposed to Fe for ten days, the carbohydrate content of the algae decreased with increasing concentrations of toxicant. However the carbohydrate contents, increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant in the *Chlorella* cultures exposed to Cd, Co, Cu, Fe, Zn, Metal complex dye and Dichlovos and also in the *Chlorella* exposed to Dichlovos and Malathion for ten days. The carbohydrate content in the culture exposed to toxicants for four and ten days ranged from 0.33 % to 4.98% and 8.13% to 42.72% respectively.

In general, the protein content in the cultures exposed to toxicant four days were higher incomparison to control cultures. However, the protein content in the cultures exposed to toxicant for ten days were markedly lower than control cultures ($p<0.05$). The results showed the protein content of the *Chlorella* exposed to Dichlovos and Malathion for four days and also in *Chlorella* exposed to Cd, Cr, Fe, Mn, Zn, Acidic dye and Basic dye for ten days, the protein content decreased with increasing concentrations of toxicant. The protein content in the culture exposed to toxicants for four and ten days ranged from 15.38 % to 42.59% and 21.01% to 67.54% respectively.

The lipid content in the algae exposed to toxicant for four and ten days, were significantly lower than control cultures ($p<0.05$). In the *Chlorella* exposed to Zn and Dichlovos for four days and also in *Chlorella* exposed to Cr, Acidic dye, Basic dye and Malathion for ten days, the lipid content decreased with increasing concentrations of toxicant. However in the four days culture of *Chlorella* exposed to Co, Cu, Fe, Mn and Malathion and also in *Chlorella* exposed to Cd, Co, Cu, Fe, Mn, Zn, Metal complex dye and Dichlovos for ten days, the lipid content increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. The lipid content in the culture exposed to toxicants for four and ten days ranged from 0.60 % to 5.51% lipid and 1.61% to 8.34% lipid respectively.

Statistical analysis using ANOVA showed there were significant difference ($p<0.05$) for all biochemical compositions in each concentration of toxicant tested compared with the control culture (Appendix 26). At the concentrations of 500 mg/L of the toxicant, the carbohydrate, protein and lipid content in the cultures were significantly low ($p<0.05$).

4.2.2 *Tetraselmis tetrahele* UMACC 144: Effects of 12 Toxicants on Biochemical Composition.

Figure 4.27 to Figure 4.32 summarizes the carbohydrate (Fig. 4.27 to Fig. 4.28), protein (Fig. 4.29 to Fig. 4.30) and lipid (Fig. 4.31 to Fig. 4.32) in *Tetraselmis tetrahele* UMACC 144 after exposure to 12 toxicants for four and ten days. Appendix 11 shows the detailed results of the biochemical compositions in *Tetraselmis tetrahele* UMACC 144.

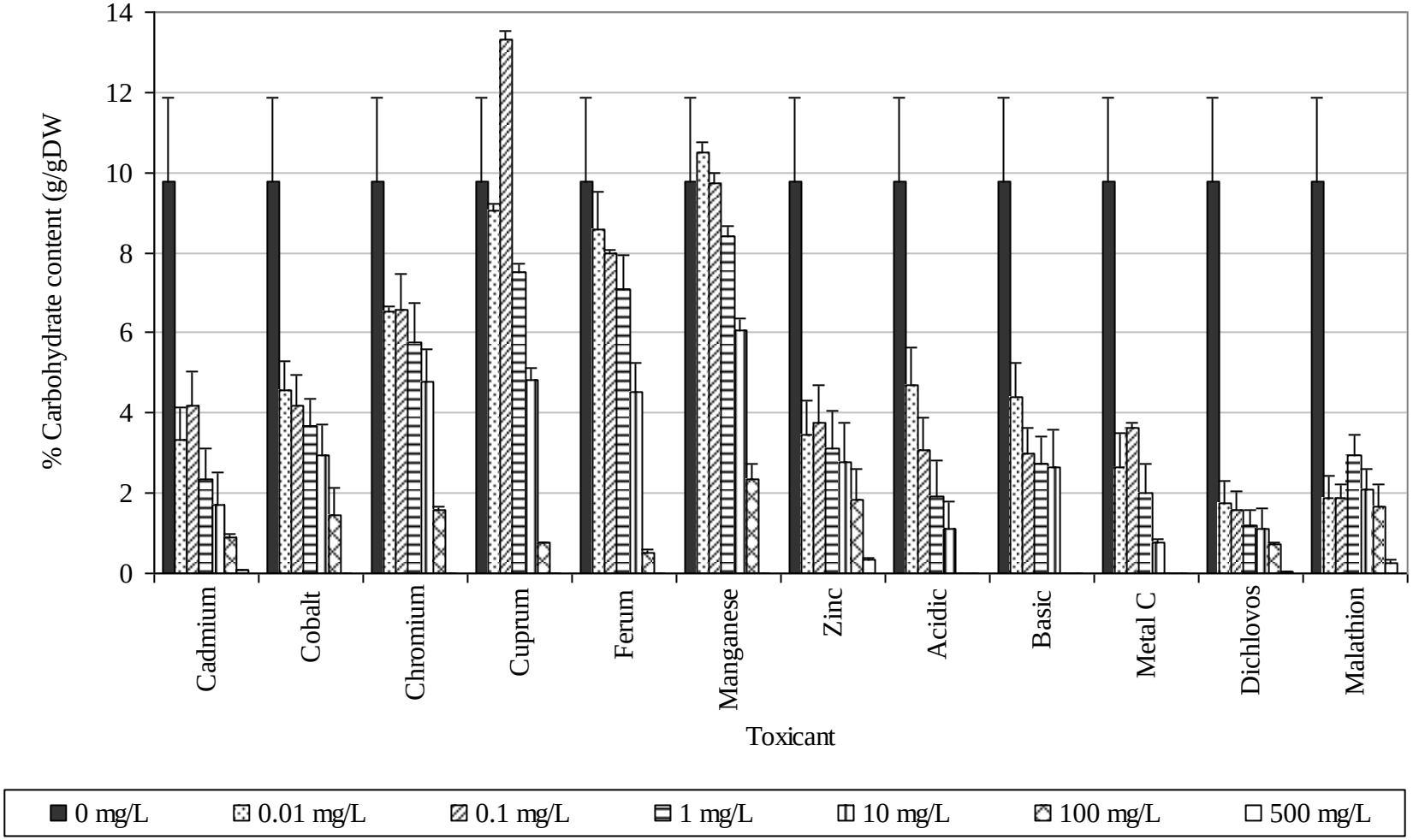


Figure 4.27: Carbohydrate content in *Tetraselmis tetrahele* UMACC 144 after exposure to selected toxicant for four days

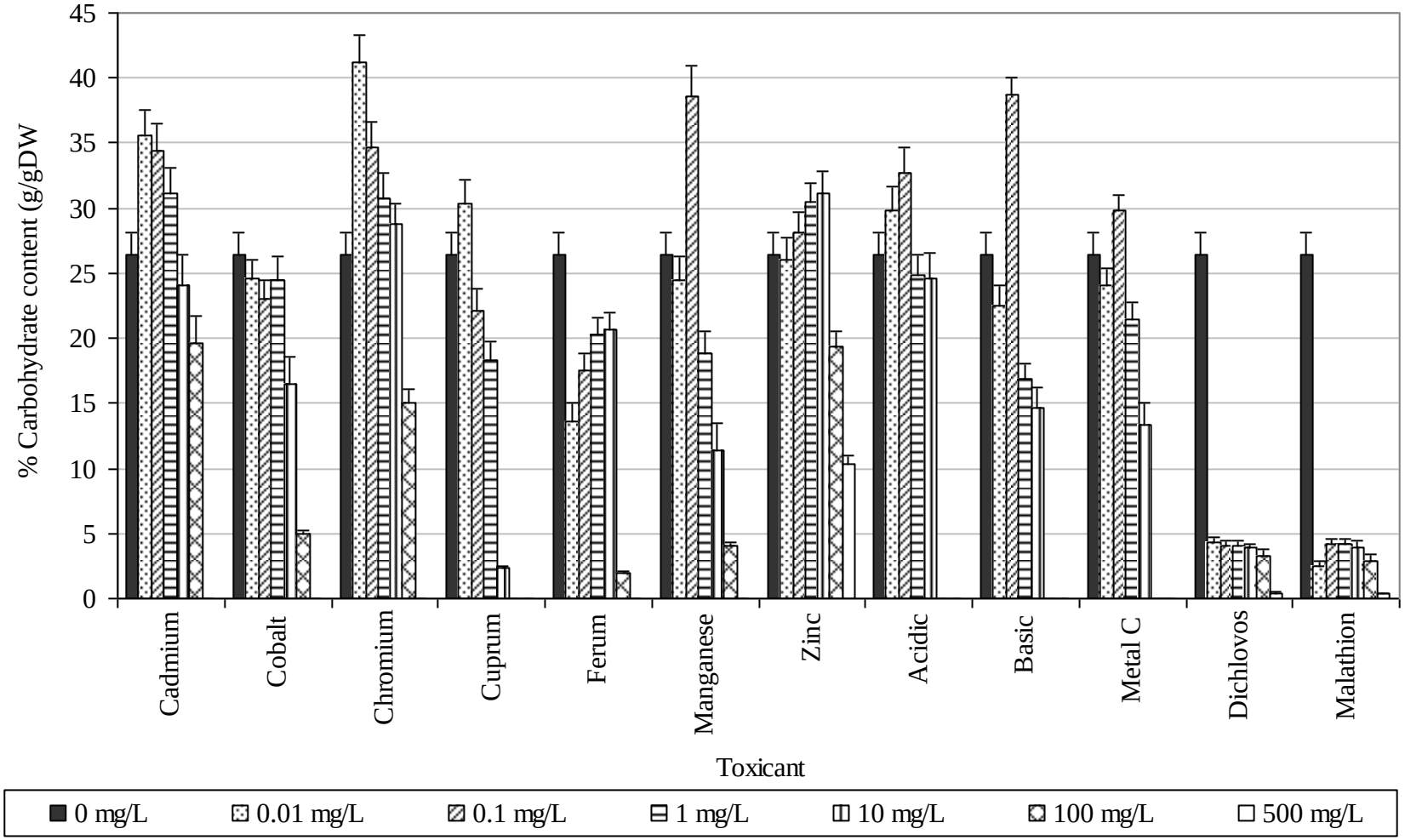


Figure 4.28: Carbohydrate content in *Tetraselmis tetrahele* UMACC 144 after exposure to selected toxicant for ten days

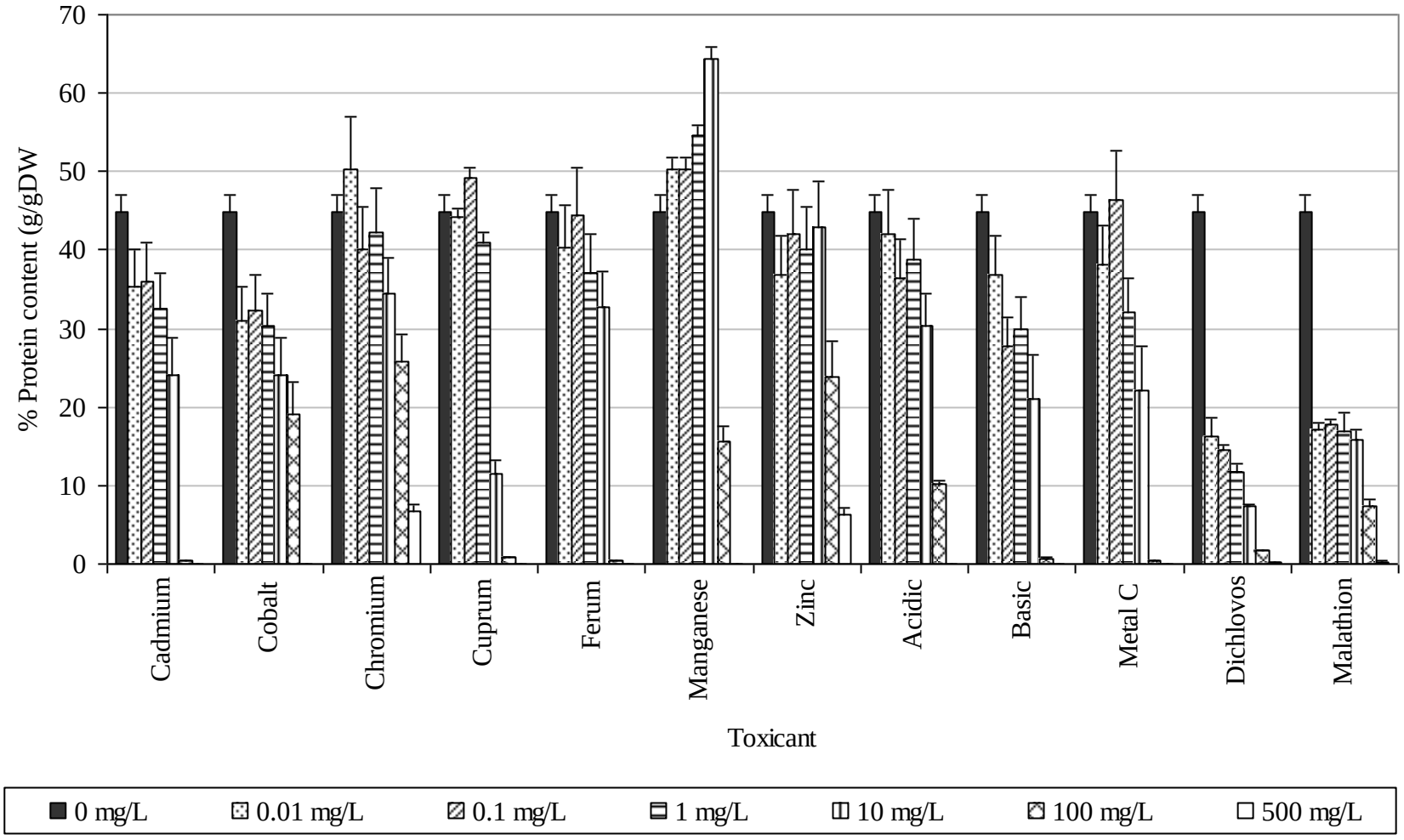


Figure 4.29: Protein content in *Tetraselmis tetrahele* UMACC 144 after exposure to selected toxicant for four days

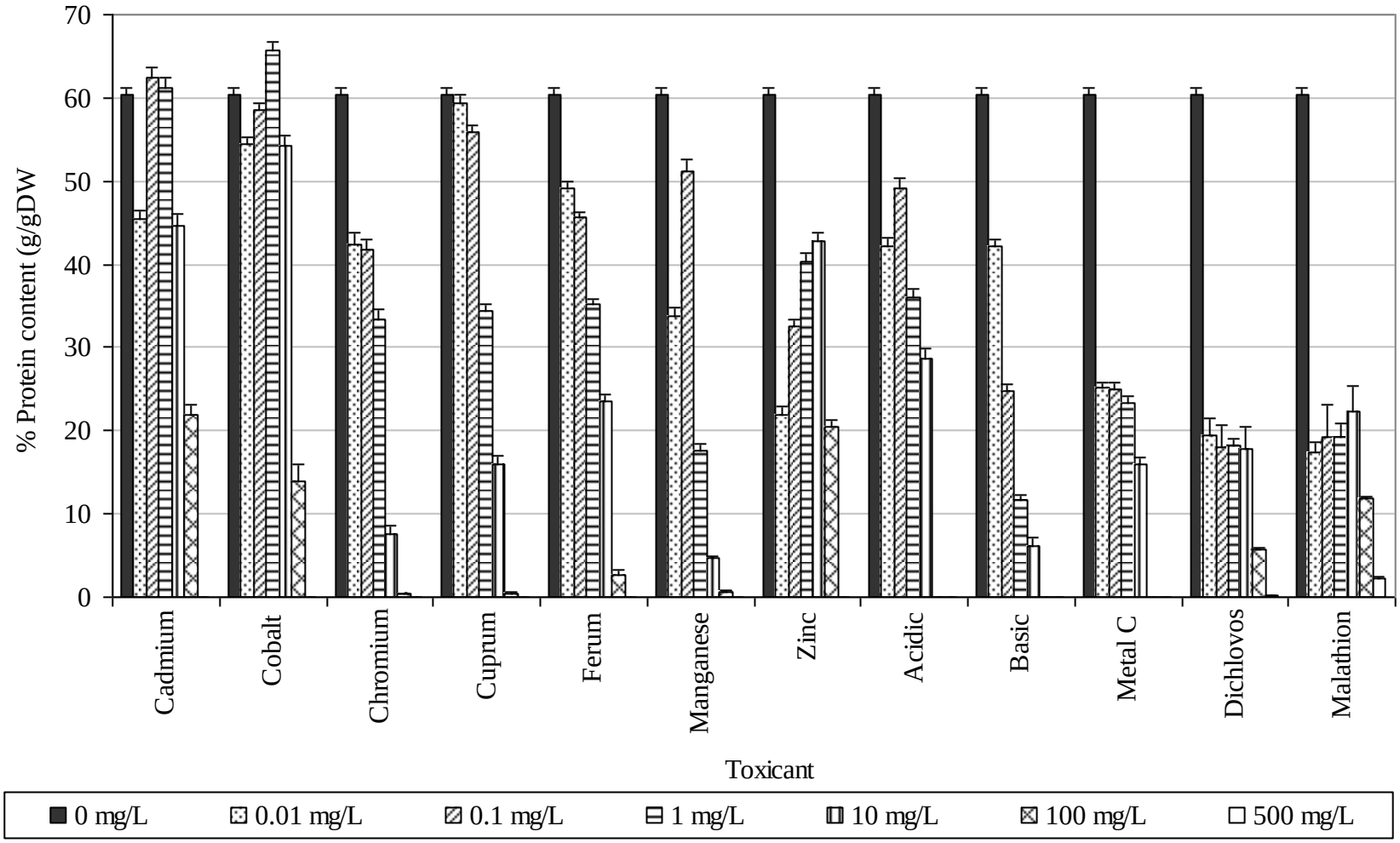


Figure 4.30: Protein content in *Tetraselmis tetrahele* UMACC 144 after exposure to selected toxicant for ten days

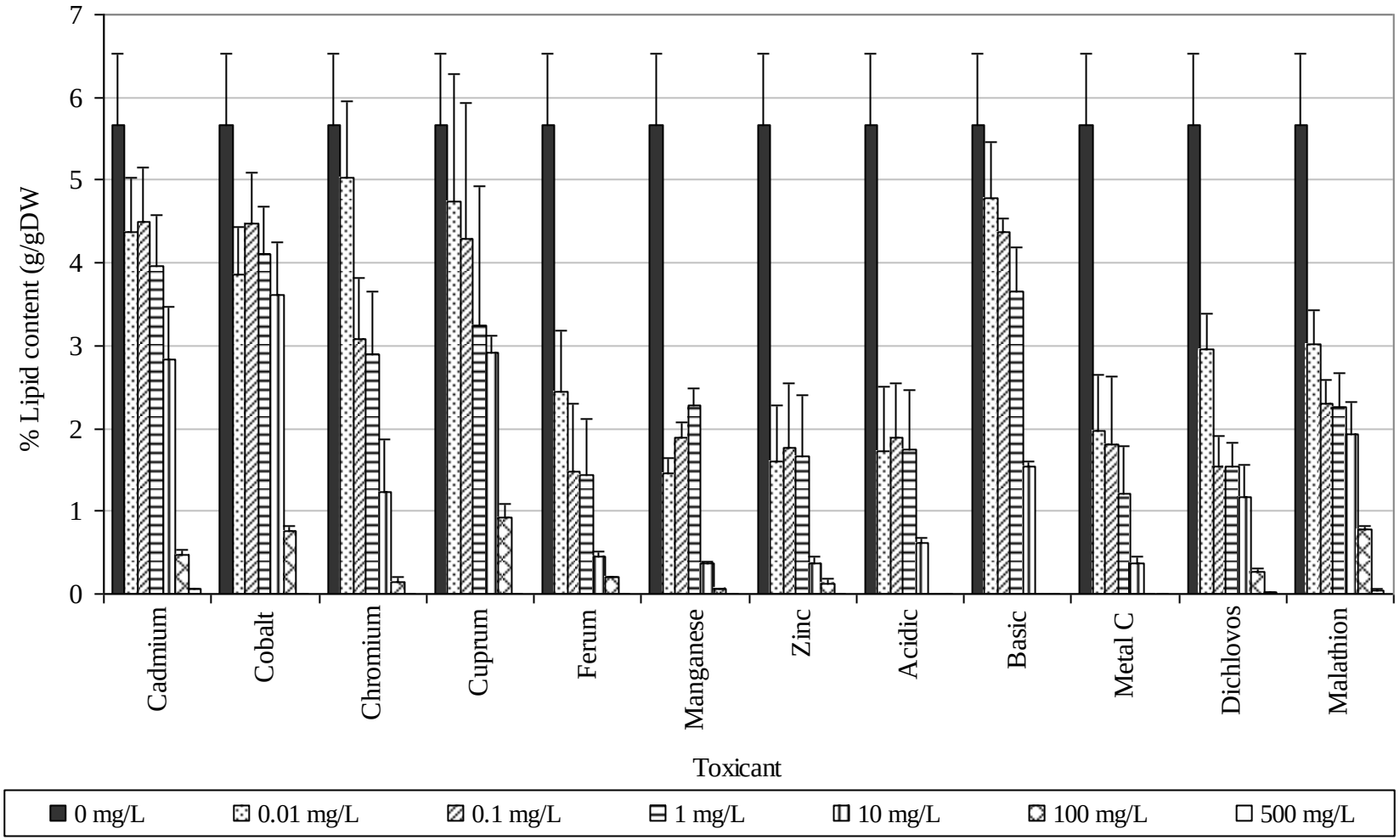


Figure 4.31: Lipid content in *Tetraselmis tetrahele* UMACC 144 after exposure to selected toxicant for four days

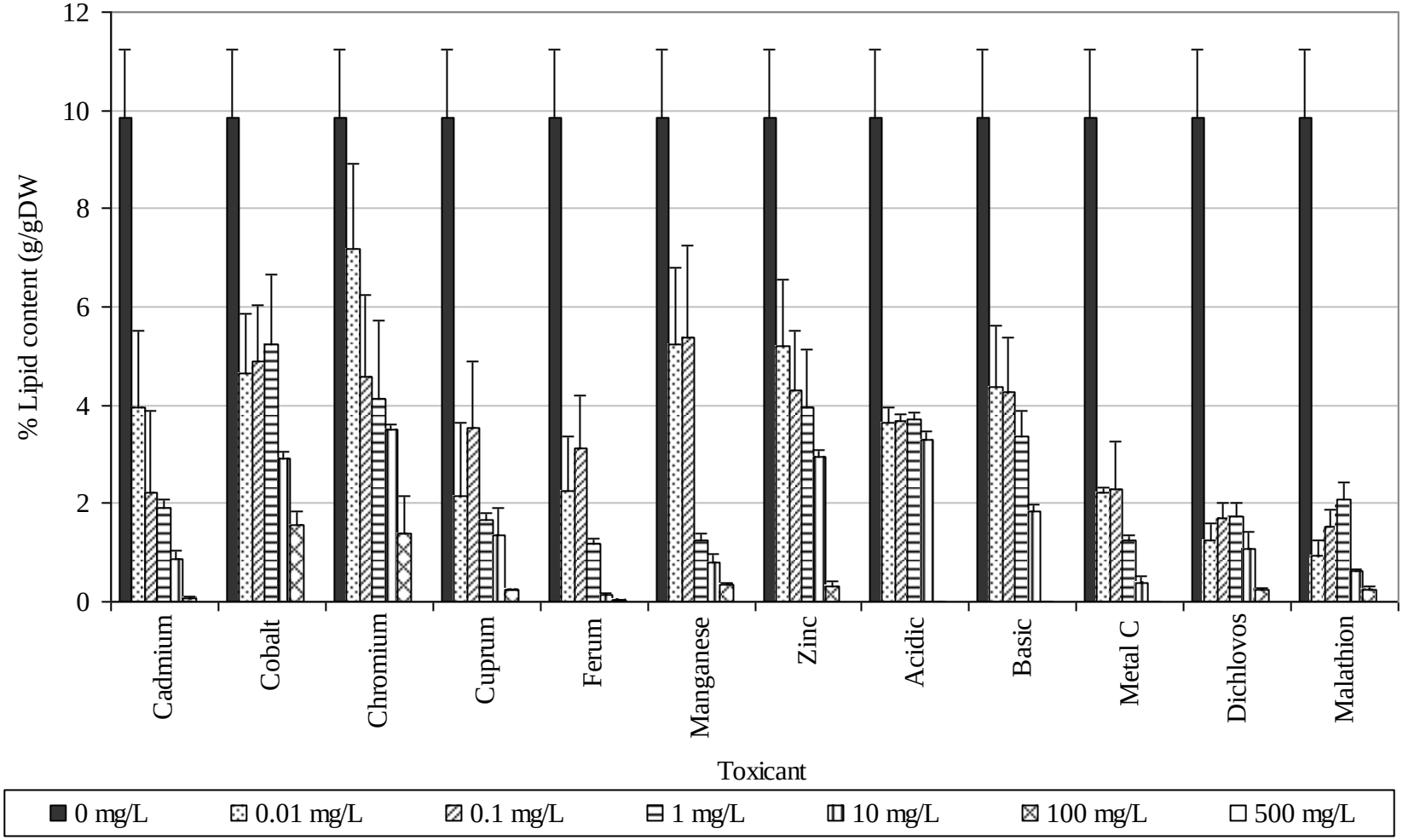


Figure 4.32: Lipid content in *Tetraselmis tetrahele* UMACC 144 after exposure to selected toxicant for ten days

The carbohydrate, protein and lipid content of the algae grown in Prov50 medium without toxicant (control cultures) for four day were 9.793±2.089% carbohydrate; 44.888±2.190 % protein; 5.668±0.855% lipid respectively. In the ten days culture, the carbohydrate, protein and lipid content of the algae were 26.389±1.736 % carbohydrate; 60.453±0.804% protein and 9.843±1.389% lipid respectively.

In general, the carbohydrate content in the cultures exposed to toxicant for four days were lower than control cultures. In contrast, in the cultures exposed to toxicant for ten days, the carbohydrate content were higher than control cultures between concentrations 0.01 mg/L to 10 mg/L toxicant except for carbohydrate content in the cultures exposed to Co, Fe, Dichlovos and Malathion. The carbohydrate content in the culture exposed to toxicants for four and ten days ranged from 1.75 % to 13.32% and 4.22% to 41.16% respectively.

After being exposed for four days, the protein content of *Tetraselmis* exposed to Acidic dye, Basic dye and Dichlovos, decreased with increasing concentrations of toxicant. Similar trend also shown in the *Tetraselmis* exposed to Cr, Cu, Fe, Basic dye, Metal complex dye and Dichlovos for ten days. However for the *Tetraselmis* exposed to Cd, Co, Fe, Zn and Malathion for four days and also in the *Tetraselmis* exposed to Mn, Zn, Acidic dye and Malathion for ten days, the protein content increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. The protein content in the culture exposed to toxicants for four and ten days ranged from 16.29 % to 64.42% and 19.34% to 65.71% respectively.

The lipid content in culture exposed to toxicant for four and ten days, were significantly lower than control cultures ($p<0.05$). The results showed, in the *Tetraselmis* exposed to Cr, Cu, Fe, Basic dye, Metal complex dye, Dichlovos and Malathion for four days and also in *Tetraselmis* exposed to Cd, Cr, Zn and Basic dye, the lipid content of the algae decreased with increasing concentrations of toxicant. However, in the four days cultures of *Tetraselmis* exposed to Cd, Co, Mn, Zn and Acidic dye and also in the ten days cultures of *Tetraselmis* exposed to Co, Cu, Fe, Mn, Acidic dye, Metal complex dye, Dichlovos and Malathion, the lipid content increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. The lipid content in the culture exposed to toxicants for four and ten days ranged from 1.77 % to 5.04% and 1.72% to 7.17% respectively

Statistical analysis using ANOVA showed there were significant difference ($p<0.05$) for all biochemical compositions in each concentration of toxicant tested compared with the control culture (Appendix 31). At the concentrations of 500 mg/L of the toxicant, the carbohydrate, protein and lipid content in the cultures were significantly low ($p<0.05$).

4.2.3 *Boergesenia forbesii*: Effects of 12 Toxicants on Biochemical Composition

Figure 4.33 to Figure 4.38 summarizes the carbohydrate (Fig. 4.33 to Fig. 4.34), protein (Fig. 4.35 to Fig. 4.36) and lipid (Fig. 4.37 to Fig. 4.38) in *Boergesenia forbesii* after exposure to 12 toxicants for four and ten days. Appendix 16 shows the detailed results of the biochemical compositions in *Boergesenia forbesii*.

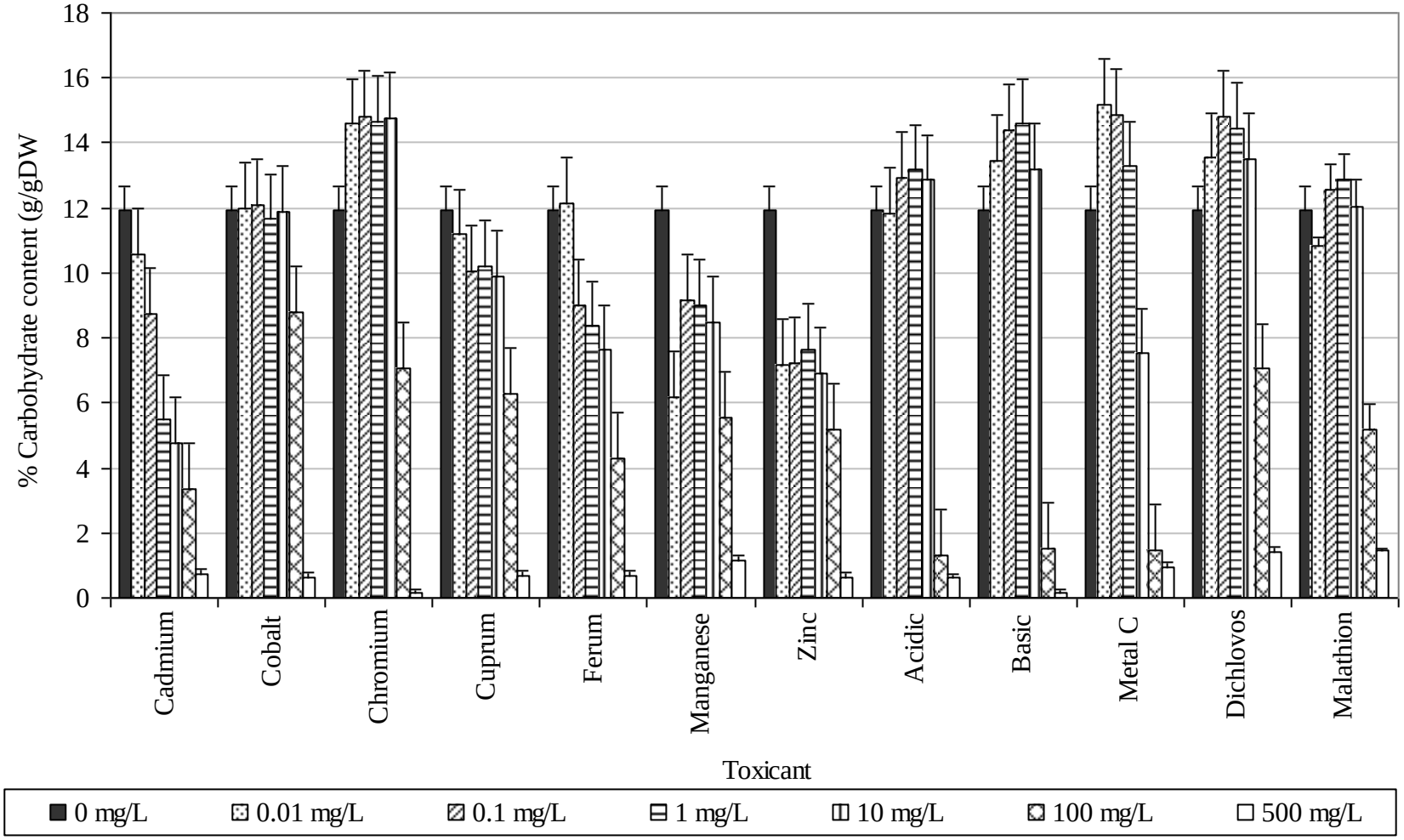


Figure 4.33: Carbohydrate content in *Boergesenia forbesii* after exposure to selected toxicant for four days

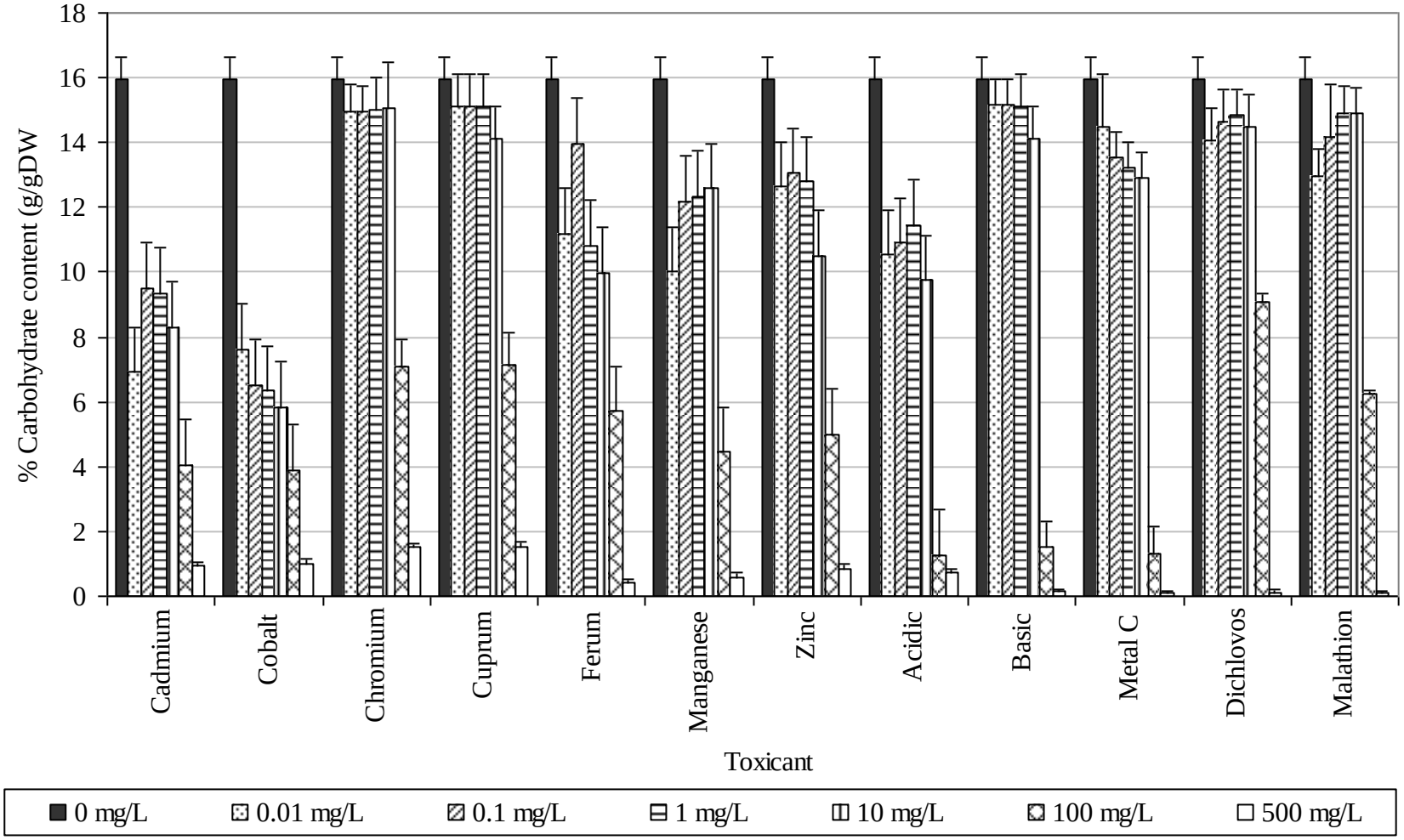


Figure 4.34: Carbohydrate content in *Boergesenia forbesii* after exposure to selected toxicant for ten days

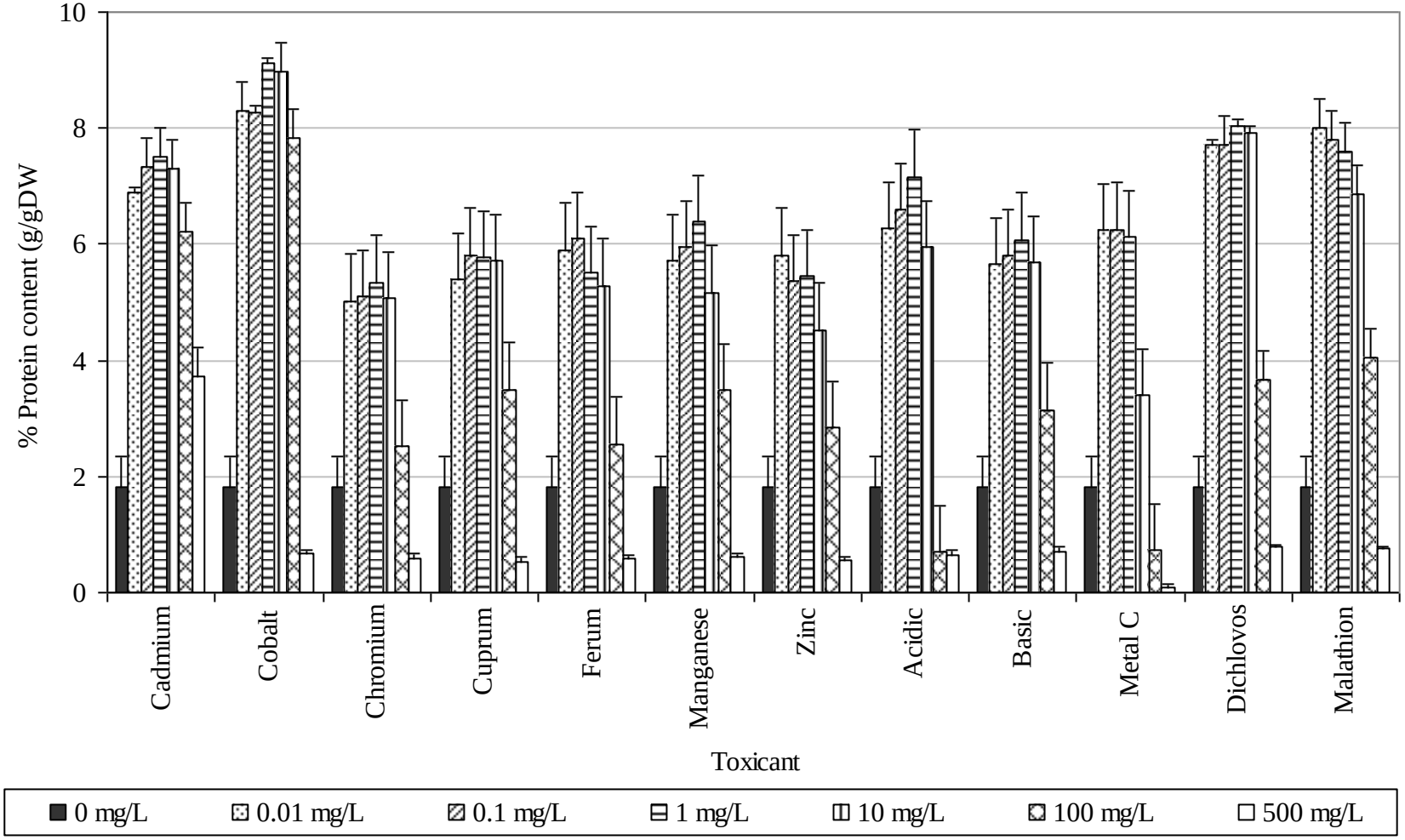


Figure 4.35: Protein content in *Boergesenia forbesii* after exposure to selected toxicant for four days

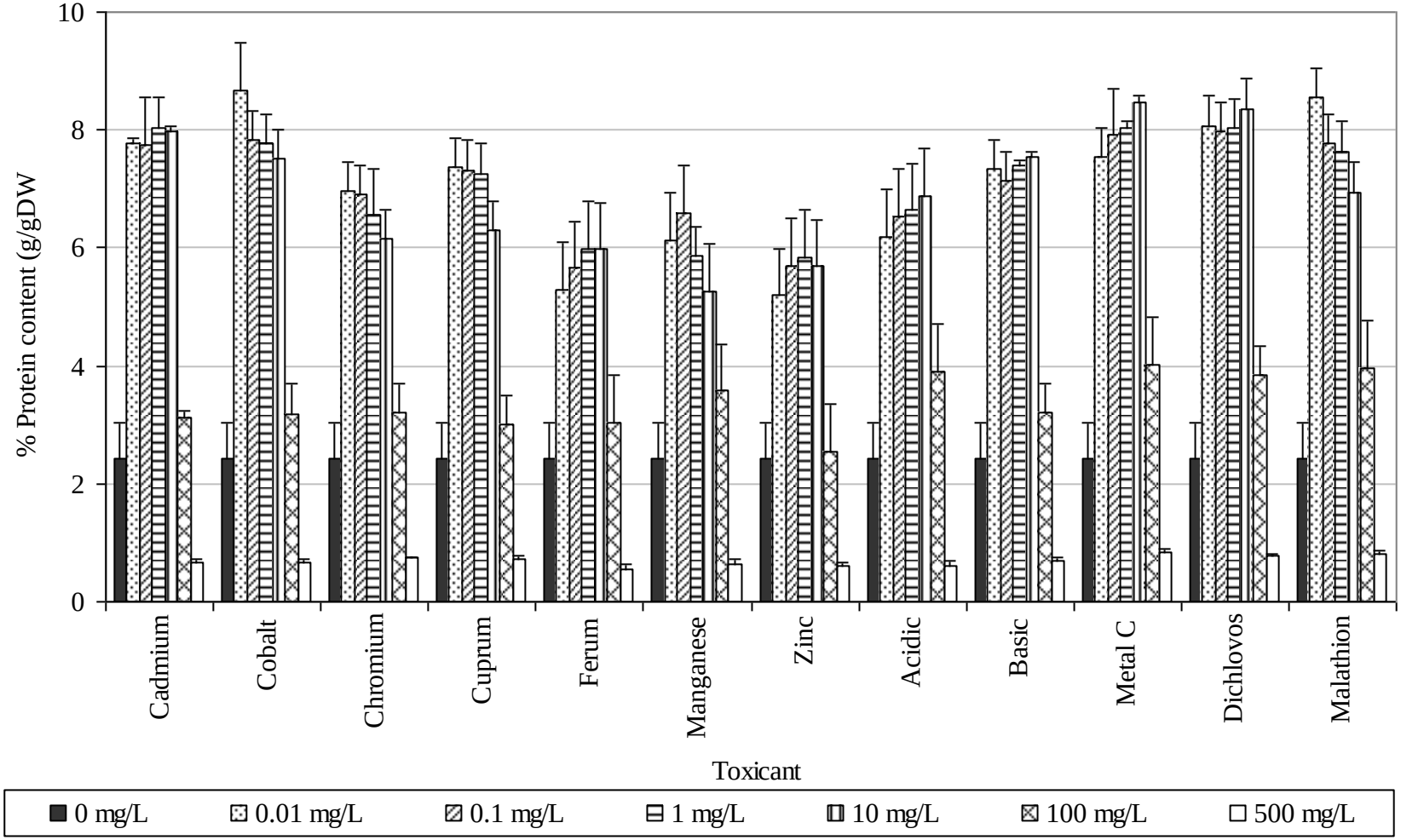


Figure 4.36: Protein content in *Boergesenia forbesii* after exposure to selected toxicant for ten days

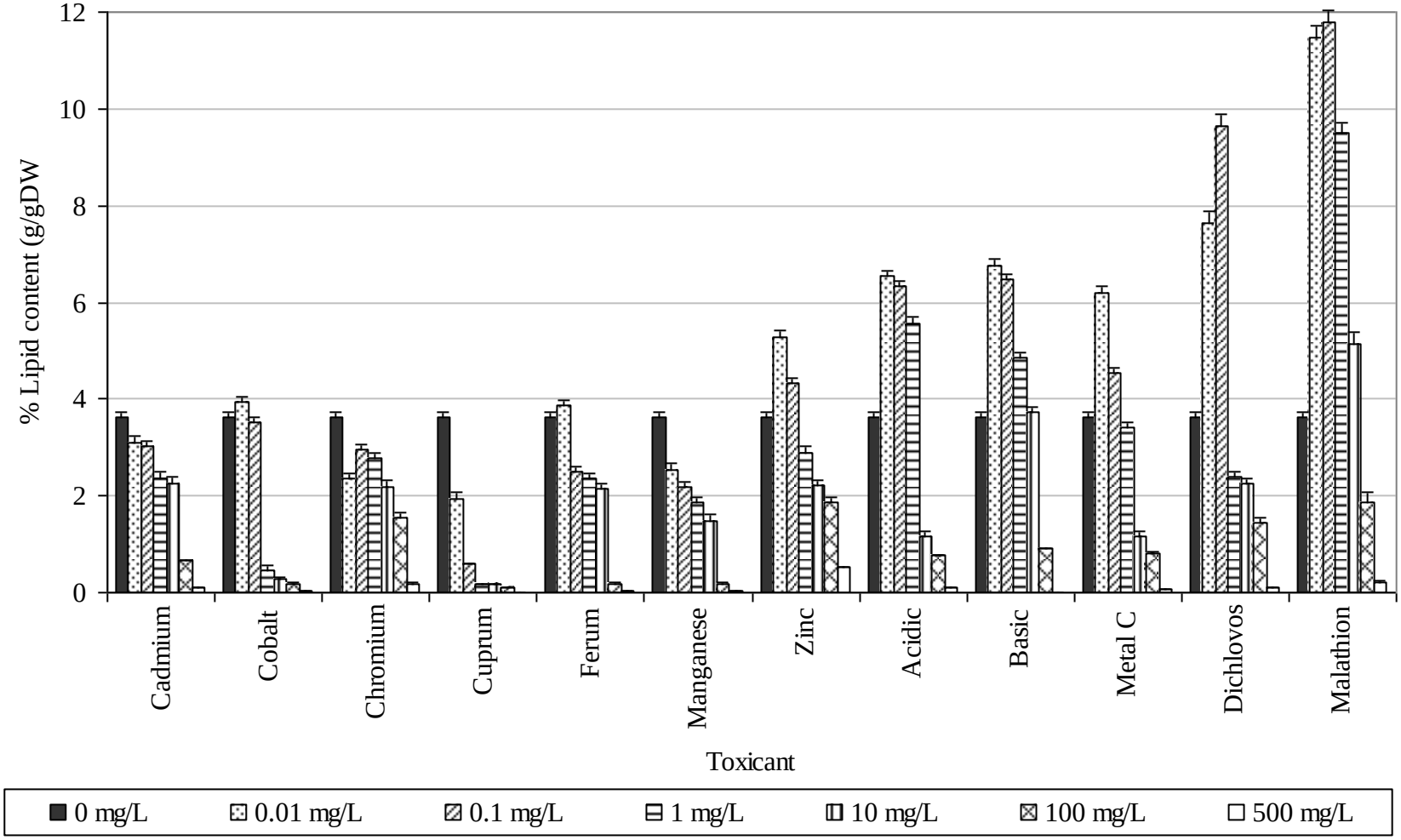


Figure 4.37: Lipid content in *Boergesenia forbesii* after exposure to selected toxicant for four days

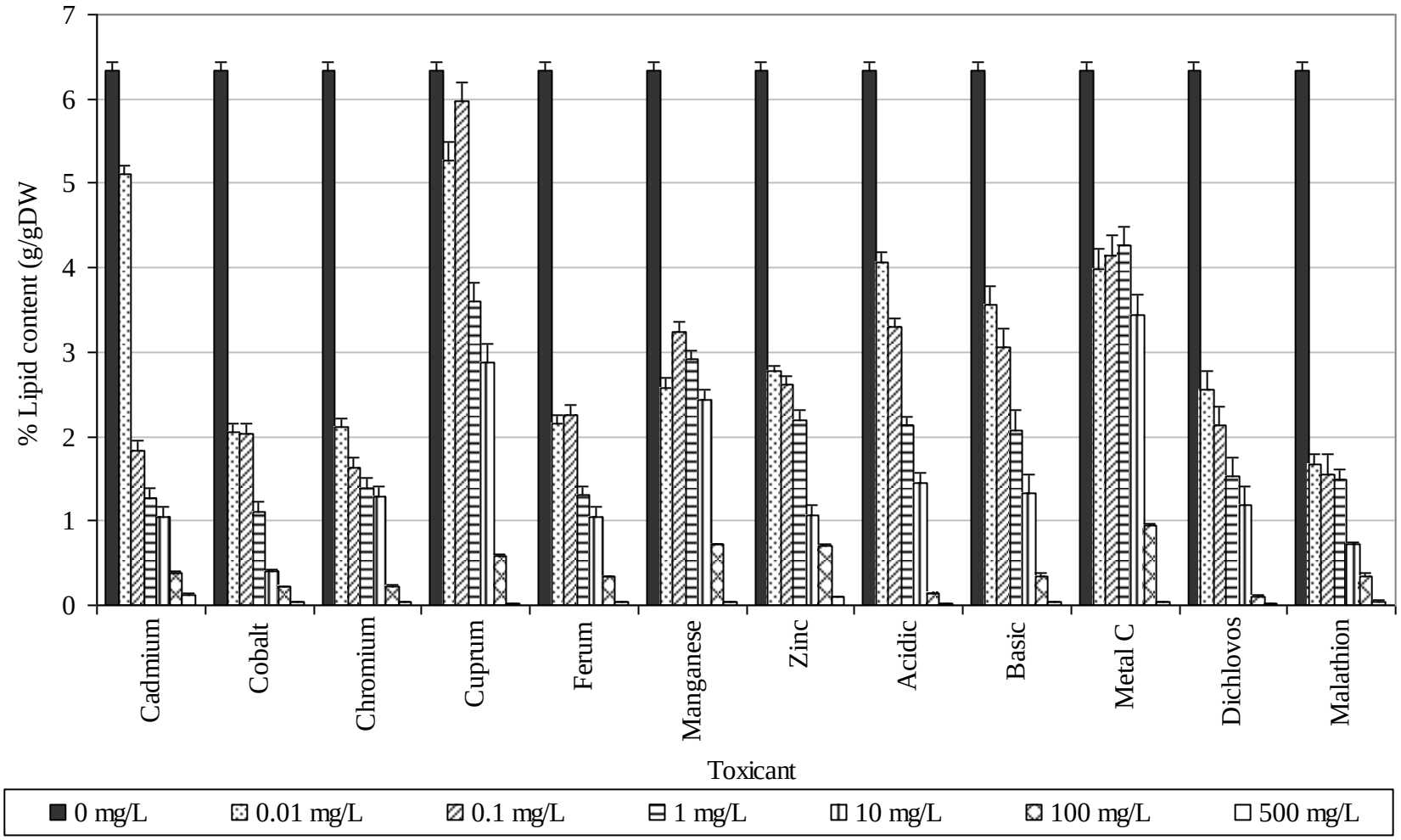


Figure 4.38: Lipid content in *Boergesenia forbesii* after exposure to selected toxicant for ten days

The carbohydrate, protein and lipid content of the algae grown in Prov50 medium without toxicant (control cultures) for four day were 11.948±0.700% carbohydrate; 1.818±0.540% protein; 3.633±0.113% lipid respectively. In the ten days culture, the carbohydrate, protein and lipid content of the algae were 15.931±0.700% carbohydrate; 2.425±0.600 % protein; 6.328±0.113 % lipid respectively.

The results showed the carbohydrate content of *Boergesenia* exposed to Cd, Cu, Fe and Metal complex dye for four days decreased with increasing concentrations of toxicant. The same trends were obtained in the *Boergesenia* exposed to Co, Basic dye and Metal complex dye. However after being exposed for four days, the carbohydrate content in the *Boergesenia* exposed to Co, Cr, Mn, Zn, Acidic dye, Basic dye, Dichlovos and Malathion, and also in the *Boergesenia* exposed to Cd, Cr, Cu, Fe, Mn, Zn, Acidic dye, Dichlovos and Malathion for ten days, the carbohydrate content in the cultures, increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. In general, the carbohydrate content in the cultures exposed to toxicant for ten days were lower than control cultures. The carbohydrate content in the culture exposed to toxicants for four and ten days ranged from 7.66% to 15.17% and 7.60% to 15.16% respectively.

For the protein, in the *Boergesenia* exposed to Co, Cr, Cu and Malathion for four days, the protein content decreased with increasing concentrations of toxicant. However for the *Boergesenia* cultures exposed to Cd, Co, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye and Dichlovos for four days, the protein content increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. Similar results also shown in the *Boergesenia* cultures

exposed to Cd, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye and Dichlovos for ten days. In general, the protein content in the cultures exposed to toxicants were higher compared with protein content in control cultures; ranged from 5.35% to 9.11% and 5.84% to 8.67% respectively.

After being exposed to toxicants for four days, results showed the lipid content of *Boergesenia* exposed to Cd, Cu and Mn; and also in the *Boergesenia* exposed to Cd, Co, Cr, Zn, Acidic dye, Basic dye, Dichlovos and Malathion for ten days, the lipid content decreased with increasing concentrations of toxicant. However, in the *Boergesenia* exposed to Cr and in the cultures exposed to Cu, Fe, Mn and Metal complex dye, the lipid content increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. The lipid content in the culture exposed to toxicants for four and ten days ranged from 1.95 % to 11.80% and 1.67% to 5.97% respectively

Statistical analysis using ANOVA showed there were significant difference ($p<0.05$) for all biochemical compositions in each concentration of toxicant tested compared with the control culture (Appendix 36).

4.2.4 *Ventricaria ventricosa* : Effects of 12 Toxicants on Biochemical Composition

Figure 4.39 to Figure 4.44 summarizes the carbohydrate (Fig. 4.39 to Fig. 4.40), protein (Fig. 4.41 to Fig. 4.42) and lipid (Fig. 4.43 to Fig. 4.44) content in *Ventricaria*

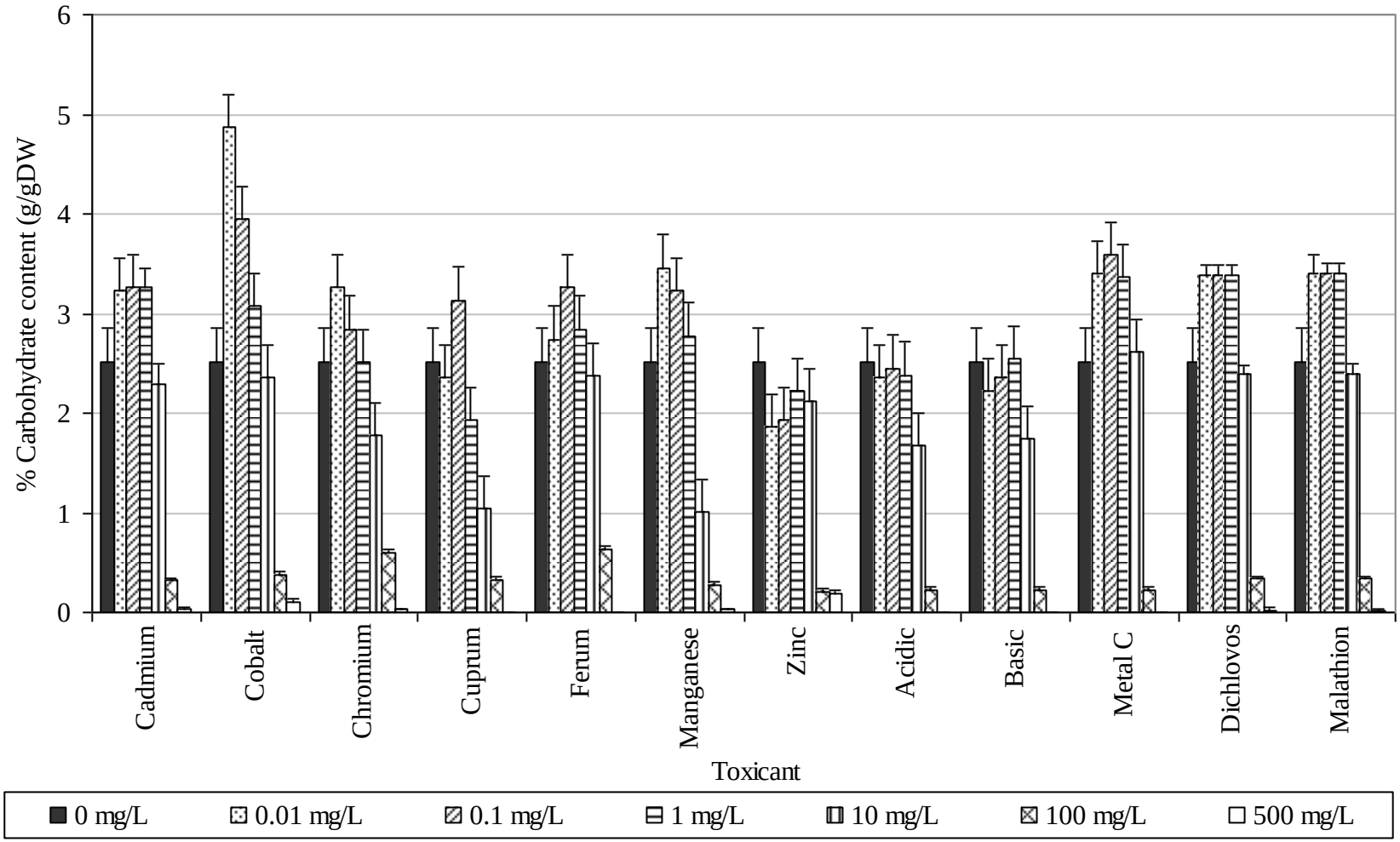


Figure 4.39: Carbohydrate content in *Ventricaria ventricosa* after exposure to selected toxicant for four days

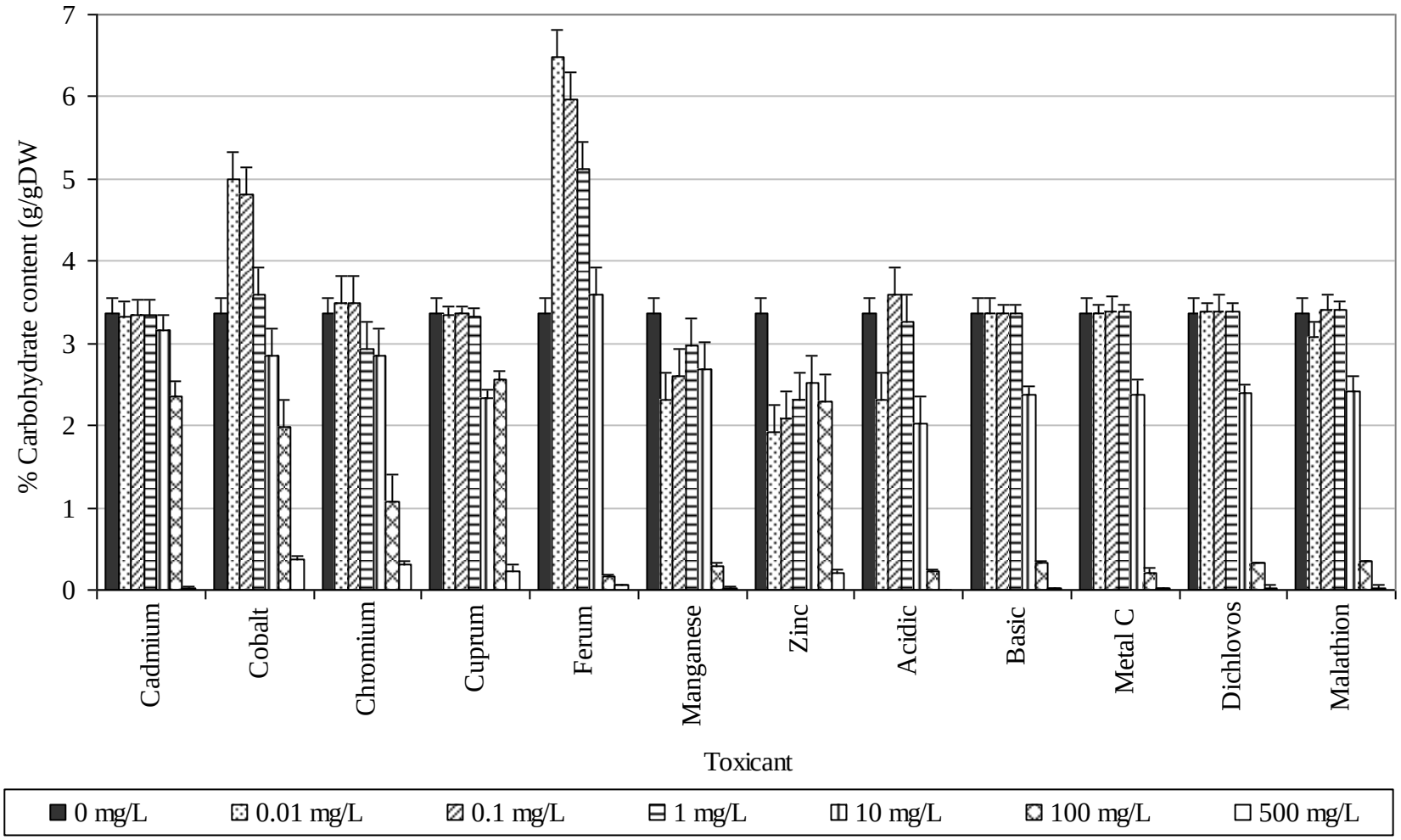


Figure 4.40: Carbohydrate content in *Ventricaria ventricosa* after exposure to selected toxicant for ten days

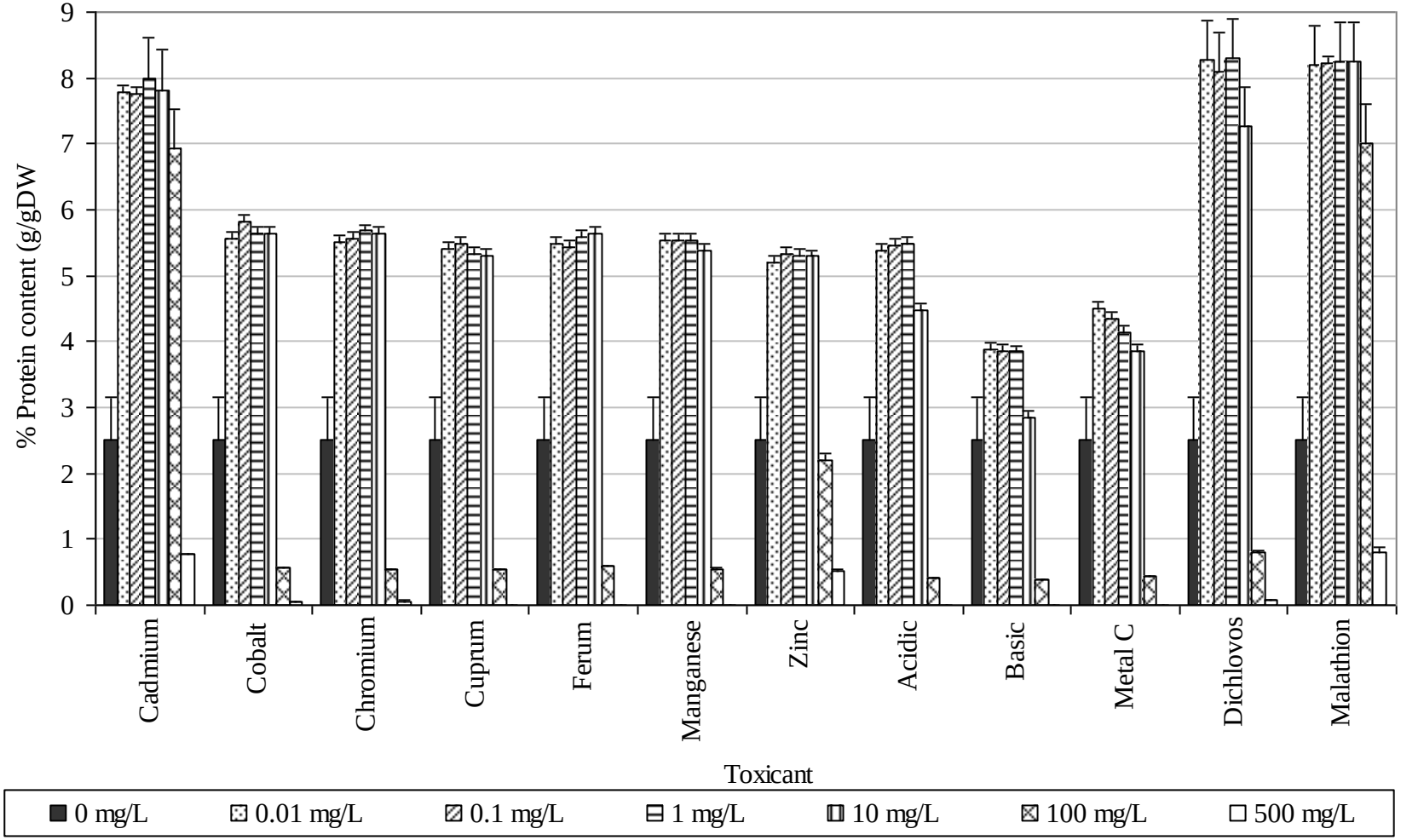


Figure 4.41: Protein content in *Ventricaria ventricosa* after exposure to selected toxicant for four days

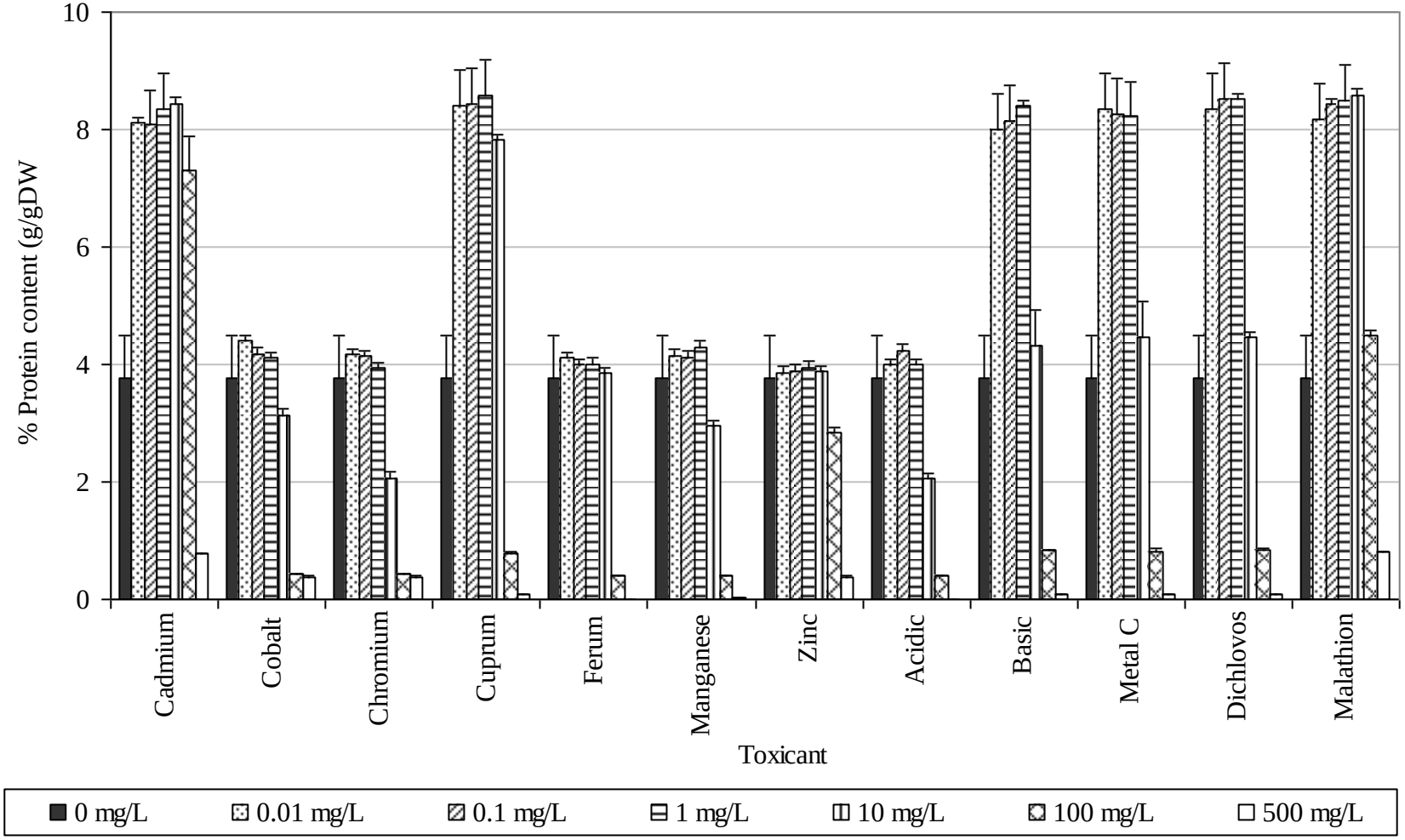


Figure 4.42: Protein content in *Ventricaria ventricosa* after exposure to selected toxicant for ten days

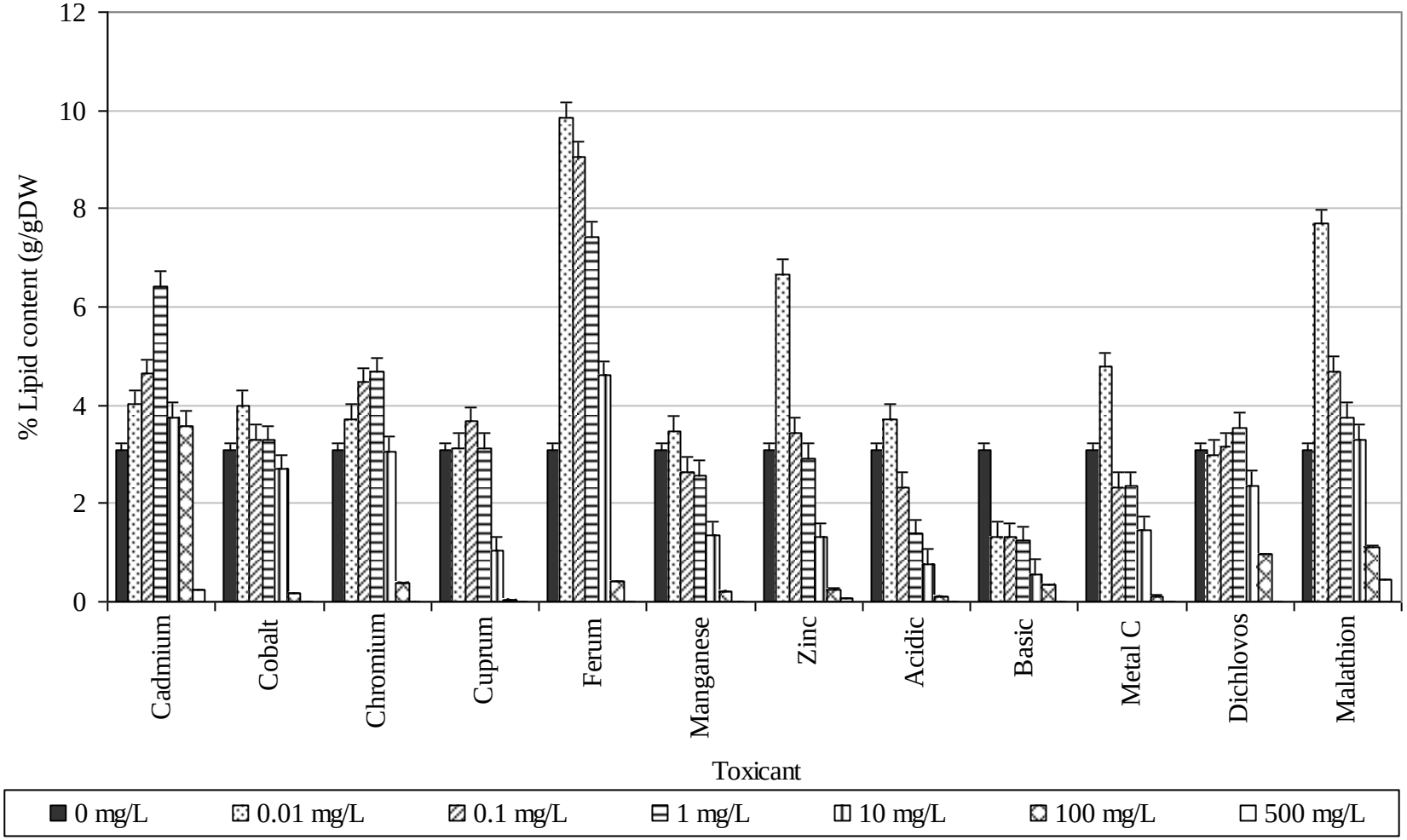


Figure 4.43: Lipid content in *Ventricaria ventricosa* after exposure to selected toxicant for four days

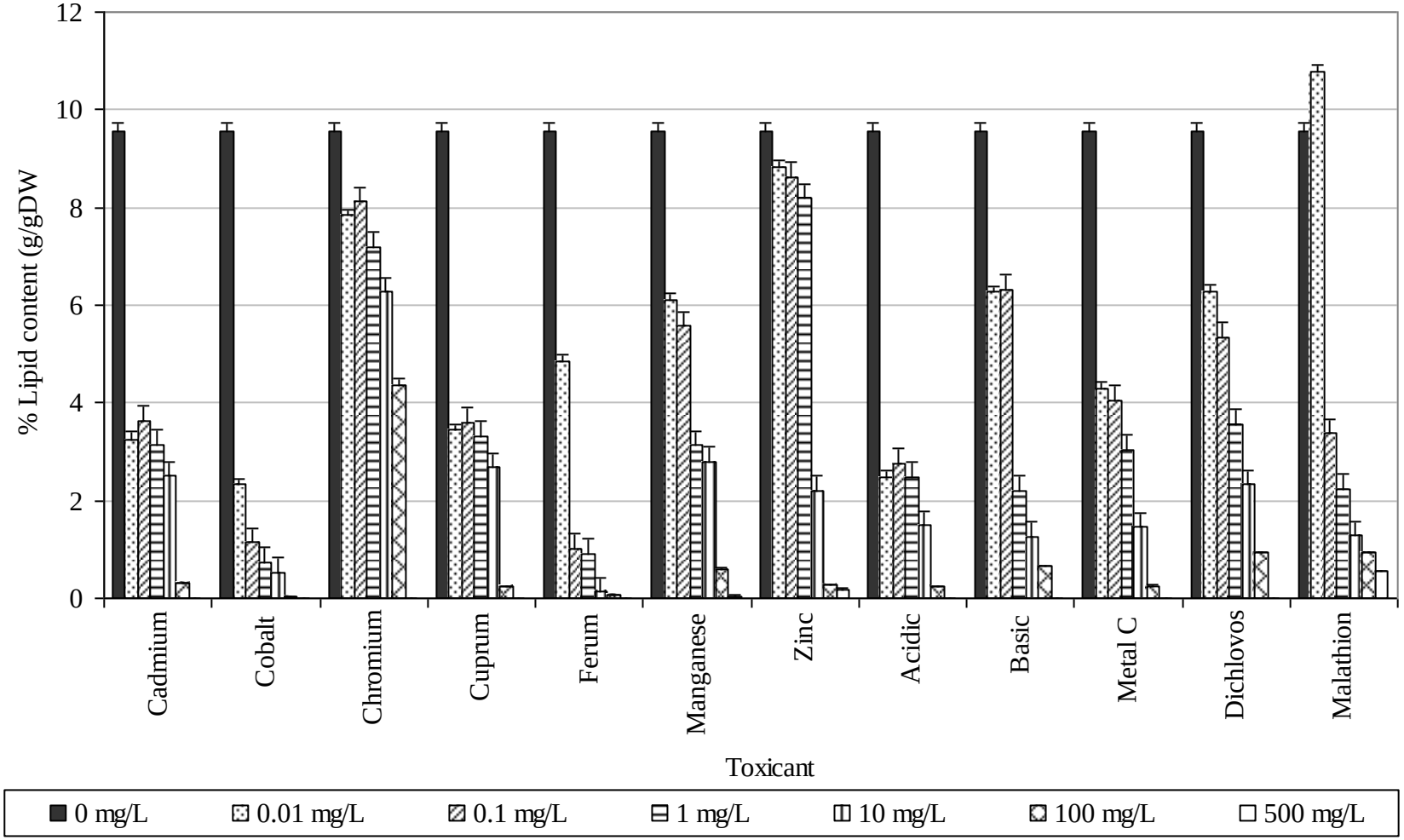


Figure 4.44: Lipid content in *Ventricaria ventricosa* after exposure to selected toxicant for ten days

ventricosa after exposure to 12 toxicants for four and ten days. Appendix 21 shows the detailed results of the biochemical compositions in *Ventricaria ventricosa*.

The carbohydrate, protein and lipid content of the algae grown in Prov50 medium without toxicant (control cultures) for four day were 2.521±0.330% carbohydrate; 2.519±0.640% protein; 3.095±0.130% lipid respectively. In the ten days culture, the carbohydrate, protein and lipid content of the algae were 3.362±0.190% carbohydrate; 3.779±0.700% protein; 9.545±0.180% lipid respectively.

In general, the carbohydrate content in the cultures exposed to all toxicants for four days were higher than in control cultures between concentrations 0.01 mg/L to 1 mg/L toxicant except for carbohydrate content in the cultures exposed to Zn, Acidic dye and Basic dye. However, in the cultures exposed to toxicant for ten days, the carbohydrate content were lower than in control cultures except for carbohydrate content in the cultures exposed to Co. The results shows, in *Ventricaria* cultures exposed to Zn and Acidic dye for four days and also in *Ventricaria* exposed to Cd, Cu, Mn and Zn for ten days, the carbohydrate content increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. The carbohydrate content in the culture exposed to toxicants for four and ten days ranged from 2.22 % to 4.86% carbohydrate and 2.51% to 6.49% carbohydrate respectively

The results also shows that, protein content in the cultures exposed to toxicants for four and ten day were higher than in control cultures and ranged from 3.87 % to 8.30% protein and 3.95% to 8.59% protein respectively. For the *Ventricaria* exposed to Mn, Basic dye and Metal complex dye for four days, and also for *Ventricaria* exposed to

Co, Cr, Fe and Metal complex dye for ten days, the protein content decreased with increasing concentrations of toxicant. However in the *Ventricaria* cultures exposed to Cd, Co, Cr, Cu, Fe, Zn, Acidic dye, Dichlovos and Malathion for four days and also in the ten days culture of *Ventricaria* cultures exposed to Cd, Cu, Mn, Zn, Acidic dye, Basic dye, Dichlovos and Malathion, the protein content increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant.

In this study, the results shows, the lipid content in the *Ventricaria* exposed to Basic dye for four days and also in the *Ventricaria* exposed to Co, Fe, Mn, Zn, Metal complex dye and Dichlovos for ten days, decreased with increasing concentrations of toxicant. However in the *Ventricaria* exposed to Cd, Cr, Cu, Acidic dye and Basic dye for ten days, the lipid content increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. In general, the lipid content in the *Ventricaria* culture exposed to toxicants for four and ten days ranged from 1.33 % to 9.06% lipid and 2.33% to 8.84% lipid respectively

Statistical analysis using ANOVA showed there were significant difference ($p<0.05$) for all biochemical compositions in each concentration of toxicant tested compared with the control culture (Appendix 41).

4.3 EFFECT OF SELECTED TOXICANTS ON DNA DAMAGE AND TOXICITY STUDIES: RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

Random Amplified Polymorphic DNA (RAPD) analysis was used to assess the DNA damage in *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa* after exposure to selected toxicants for four and ten days. Three metals including Cadmium (as reference metal) and two others that represent the most and the least toxic metal to the algae, three textile dyes [Supranol Br. Red 3Bur (Acidic dye); Astrazon Red FBL (Basic dye); Lanaset Red 2GA (Metal complex dye)] and two organophosphate pesticides [Malathion; Dichlovos] in different range of concentrations were exposed individually to each algal species.

Appendix 45 summarizes the detailed results of the study which include:

(i) Genomic DNA extraction: Quantification of genomic DNA concentrations in the algae

(ii) Selecting suitable primers for the RAPD analysis

(iii) Optimization of primer annealing temperature for RAPD

Appendix 7, 12, 17 and 22 shows the RAPD DNA profiles of the algae, for each type of primer used, where the base-pair size of each band were measured based on the DNA molecular marker [1kb and 100bp] and the intensity of the band in comparison with the control DNA band (set as 100%) were analysed using Alpha ImagerTM 2200 Image Analysis Software (Alpha Innotech, USA). Due to limitation of space, only few example of RAPD DNA profile obtained for each algal species used in this study is

shown in the Appendix 7a, 12a, 17a and 22a. The results shows, RAPD patterns generated by the algae exposed to selected toxicant were different from those obtained using control DNA. DNA pattern generated by each concentrations group were reproducible, although each RAPD profile was obtained from individual alga. The principal events observed following the toxicant exposure were the appearance of new bands, disappearance of band, similiarity of band and intensity of band in comparison to control.

Statistical analysis using ANOVA showed there were significant difference ($p<0.05$) for all the RAPD pattern in each concentration of toxicant tested compared with the control cultures (Appendix 27, 32, 37, 42).

4.3.1 *Chlorella vulgaris* UMACC 245: Effects of Selected Toxicants on DNA Damage (RAPD profiles)

In total eight 10-mer priming oligonucleotides (OPA13, OPN13, S17, S67,S112, S118, S124, S125) were used to analyses the result for each types of chemical contaminant tested. In this sudy, the RAPD profiles in the *Chlorella vulgaris* UMACC 245 grown in Prov50 medium without toxicant (control cultures) were set as 100%.

Figure 4.45 to Figure 4.46 shows the variation of appearance of new bands, disappearance of band (Fig. 4.47 to Fig. 4.48), similiarity of band (Fig. 4.49 to Fig. 4.50), intensity of band (Fig. 4.51 to Fig. 4.52) and genomic template stability (Fig. 4.53 to Fig. 4.54) in comparison to control (set as 100%), obtained in the RAPD profiles of *Chlorella vulgaris* UMACC 245 exposed to Cadmium (Cd), Iron (Fe), Manganese (Mn),

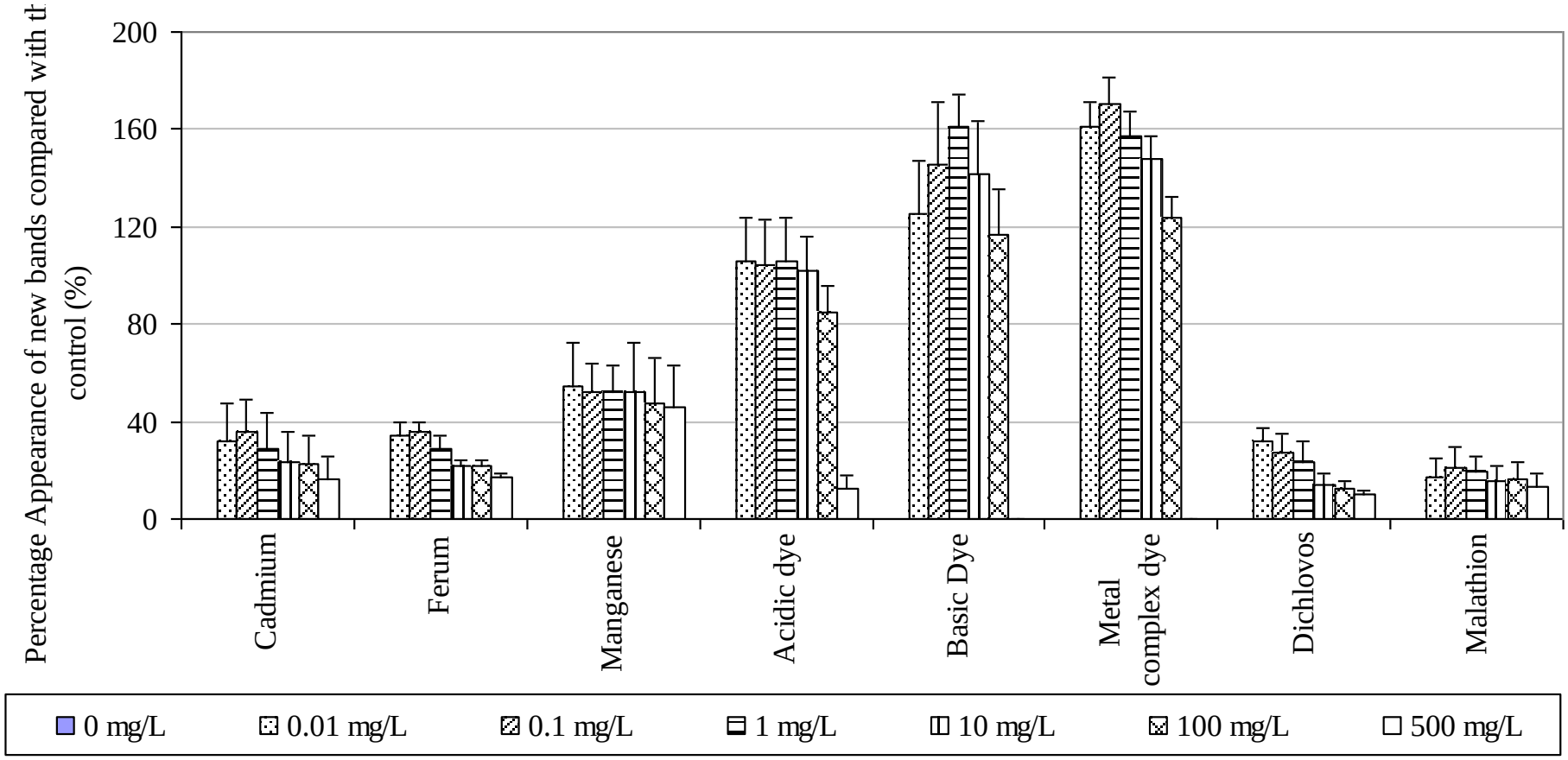


Figure 4.45: Variation of Appearance of new bands in comparison to the control (set as 100%) in RAPD profiles of *Chlorella vulgaris* UMACC 245 exposed to different concentrations of toxicant for four days

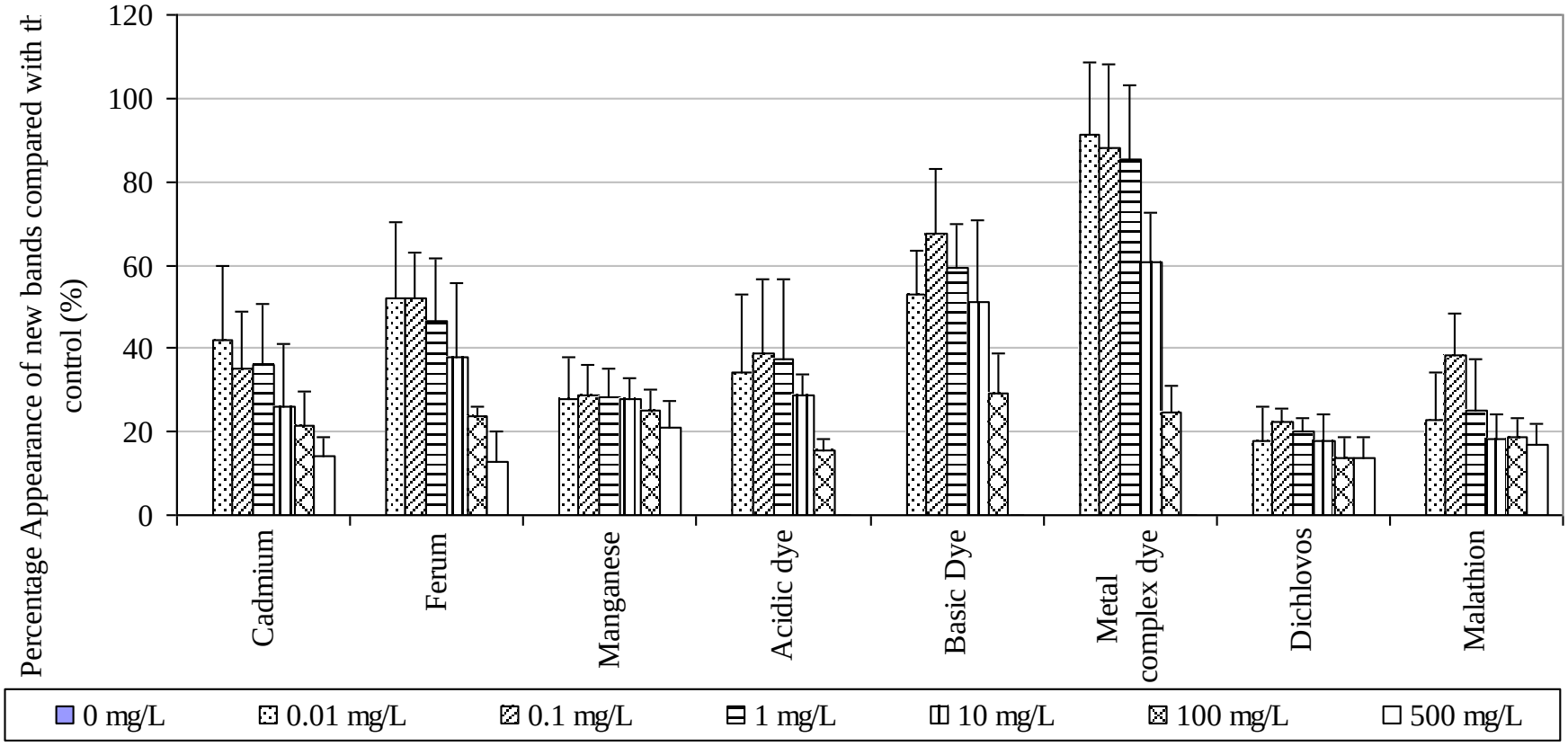


Figure 4.46: Variation of Appearance of new bands in comparison to the control (set as 100%) in RAPD profiles of *Chlorella vulgaris* UMACC 245 exposed to different concentrations of toxicant for for ten days

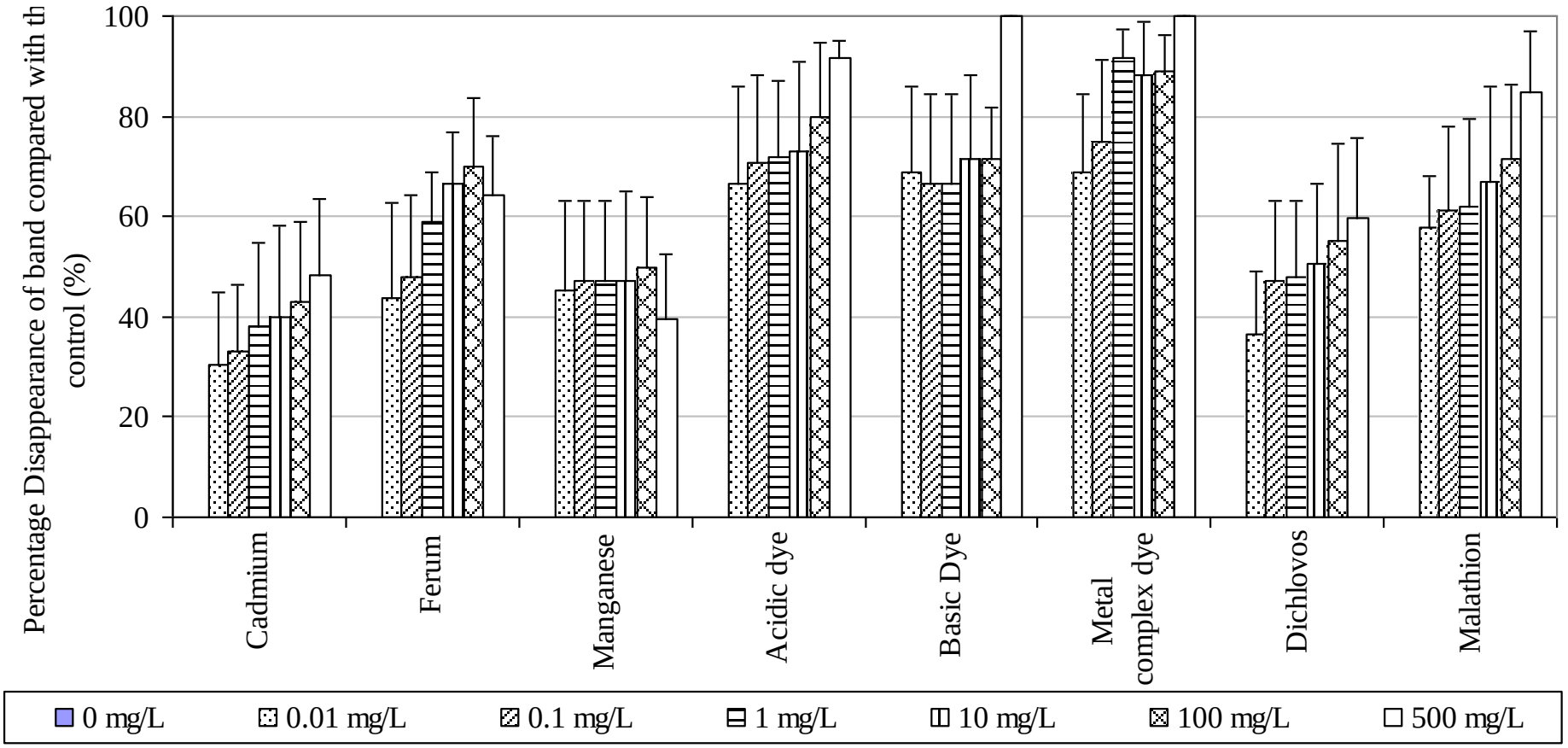


Figure 4.47: Variation of Disappearance of bands in comparison to the control (set as 100%) in RAPD profiles of *Chlorella vulgaris* UMACC 245 exposed to different concentrations of toxicant for four days

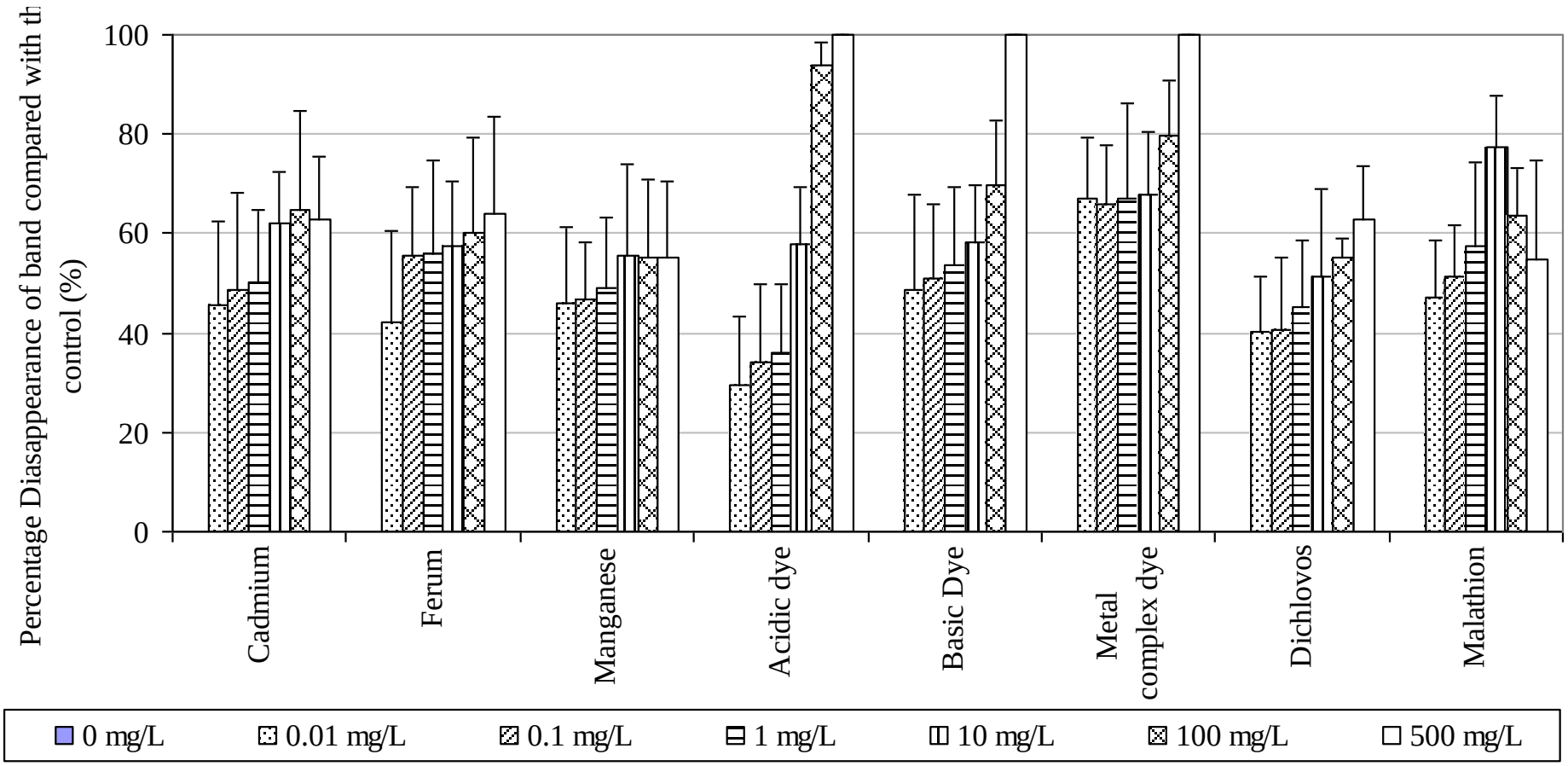


Figure 4.48: Variation of Disappearance of bands in comparison to the control (set as 100%) in RAPD profiles of *Chlorella vulgaris* UMACC 245 exposed to different concentrations of toxicant for ten days

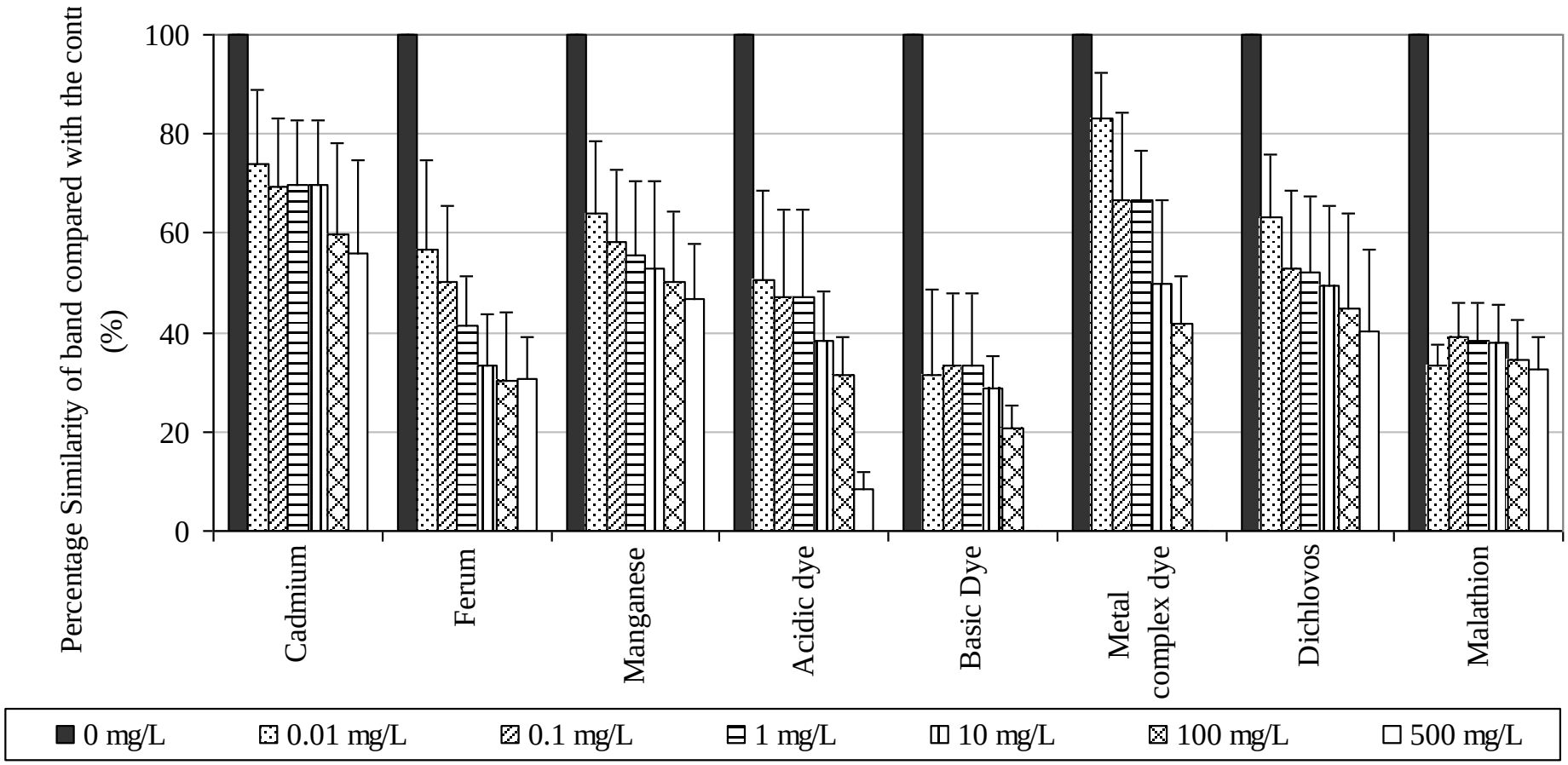


Figure 4.49: Variation of similiarity of bands in comparison to the control (set as 100%) in RAPD profiles of *Chlorella vulgaris* UMACC 245 exposed to different concentrations of toxicant for four days

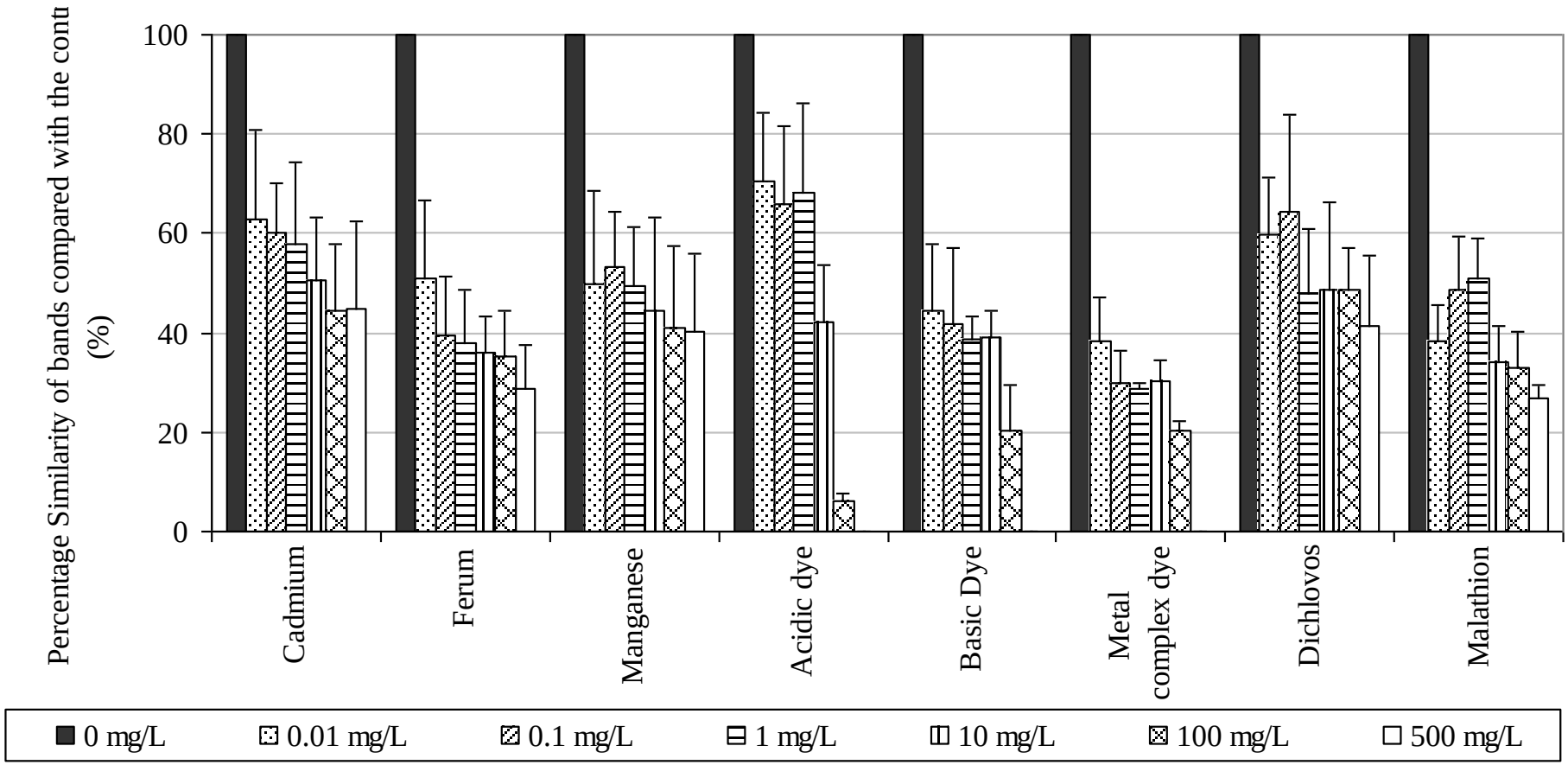


Figure 4.50: Variation of similiarity of in comparison to the control (set as 100%) in RAPD profiles of *Chlorella vulgaris* UMACC 245 exposed to different concentrations of toxicant for ten days

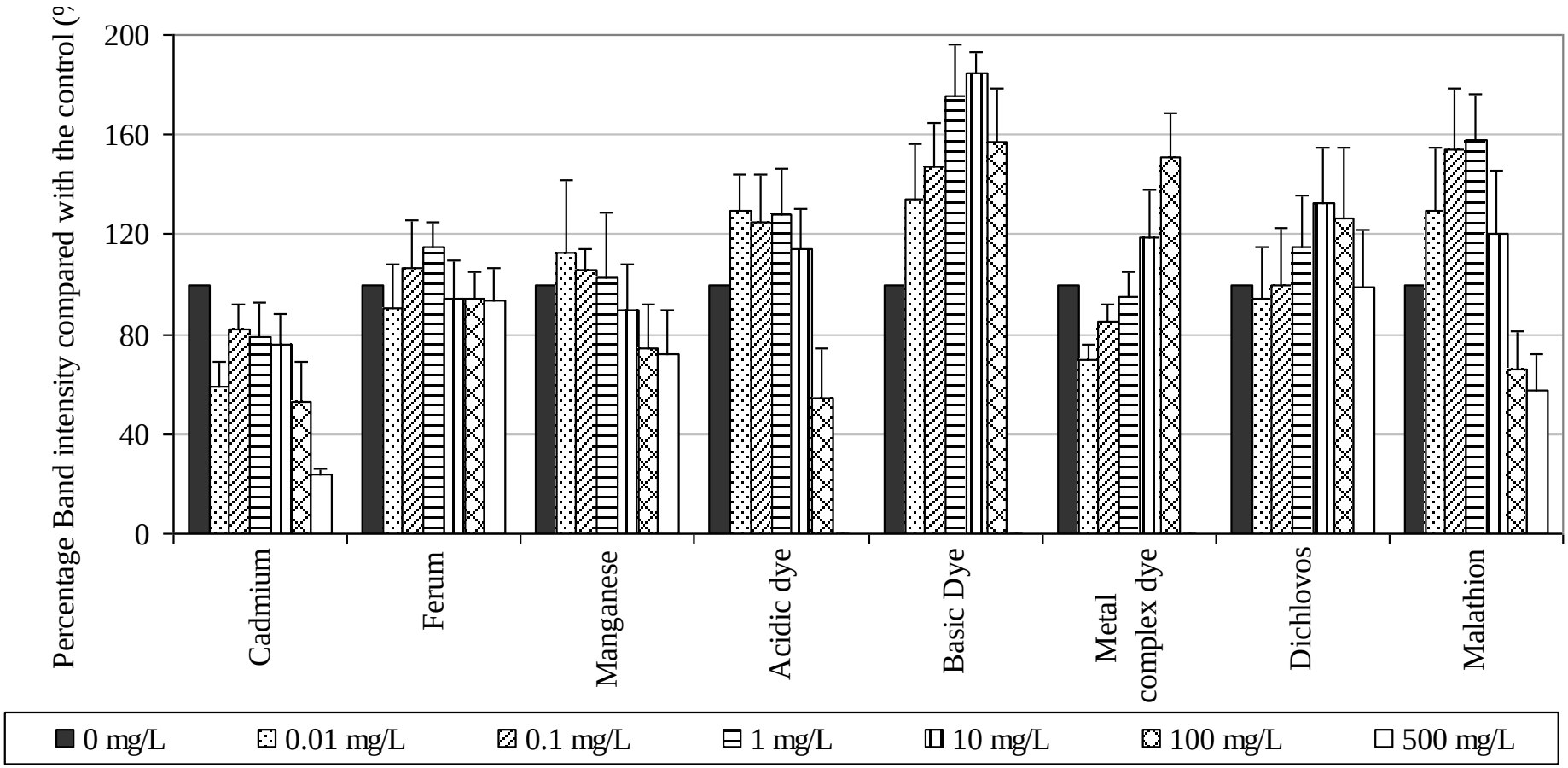


Figure 4.51: Variation of band intensity in comparison to the control (set as 100%) in RAPD profiles of *Chlorella vulgaris* UMACC 245 exposed to different concentrations of toxicant four days

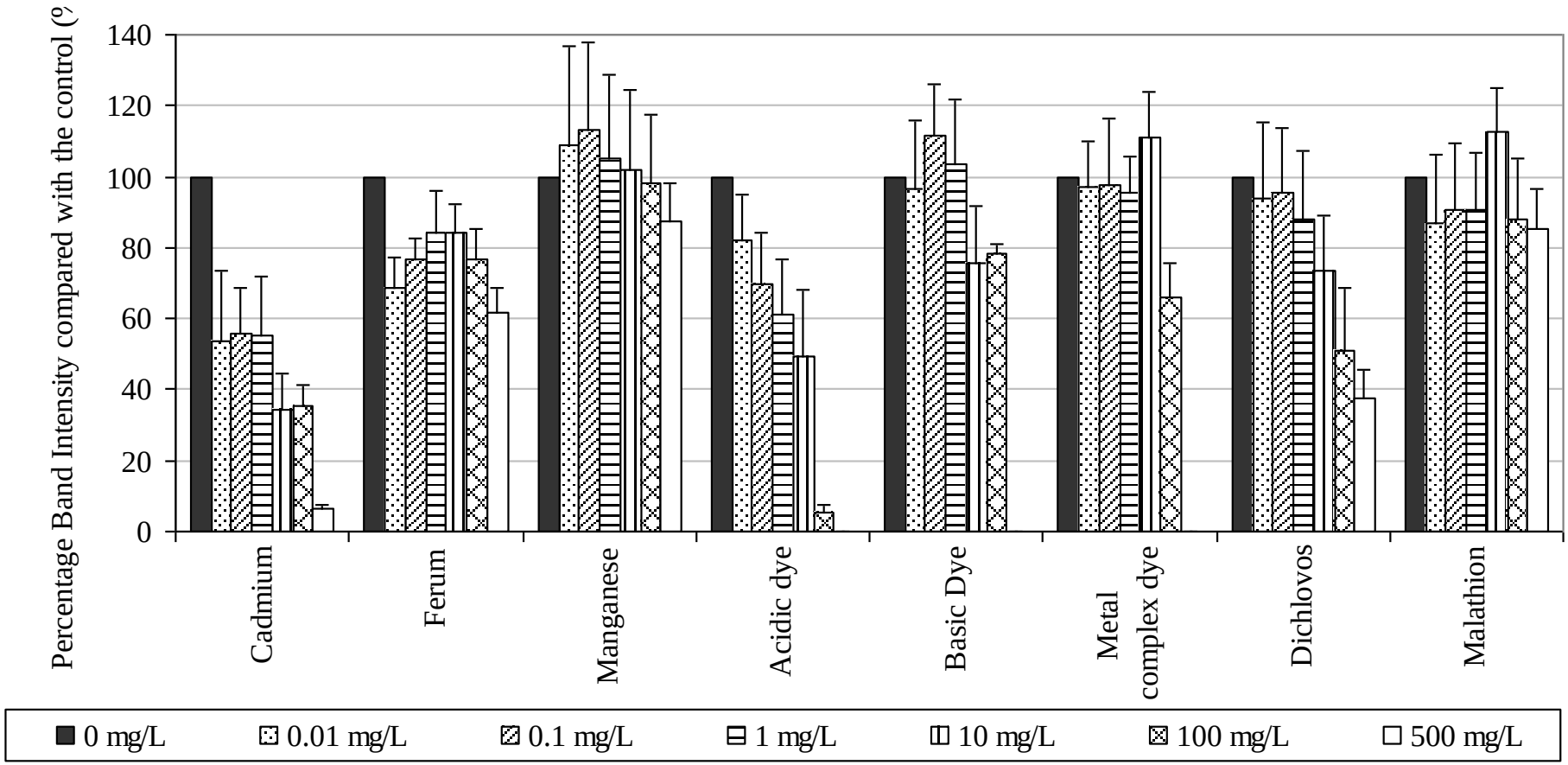


Figure 4.52: Variation of band intensity in comparison to the control (set as 100%) in RAPD profiles of *Chlorella vulgaris* UMACC 245 exposed to different concentrations of toxicant for ten days

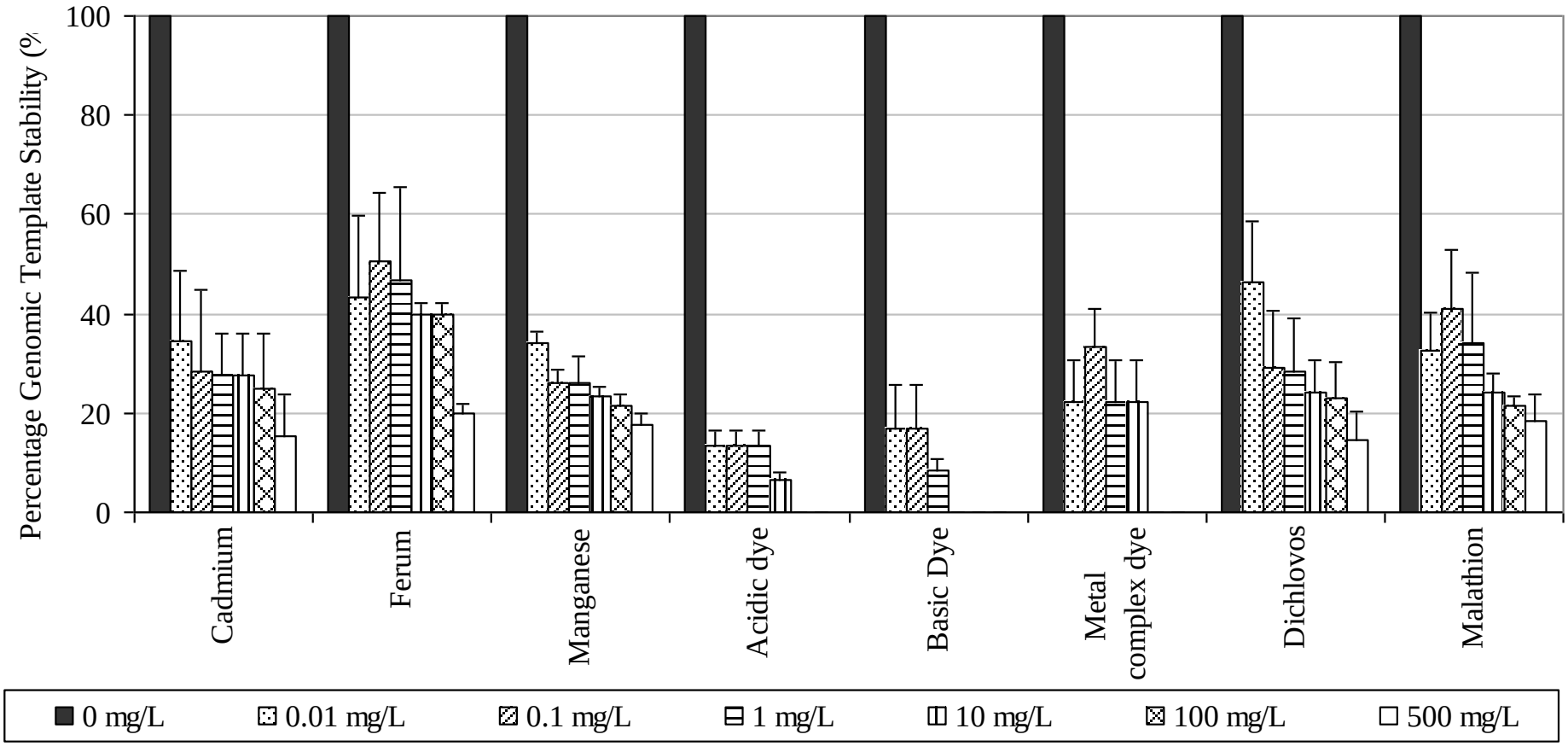


Figure 4.53: Genomic Template Stability of *Chlorella vulgaris* UMACC 245 after exposure to different concentrations of toxicant for four days

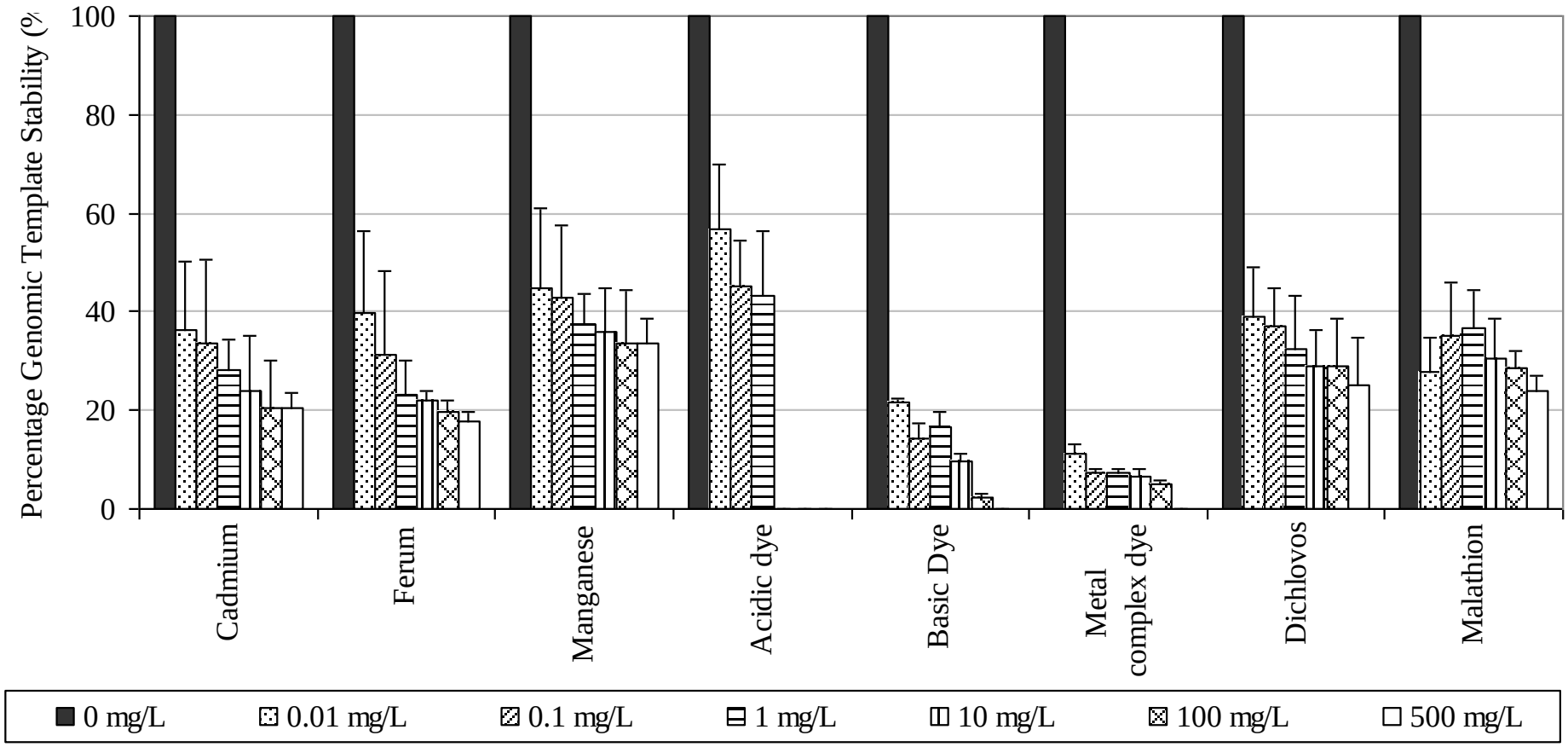


Figure 4.54: Genomic Template Stability of *Chlorella vulgaris* UMACC 245 after exposure to different concentrations of toxicant for ten days

Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion for four and ten days. Appendix 7 summarizes the detailed results of the study.

The results shows, the appearance of new bands in RAPD profiles of *Chlorella vulgaris* UMACC 245 cultures exposed to toxicant for four and ten days decreased with increasing concentrations of toxicant. However in the cultures exposed to Basic dye and Malathion for four and ten days, the appearance of new bands in the RAPD profiles increased with increasing concentrations of toxicant up to 1 mg/L toxicants and then decreased at higher concentrations of toxicants. In contrast, the disappearance of bands in RAPD profiles of the cultures exposed to toxicant for four and ten days increased with increasing concentrations of toxicant.

The decreased in intensity of band following increasing concentrations of toxicant was particularly obvious in the cultures exposed to Acidic dye for four and ten days. In spite of that, in the *Chlorella* exposed to Cd, Fe, Basic dye, Dichlovos and Malathion for four days and also in *Chlorella* exposed to Cd, Fe, Mn, Basic dye, Metal complex dye, Dichlovos and Malathion, the intensity of band in the RAPD profiles, increased with increasing concentrations of toxicant up to 1 mg/L toxicant and then decreased at higher concentrations of toxicant.

The results shows, the genomic template stability, a qualitative measurement reflecting changes in RAPD, and also similarity of the band with the control, in the *Chlorella* exposed to toxicant for four and ten days decreased with increasing concentrations of toxicant.

4.3.2 *Tetraselmis tetrahele* UMACC 144: Effects of Selected Toxicants on DNA Damage (RAPD profiles)

In total eight 10-mer priming oligonucleotides (OPA13, OPN13, OPN16, S17, S67, S68, S87 and S118) were used to analyses the results for each types of chemicals contaminant tested. For the present study, the RAPD profiles in the *Tetraselmis tetrahele* UMACC 144 control cultures (which grown in Prov50 medium without EDTA) were set as 100%.

Figure 4.55 to Figure 4.56 shows the variation of appearance of new bands, disappearance of band (Fig. 4.57 to Fig. 4.58), similiarity of band (Fig. 4.59 to Fig. 4.60), intensity of band (Fig. 4.61 to Fig. 4.62) and genomic template stability (Fig. 4.63 to Fig. 4.64) in comparison to control (set as 100%), obtained in the RAPD profiles of *Tetraselmis tetrahele* UMACC 144 to exposed to Cadmium (Cd), Chromium (Cr), Copper (Cu), Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion for four and ten days. Appendix 12 summarizes the detailed results of the study.

The results shows, after being exposed to toxicants for four and ten days, the appearance of new bands, similarity of the band and genomic template stability in RAPD profiles of *Tetraselmis tetrahele* UMACC 144 cultures decreased with increasing concentrations of toxicant. However, the band intensity of the RAPD profiles increased with increasing concentrations of toxicant up to 10 mg/L toxicant and then decreased at higher concentrations of toxicant. In contrast, we also observed that, the disappearance of bands in RAPD profiles of *Tetraselmis* exposed to all toxicants for four and ten days, increased with increasing concentrations of toxicant.

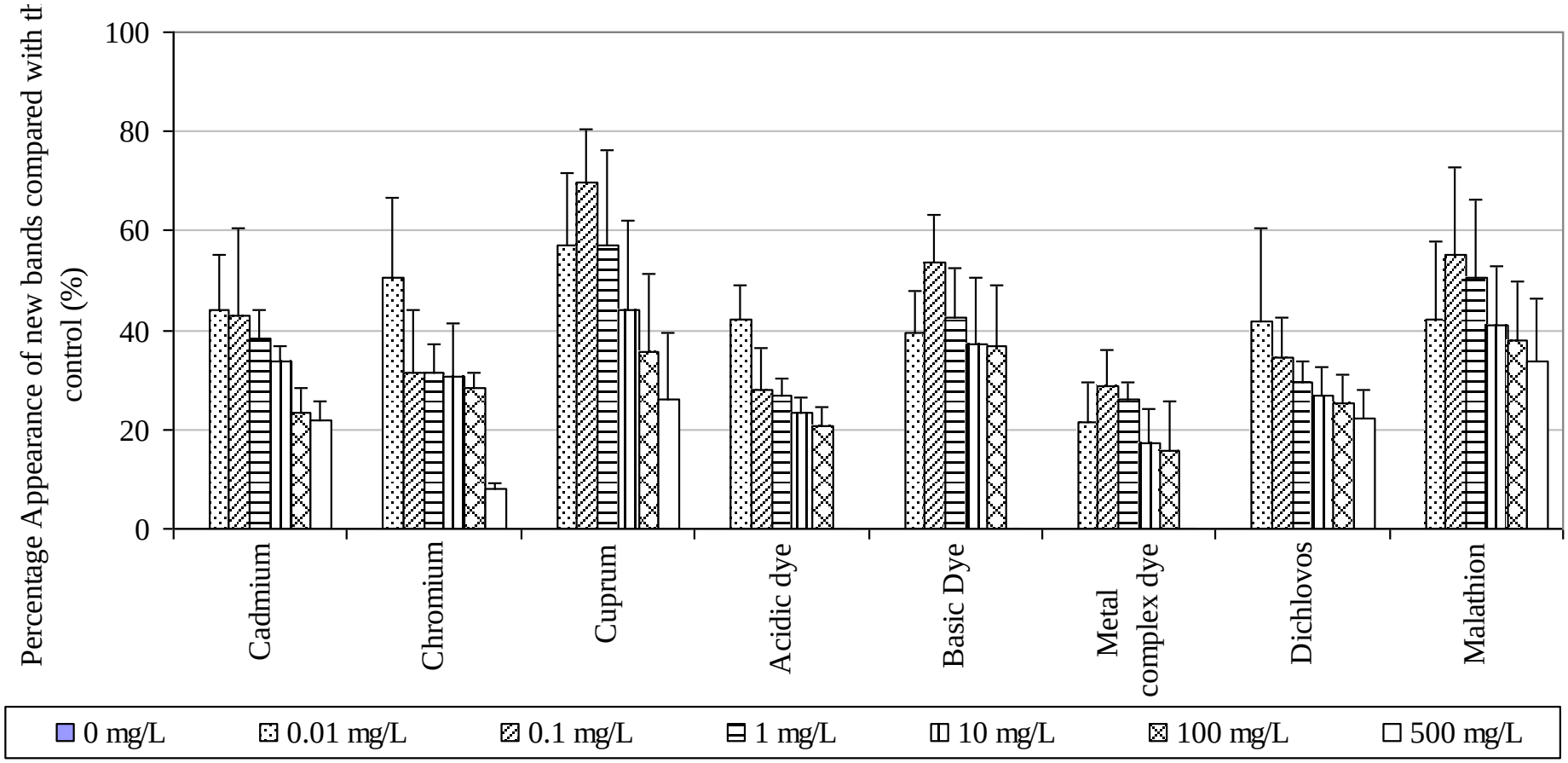


Figure 4.55: Variation of appearance of new bands in comparison to the control (set as 100%) in RAPD profiles of *Tetraselmis tetrahele* UMACC 144 exposed to different concentrations of toxicants for four days

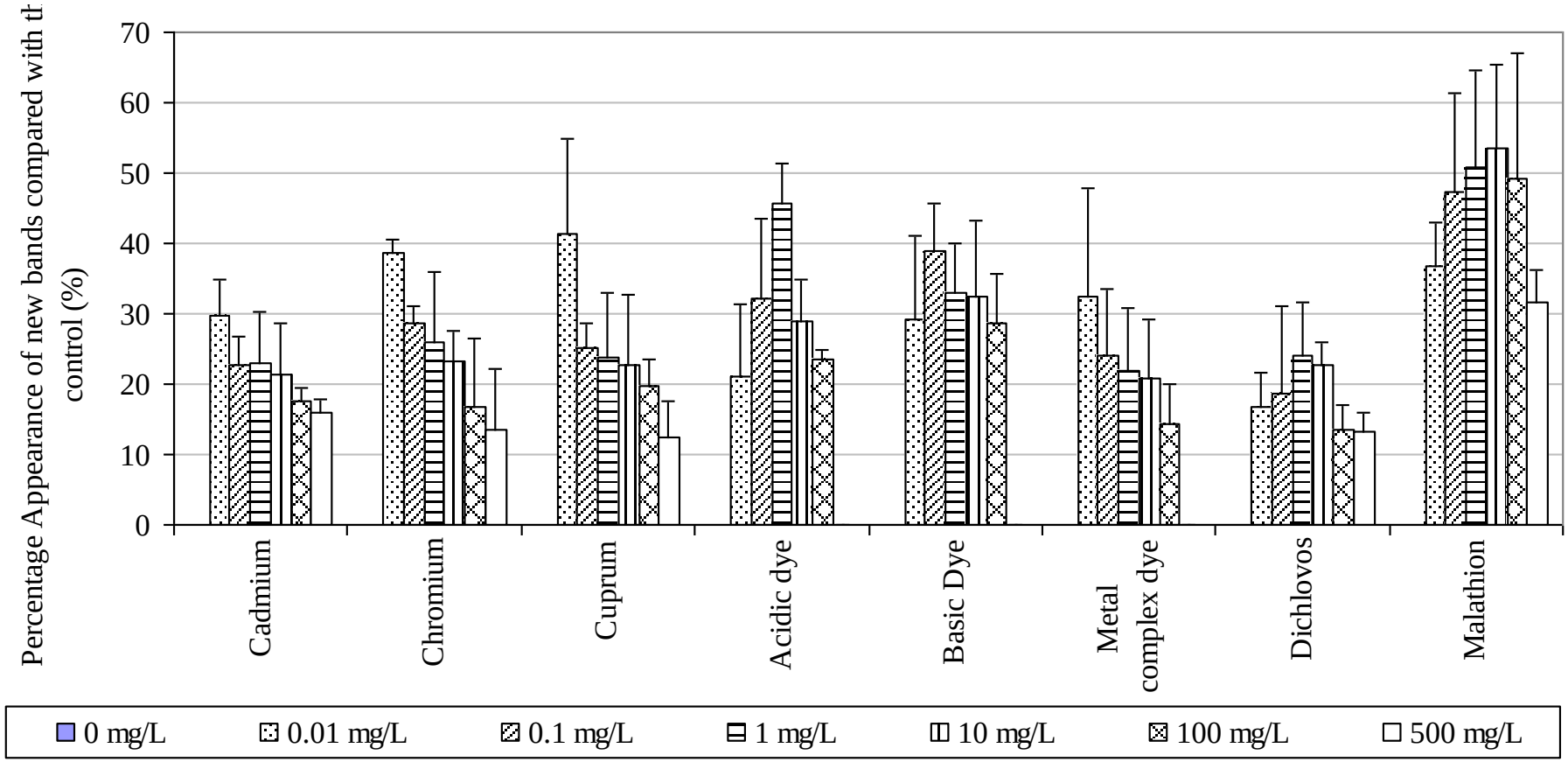


Figure 4.56: Variation of appearance of new bands in comparison to the control (set as 100%) in RAPD profiles of *Tetraselmis tetrahele* UMACC 144 exposed to different concentrations of toxicants for ten days

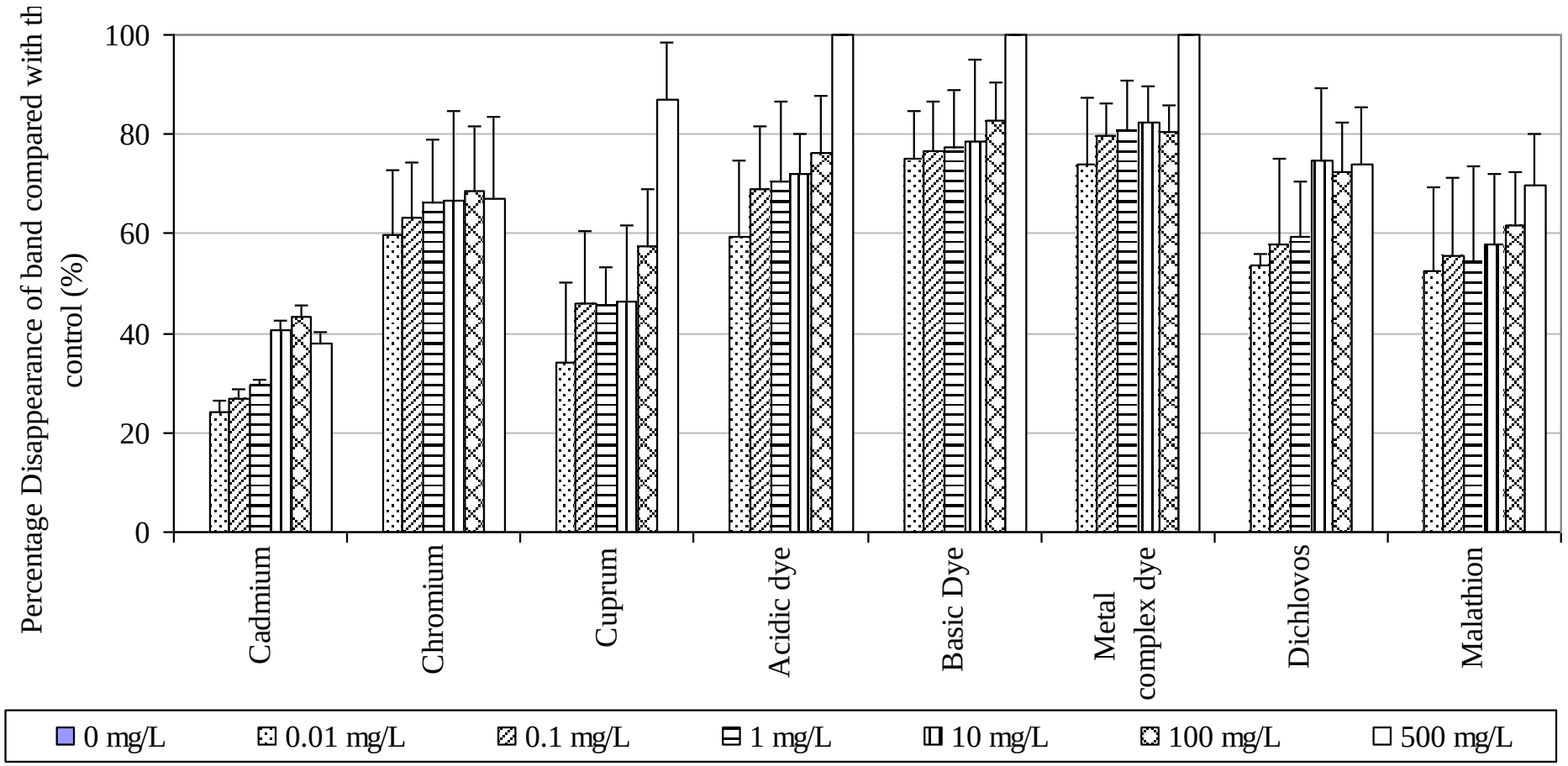


Figure 4.57: Variation of disappearance of bands in comparison to the control (set as 100%) in RAPD profiles of *Tetraselmis tetrahele* UMACC 144 exposed to different concentrations of toxicants for four days

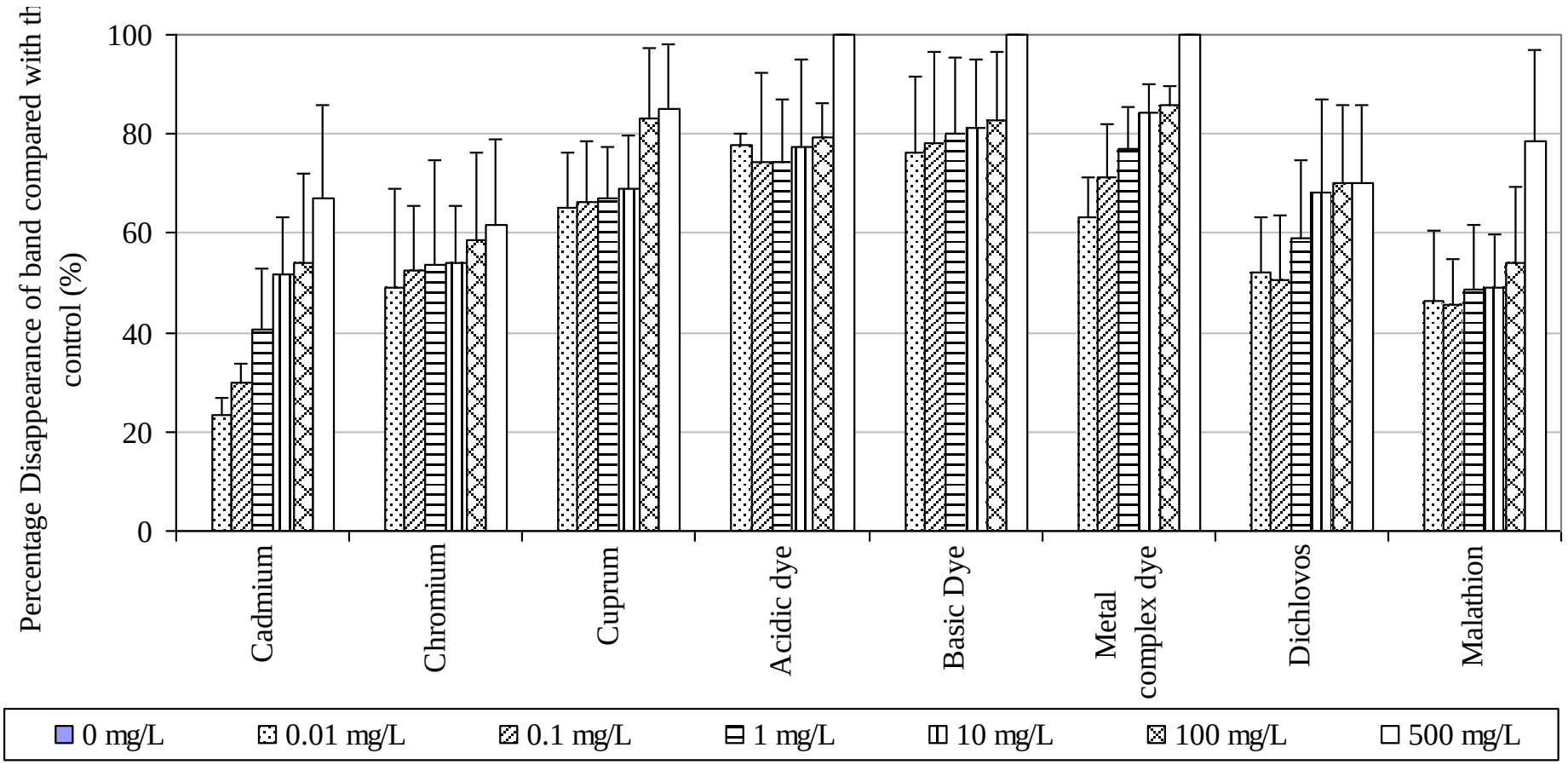


Figure 4.58: Variation of disappearance of bands in comparison to the control (set as 100%) in RAPD profiles of *Tetraselmis tetrahele* UMACC 144 exposed to different concentrations of toxicants ten days

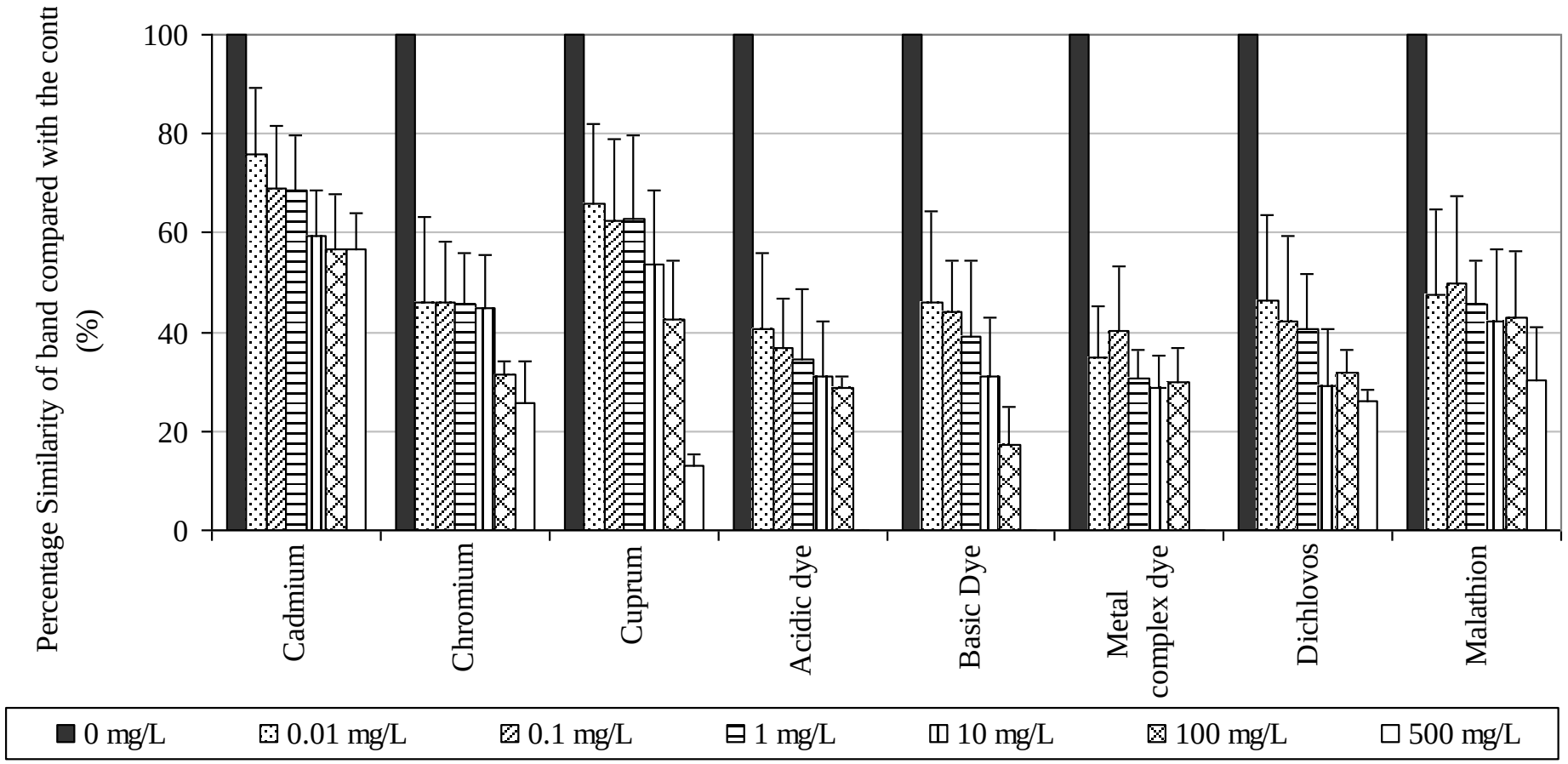


Figure 4.59: Variation of similiarity of bands in comparison to the control (set as 100%) in RAPD profiles of *Tetraselmis tetrahele* UMACC 144 exposed to different concentrations of toxicants four days

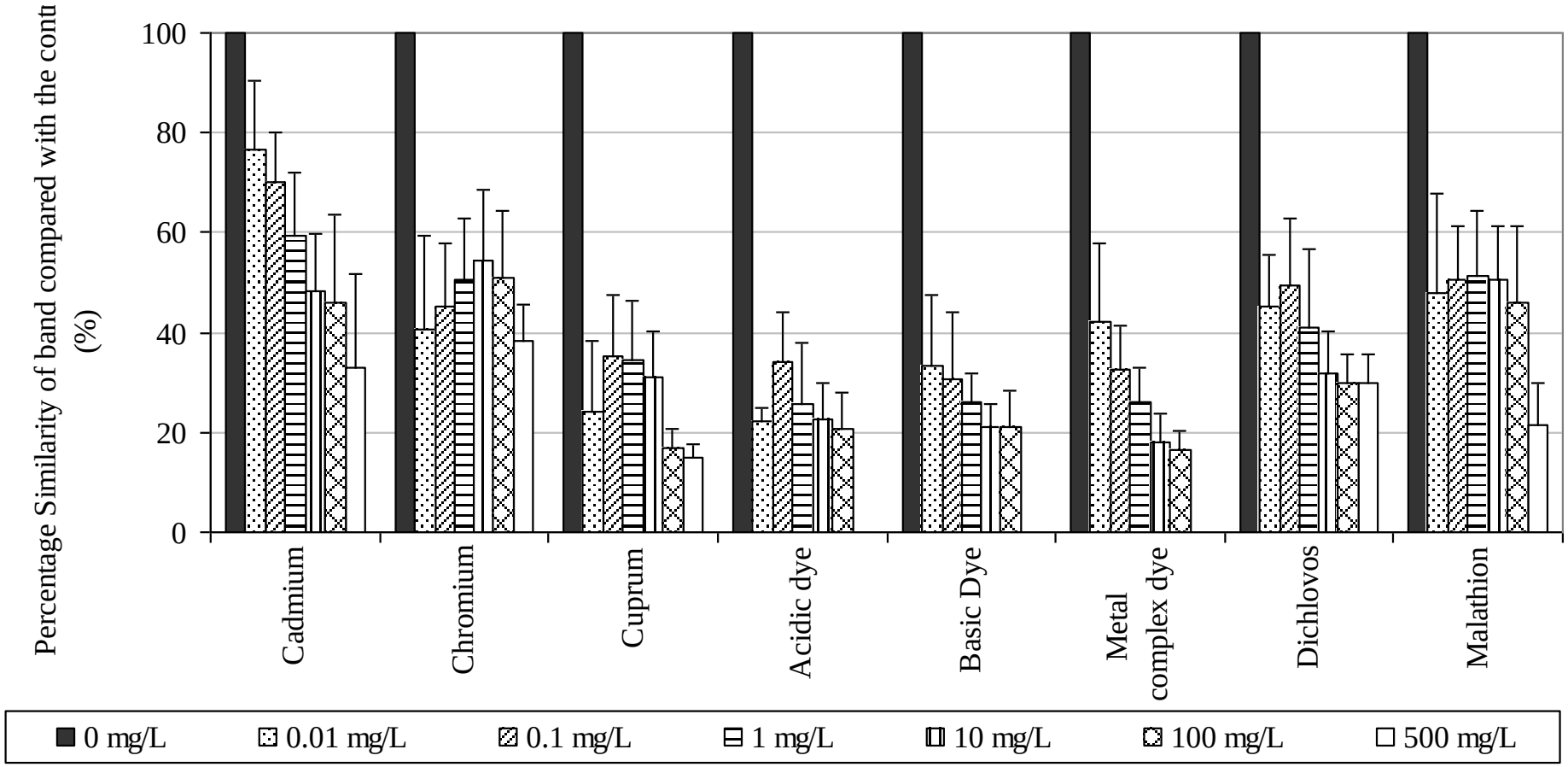


Figure 4.60: Variation of similiarity of bands in comparison to the control (set as 100%) in RAPD profiles of *Tetraselmis tetrahele* UMACC 144 exposed to different concentrations of toxicants for ten days

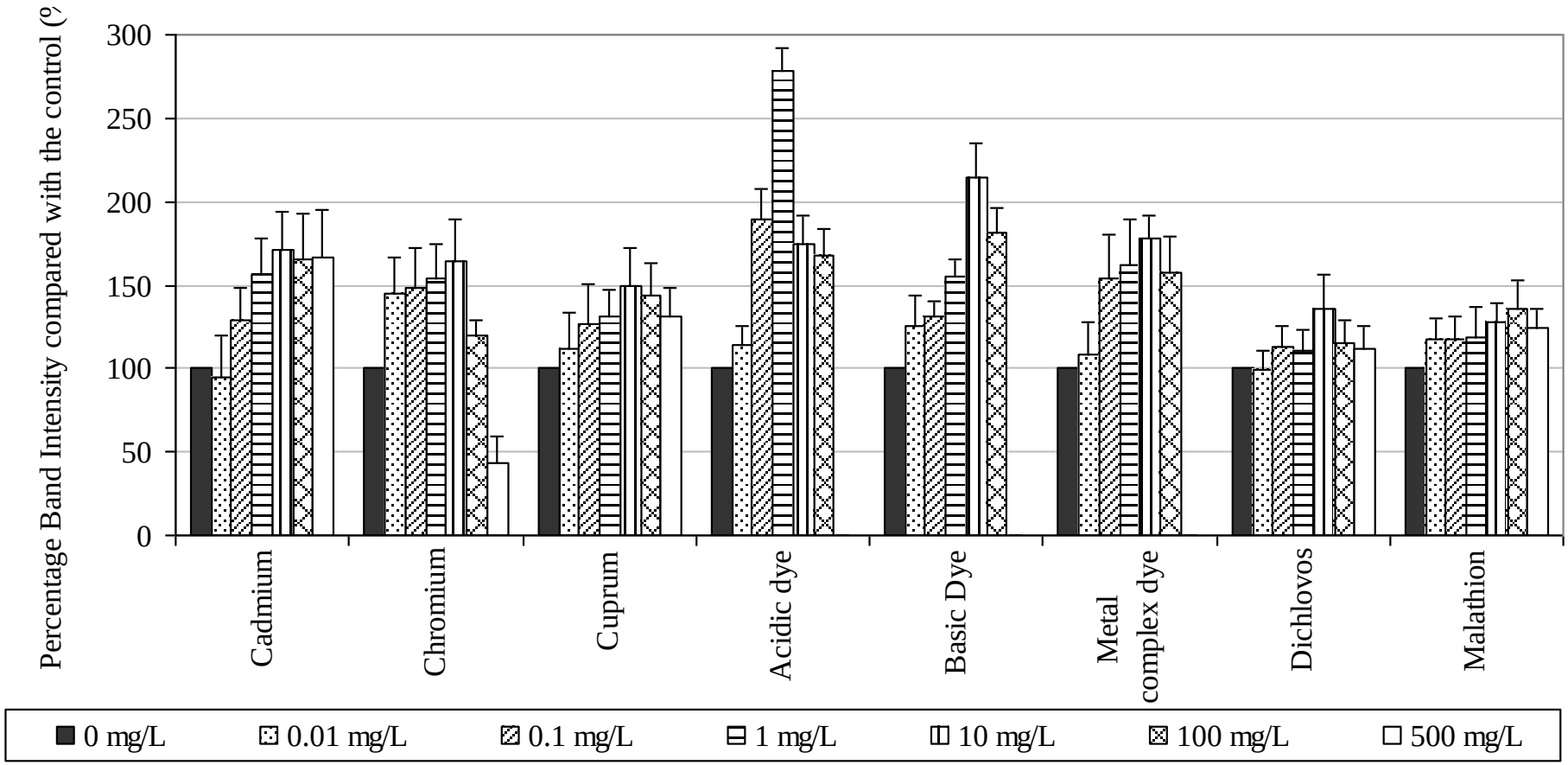


Figure 4.61: Variation of band intensity in comparison to the control (set as 100%) in RAPD profiles of *Tetraselmis tetrahele* UMACC 144 exposed to different concentrations of toxicants for four days

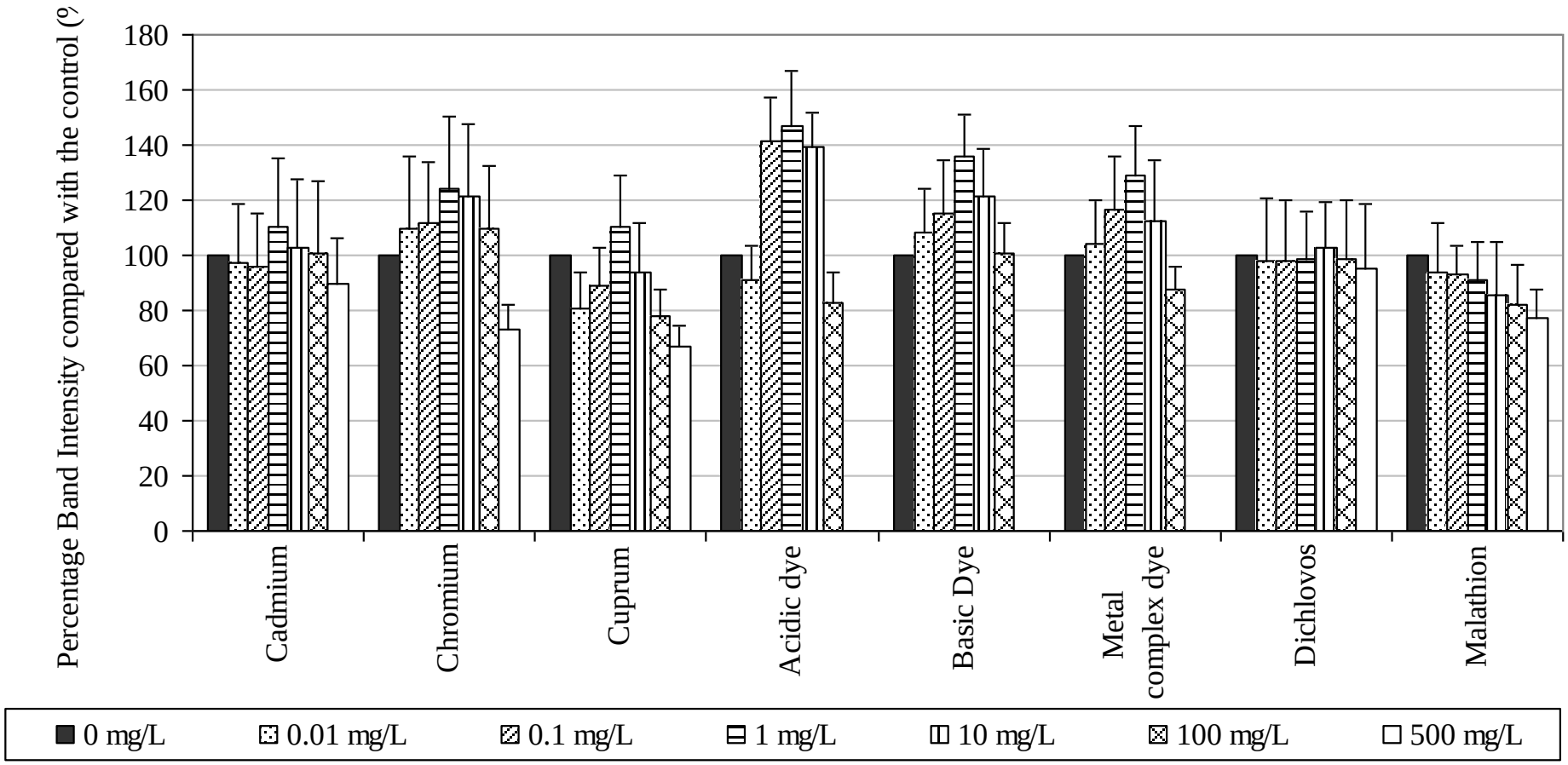


Figure 4.62: Variation of band intensity in comparison to the control (set as 100%) in RAPD profiles of *Tetraselmis tetrahele* UMACC 144 exposed to different concentrations of toxicants ten days

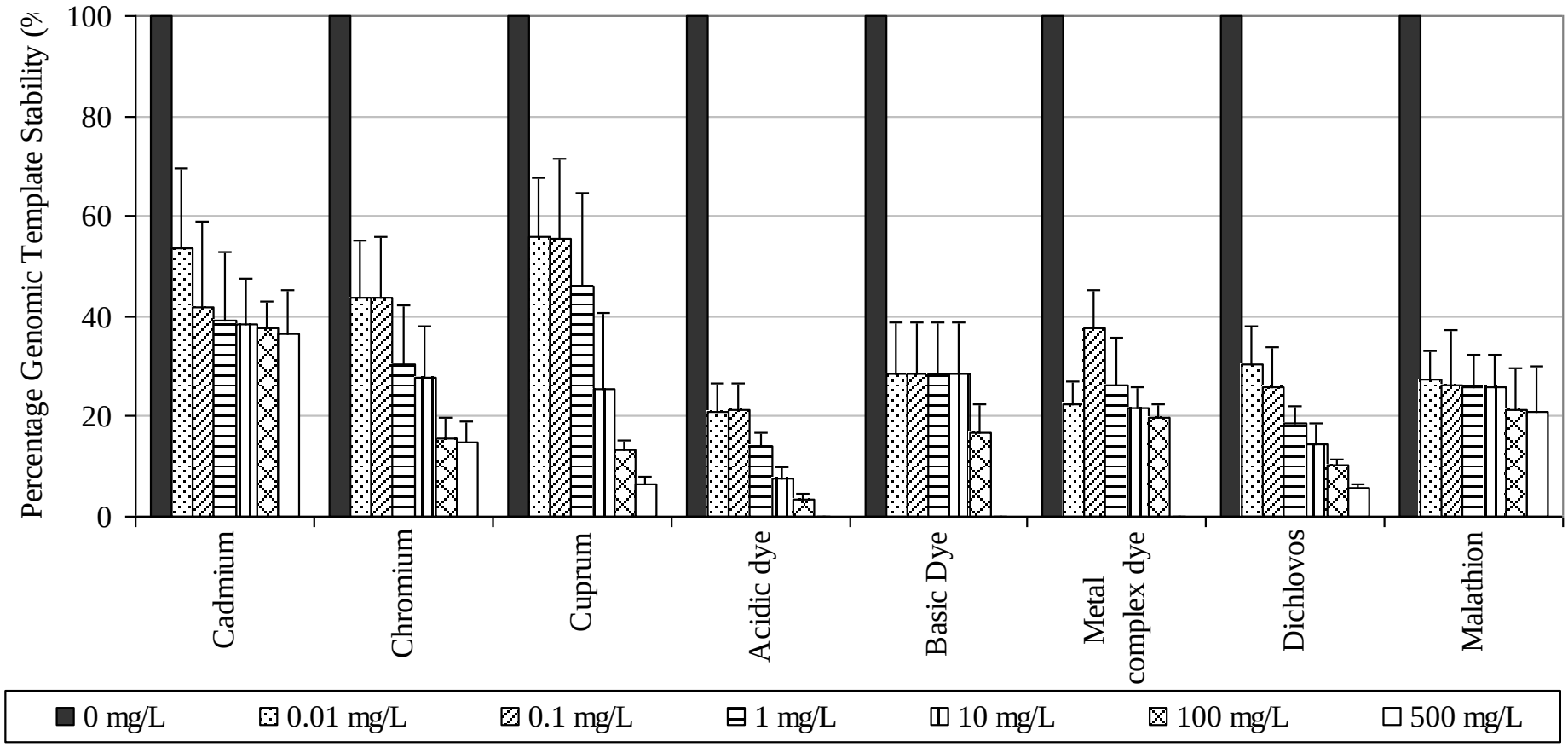


Figure 4.63: Genomic Template Stability of *Tetraselmis tetrahele* UMACC 144 after being exposed to different concentrations of selected toxicant for four days

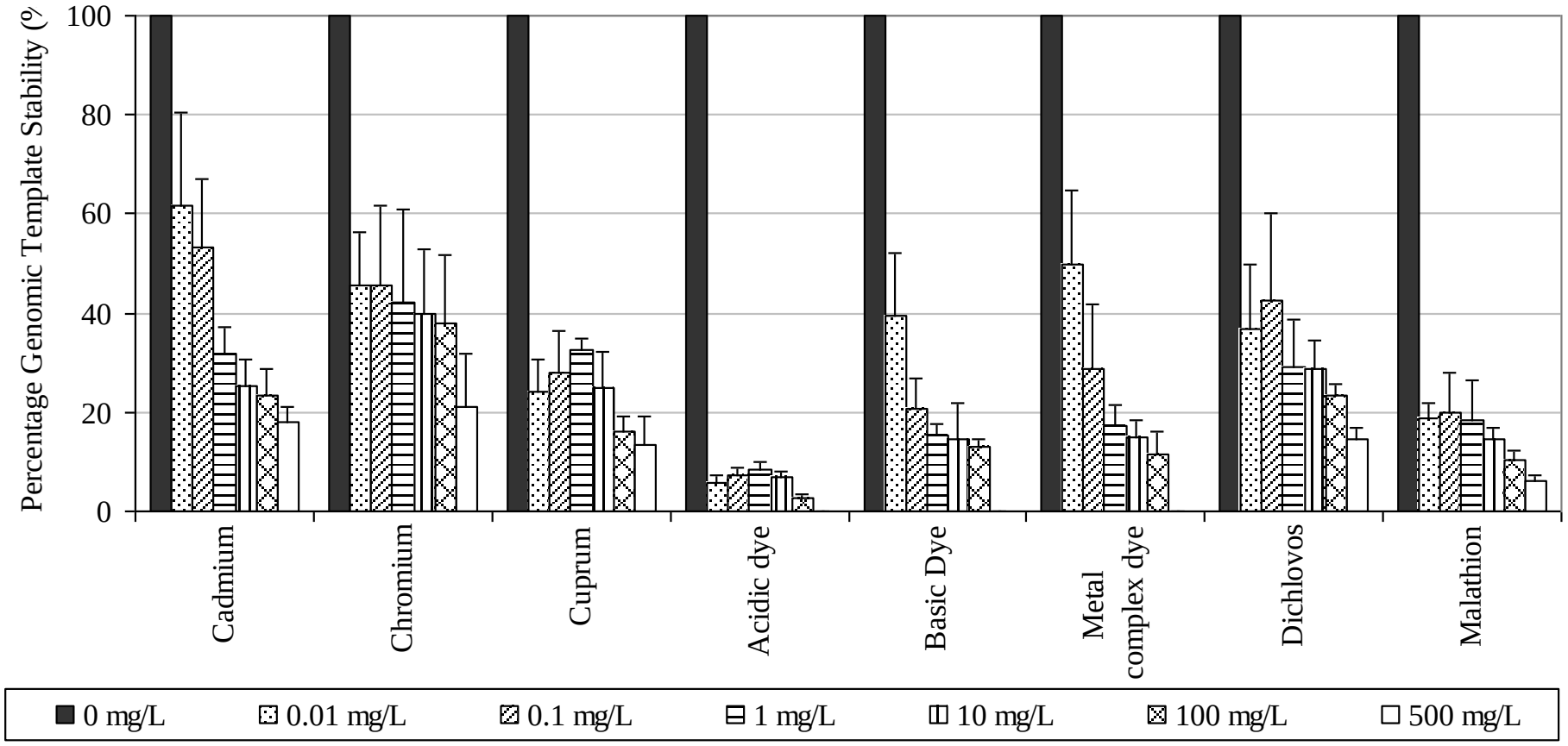


Figure 4.64: Genomic Template Stability of *Tetraselmis tetrahele* UMACC 144 after been exposed to different concentrations of selected toxicant for ten days

4.3.3 *Boergesenia forbesii* : Effects of Selected Toxicants on DNA Damage (RAPD profiles)

For *Boergesenia forbesii*, in total eight 10-mer priming oligonucleotides (OPA13, OPK14, OPN6, OPN13, S17, S67,S105, S124) were used to analyses the results for each types of chemicals contaminant tested. For the present study, the RAPD profiles *Boergesenia forbesii* in the control cultures (which grown in Prov50 medium without EDTA) were set as 100%.

Figure 4.65 to Figure 4.66 shows the variation of appearance of new bands, disappearance of band (Fig. 4.67 to Fig. 4.68), similiarity of band (Fig. 4.69 to Fig. 4.70), intensity of band (Fig. 4.71 to Fig. 4.72) and genomic template stability (Fig. 4.73 to Fig. 4.74) in comparison to control (set as 100%), obtained in the RAPD profiles of *Boergesenia forbesii* to exposed to Cadmium (Cd), Copper (Cu), Zinc (Zn), Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion for four and ten days. Appendix 17 summarizes the detailed results of the study.

In general, the results showed the appearance of new bands in RAPD profiles of *Boergesenia forbesii* cultures exposed to Basic dye for four and ten days decreased with increasing concentrations of toxicant. However in the cultures exposed to Cu for four and ten days, the appearance of new bands in the RAPD profiles increased with increasing concentrations of toxicant up to 0.1 mg/L Cu and then decreased at higher concentrations of Cu.

The disappearance of bands in RAPD profiles of the cultures exposed to all chemicals contaminants tested in this study, for four and ten days increased with

increasing concentrations of toxicant except for the cultures exposed to Cd for four days where the disappearance of band of the RAPD profiles, increased with increasing concentrations of Cd up to 10 mg/L Cd and then decreased at higher concentrations of Cd.

The decreased in the similiarity of bands in RAPD profiles following concentrations of toxicant was particularly obvious in the cultures exposed to Cu, Zn, Metal complex dye and Malathion for four and ten days. However, in the *Boergesenia* exposed to Cd, Acidic dye, Basic dye and Dichlovos for four days and in the *Boergesenia* exposed to Dichlovos for ten days, the similarity of the band increased with increasing concentrations of toxicant up to 1 mg/L of toxicant and then decreased at higher concentrations of toxicant.

The results also showed that, band intensity of the *Boergesenia* exposed to Cd for four days decreased with increasing concentrations of toxicant. However in the *Boergesenia* (exposed to Basic dye and Malathion) for four days and also in the cultures exposed to Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion for ten days, the band intensity in the RAPD profiles increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant

The genomic template stability, a qualitative measurement reflecting changes in RAPD pattern shows that the genomic template stability in the cultures exposed to Cd, Cu, Zn, Acidic dye, Basic dye and Malathion for four and ten days, decreased with increasing concentrations of toxicant.

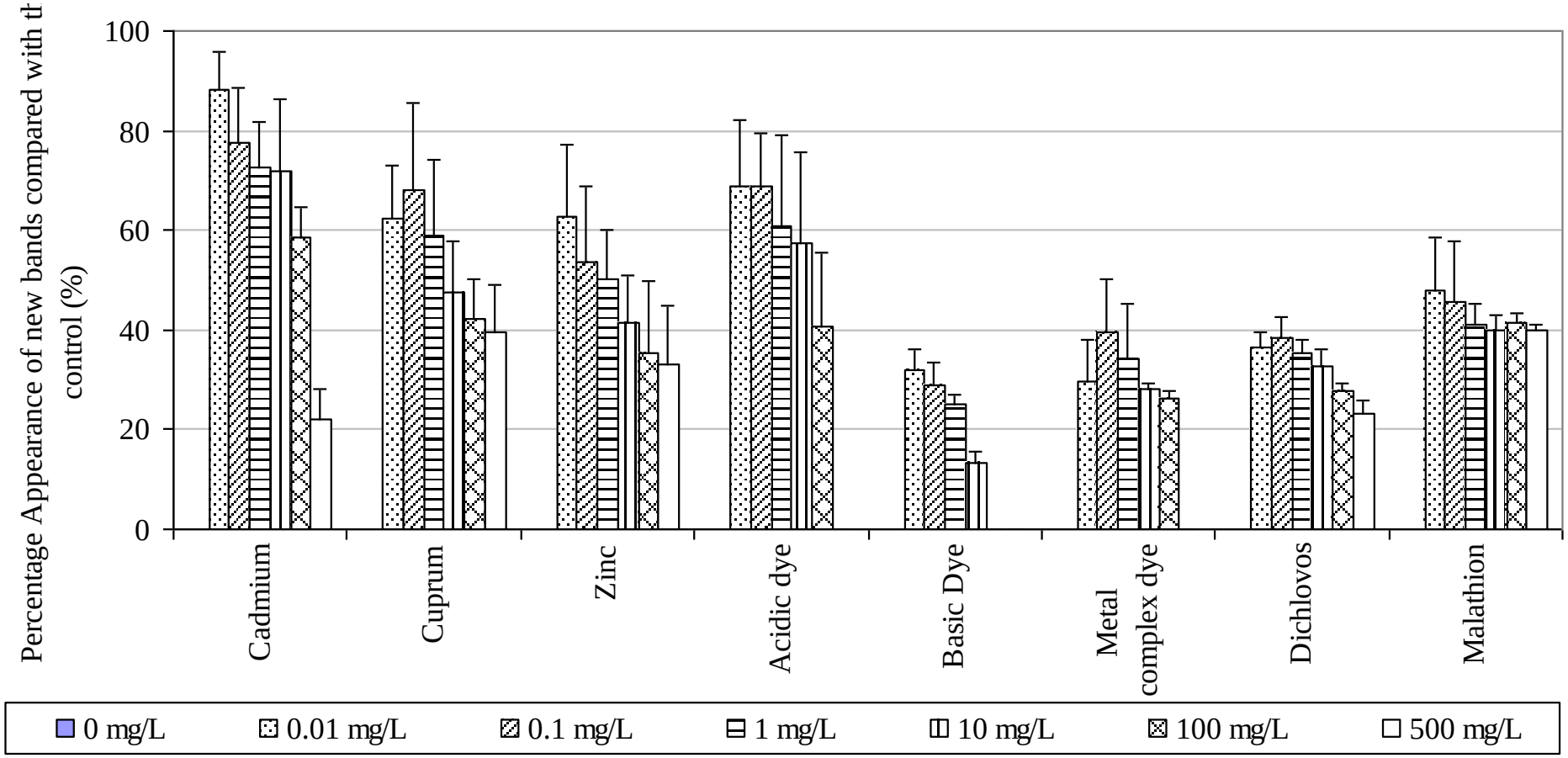


Figure 4.65: Variation of appearance of new bands in comparison to the control (set as 100%) in RAPD profiles of *Boergesenia forbesii* exposed to different concentrations of selected toxicant for four days

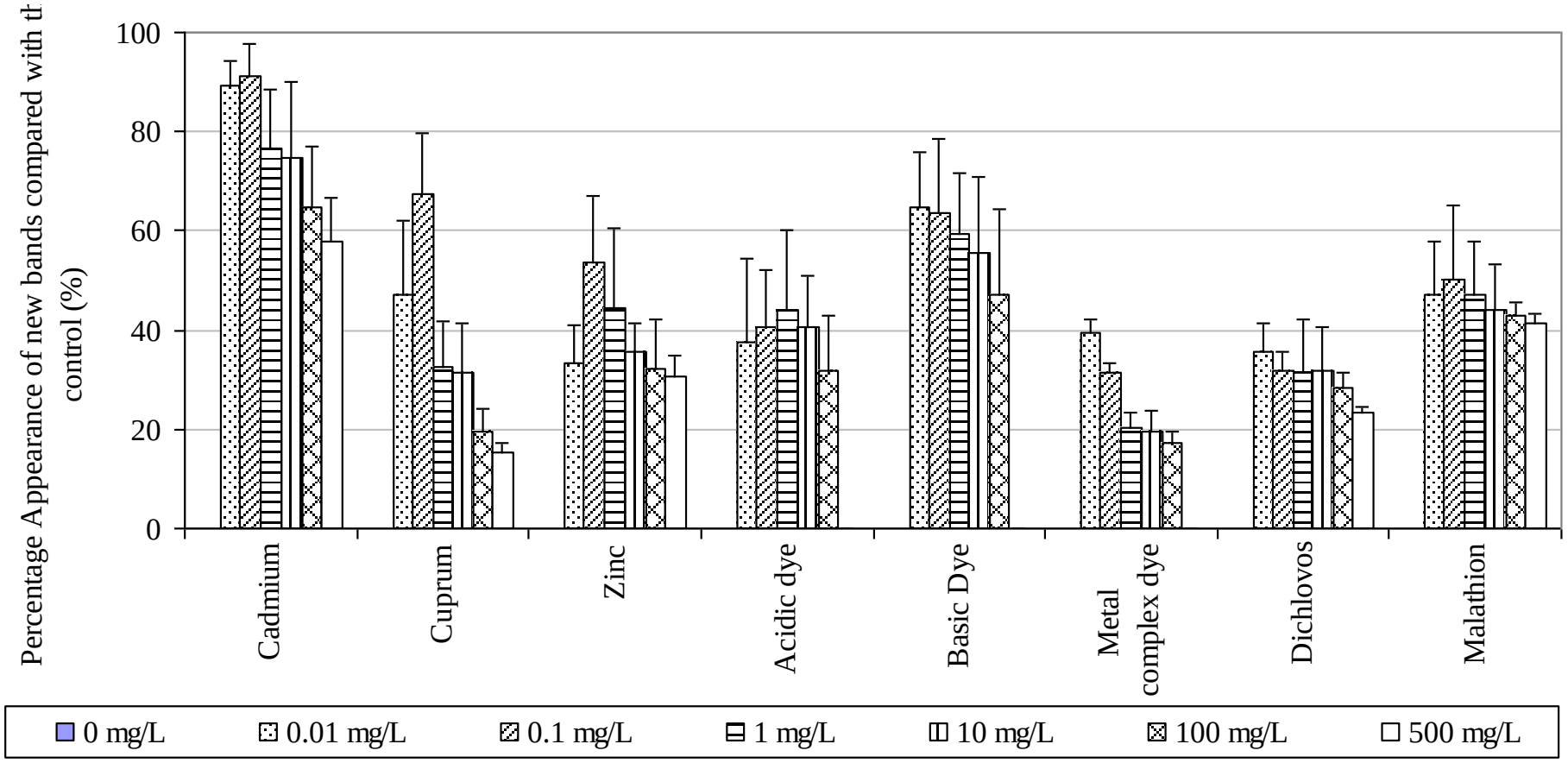


Figure 4.66: Variation of appearance of new bands in comparison to the control (set as 100%) in RAPD profiles of *Boergesenia forbesii* exposed to different concentrations of selected toxicant for for ten days

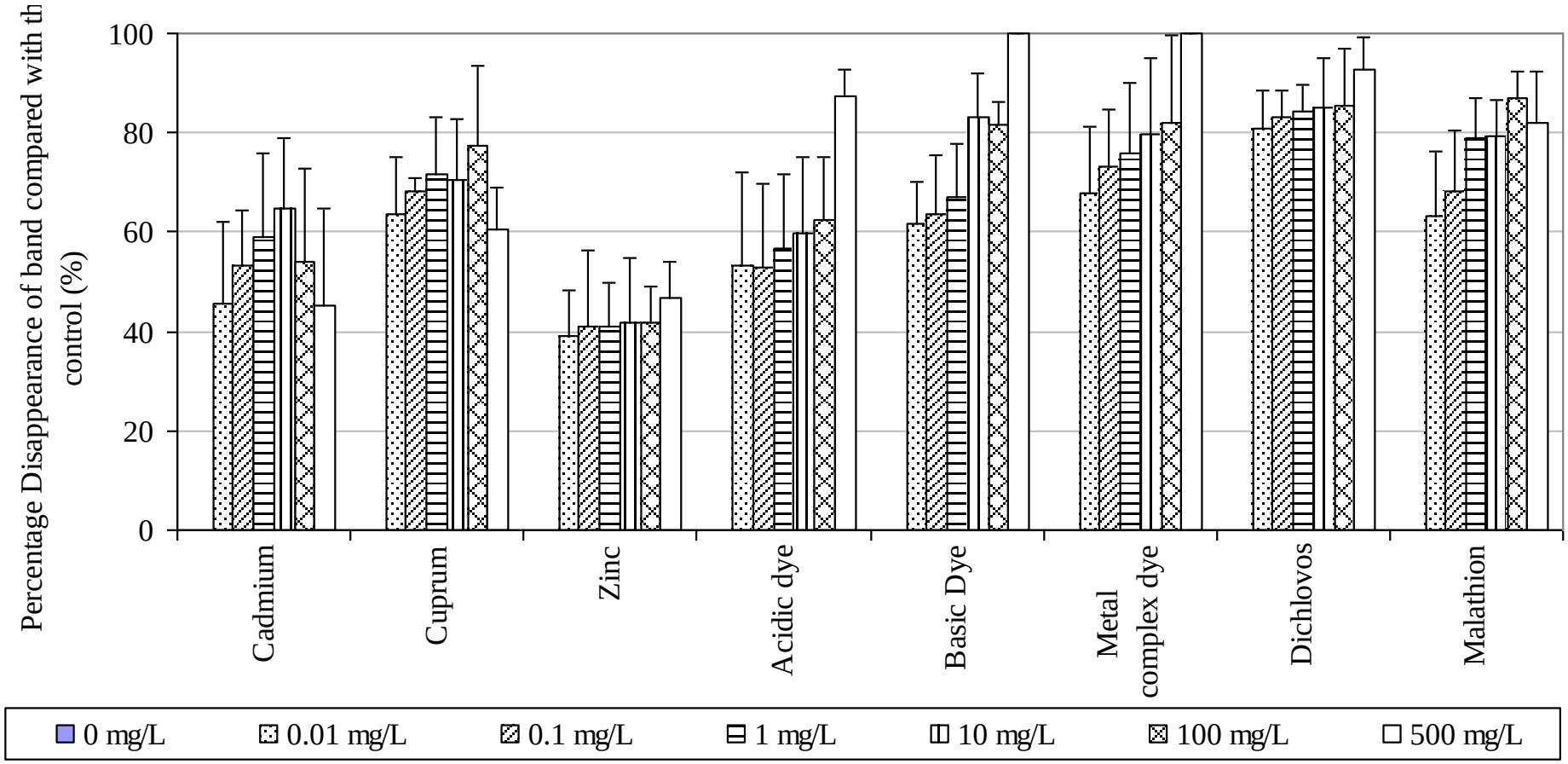


Figure 4.67: Variation of disappearance of bands in comparison to the control (set as 100%) in RAPD profiles of *Boergesenia forbesii* exposed to different concentrations of selected toxicant for four days

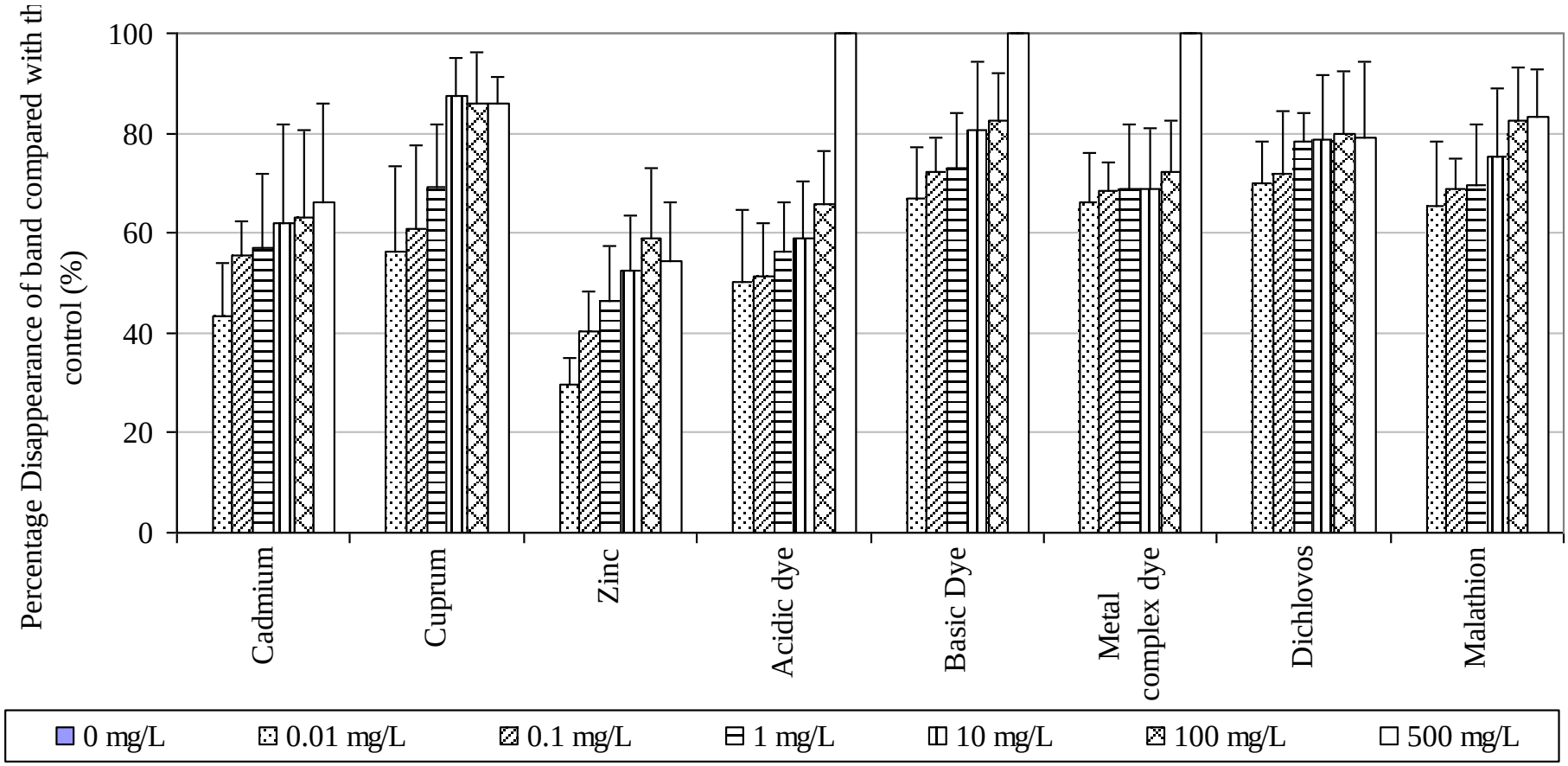


Figure 4.68: Variation of disappearance of bands in comparison to the control (set as 100%) in RAPD profiles of *Boergesenia forbesii* exposed to different concentrations of selected toxicant for ten days

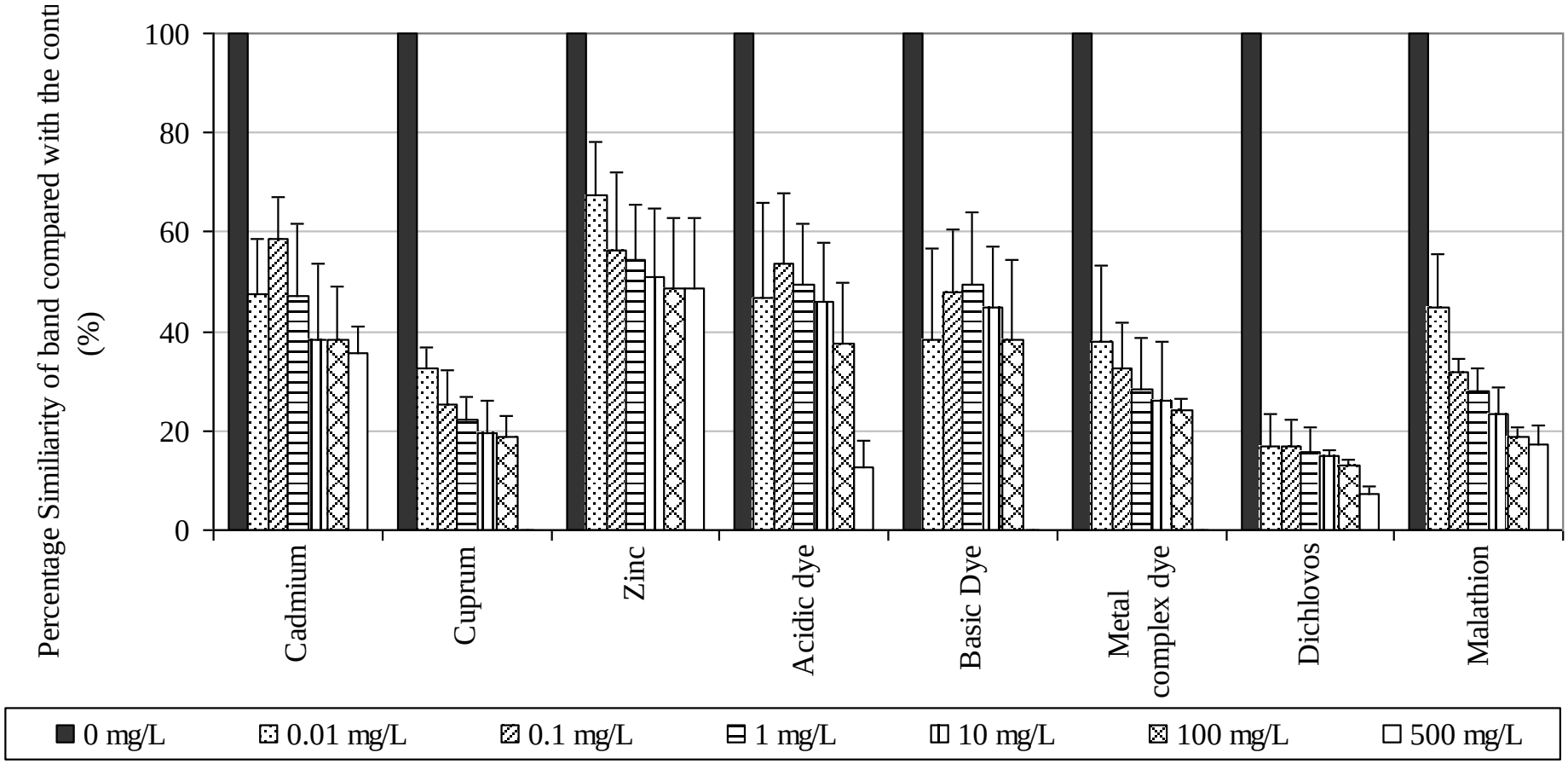


Figure 4.69: Variation of similiarity of bands in comparison to the control (set as 100%) in RAPD profiles of *Boergesenia forbesii* exposed to different concentrations of selected toxicant for four days

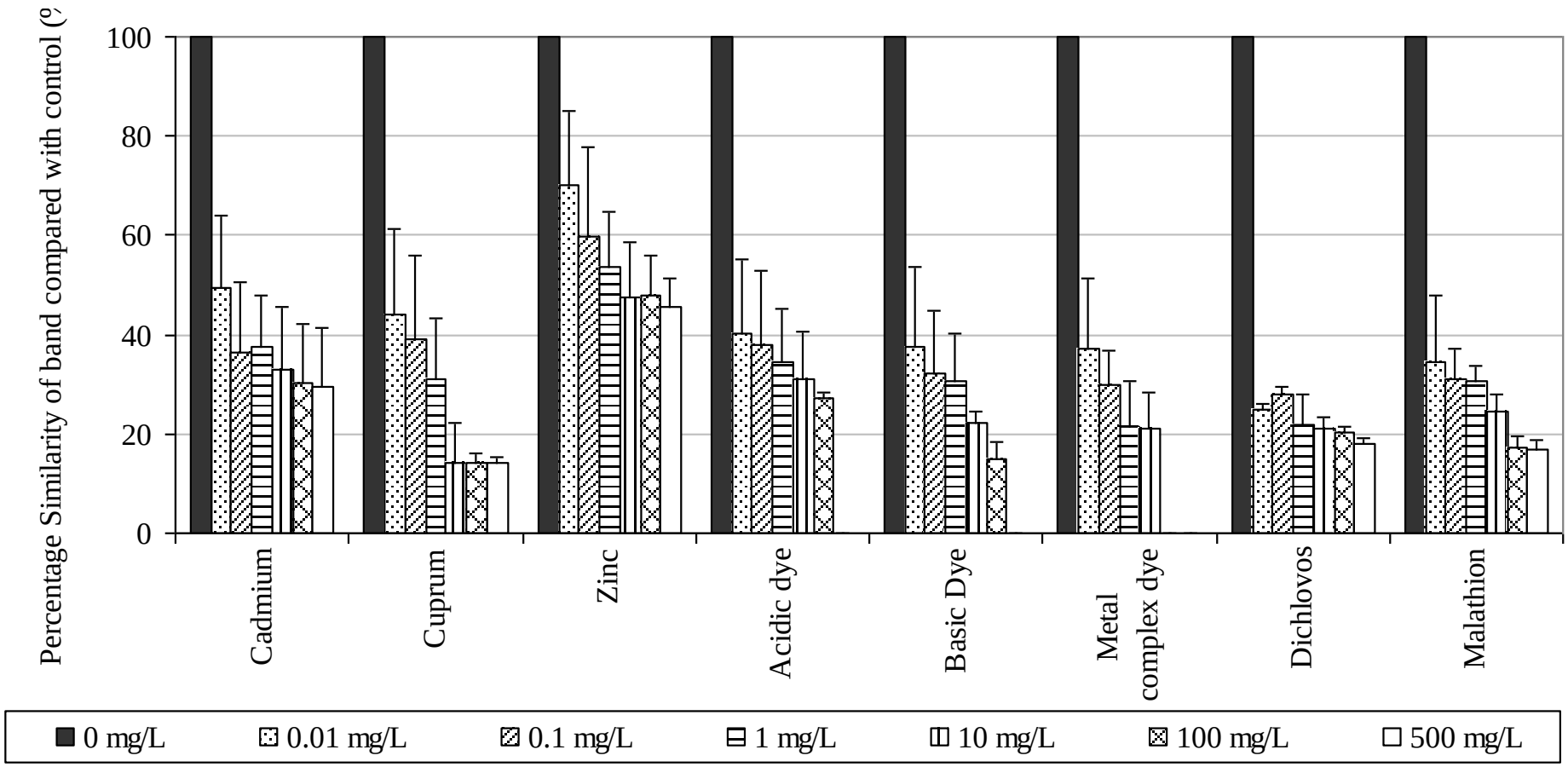


Figure 4.70: Variation of similiarity of bands in comparison to the control (set as 100%) in RAPD profiles of *Boergesenia forbesii* exposed to different concentrations of selected toxicant for ten days

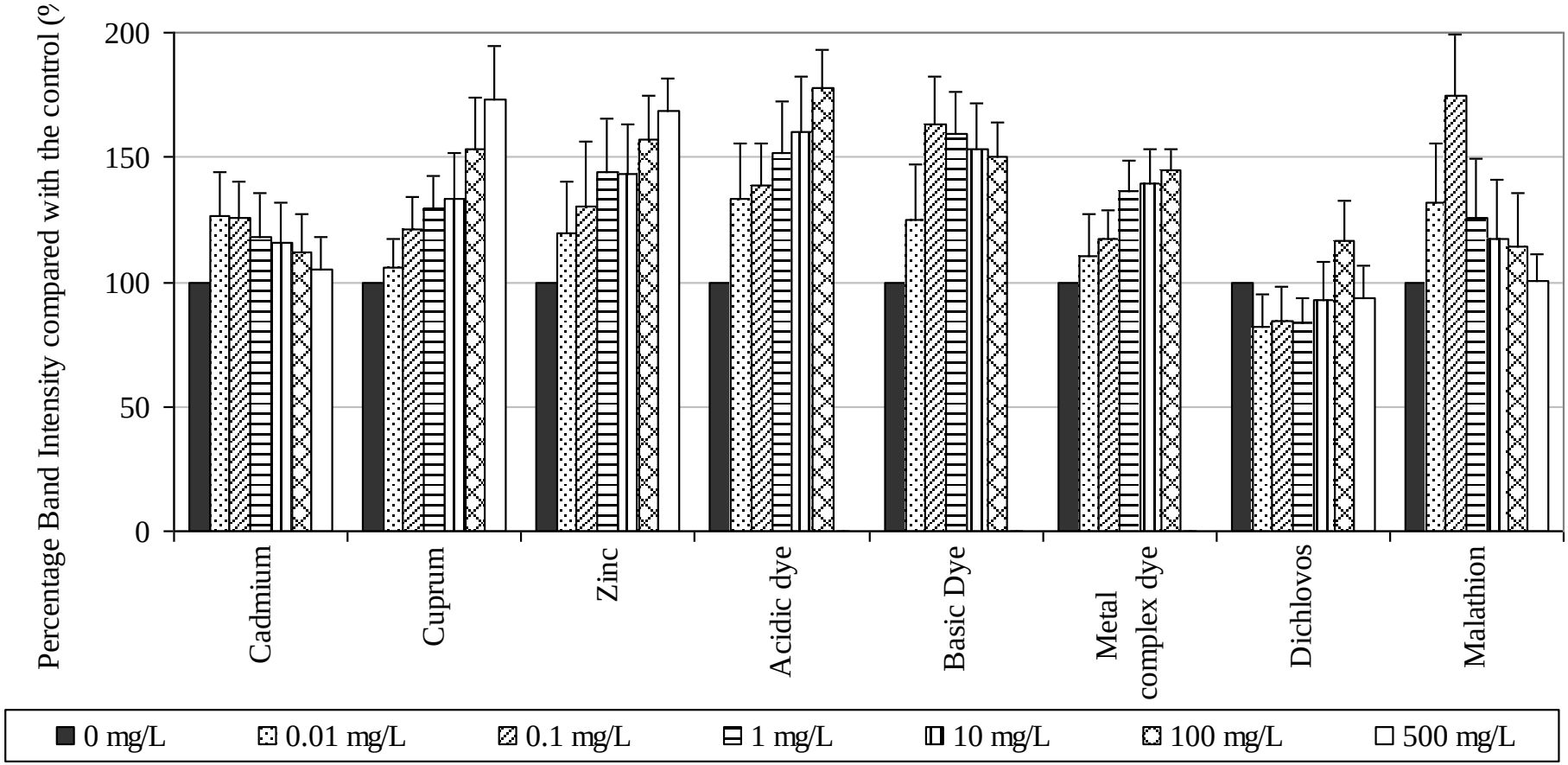


Figure 4.71: Variation of band intensity in comparison to the control (set as 100%) in RAPD profiles of *Boergesenia forbesii* exposed to different concentrations of selected toxicant for four days

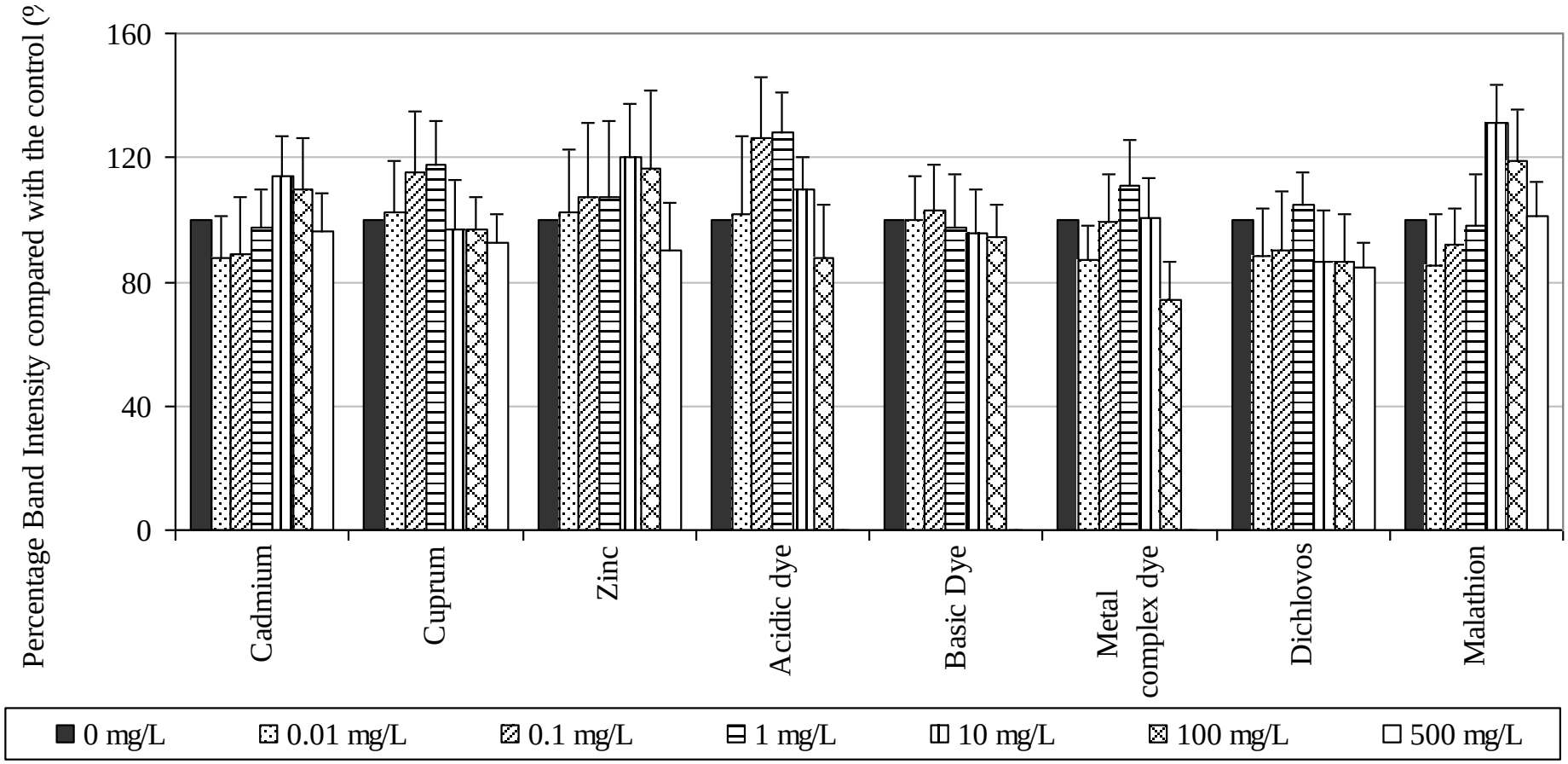


Figure 4.72: Variation of band intensity in comparison to the control (set as 100%) in RAPD profiles of *Boergesenia forbesii* exposed to different concentrations of selected toxicant for ten days

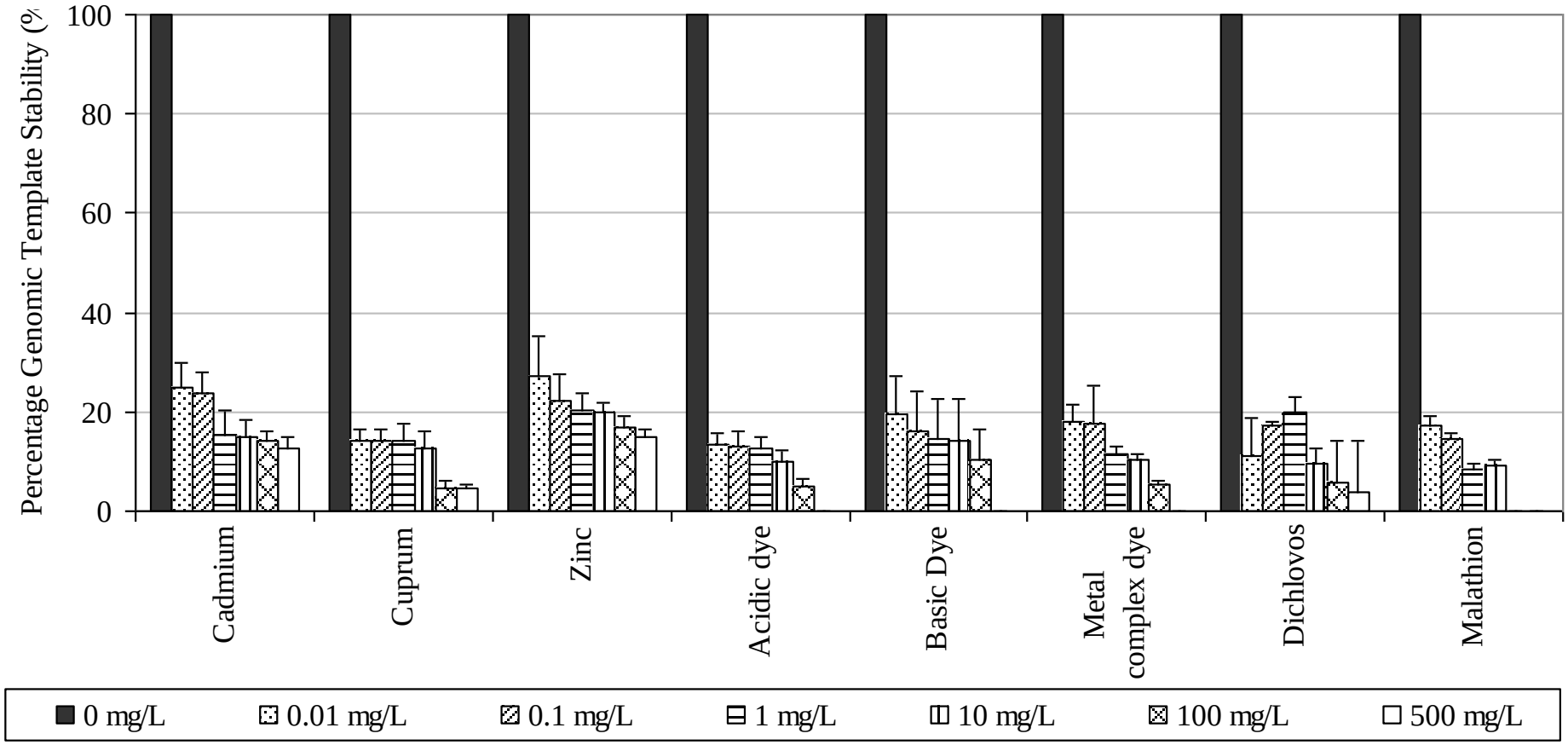


Figure 4.73: Genomic Template Stability of *Boergesenia forbesii* after being exposed to different concentrations of selected toxicant for four days

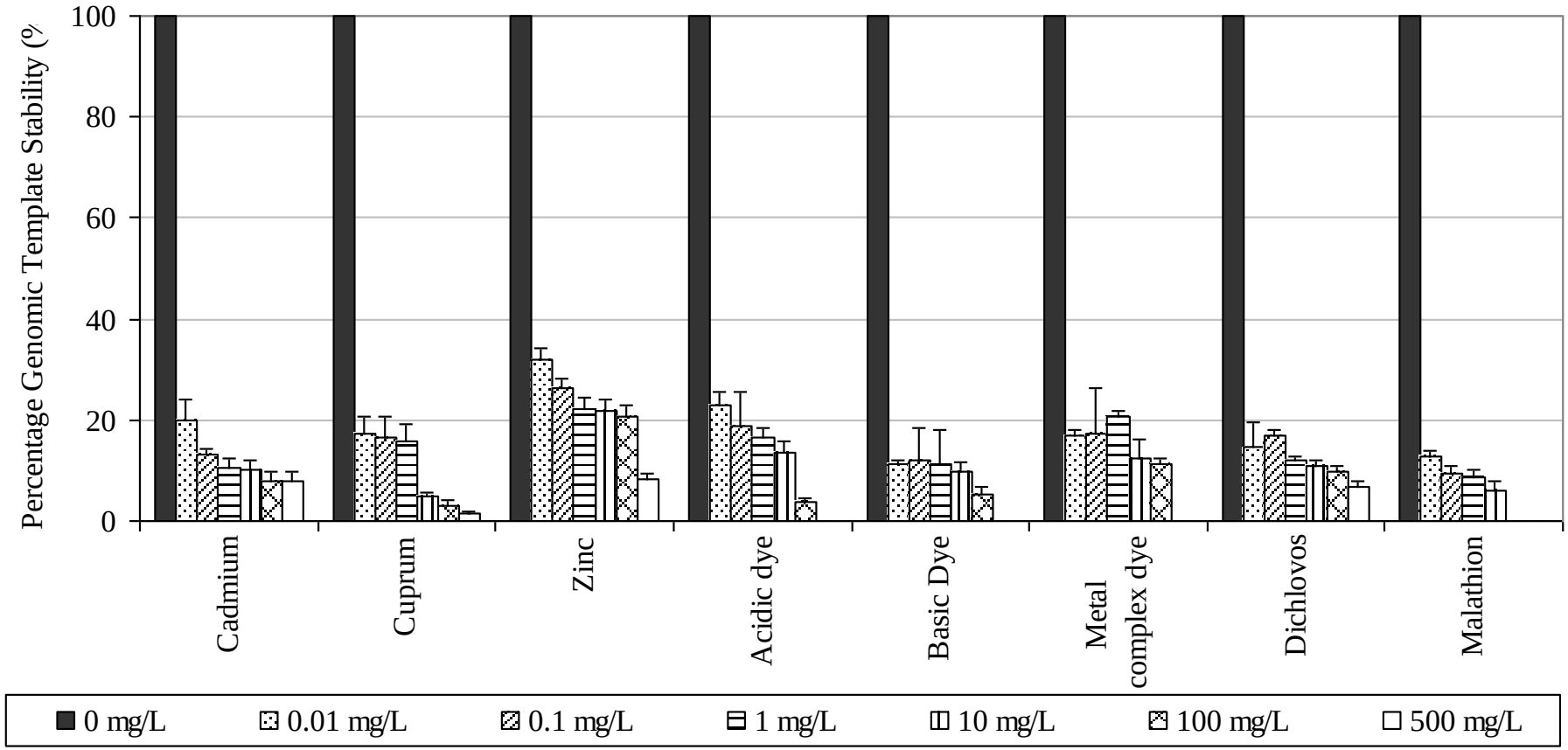


Figure 4.74: Genomic Template Stability of *Boergesenia forbesii* after being exposed to different concentrations of selected toxicant for ten days

4.3.4 *Ventricaria ventricosa*: Effects of Selected Toxicants on DNA Damage (RAPD profiles

In total eight 10-mer priming oligonucleotides (OPA13, OPK14, OPN12, OPN13, S17, S20, S67, S86) were used to analyses the results for each types of chemicals contaminant tested. For the present study, the RAPD profiles *Ventricaria ventricosa* in the control cultures (which grown in Prov50 medium without EDTA) were set as 100%.

Figure 4.75 to Figure 4.76 shows the variation of appearance of new bands, disappearance of band (Fig. 4.77 to Fig. 4.78), similiarity of band (Fig. 4.79 to Fig. 4.80), intensity of band (Fig. 4.81 to Fig. 4.82) and genomic template stability (Fig. 4.83 to Fig. 4.84) in comparison to control (set as 100%), obtained in the RAPD profiles of genomic DNA from *Ventricaria ventricosa* exposed to Cadmium (Cd), Cuprum (Cu), Zinc (Zn), Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion for four and ten days. Appendix 22 summarizes the detailed results of the study.

In general, the results showed the appearance of new bands in RAPD profiles of *Ventricaria ventricosa* cultures exposed to Basic dye for four and ten days decreased with increasing concentrations of toxicant. However in the cultures exposed to Cd, Cu, Acidic dye and Dichlovos for four days, the appearance of new bands in the RAPD profiles increased with increasing concentrations of toxicant up to 1 mg/L toxicant and then decreased at higher concentrations of toxicant and in the ten days cultures, the appearance of new bands in the RAPD profiles decreased with increasing concentrations.

The disappearance of bands in RAPD profiles of the cultures exposed to all chemical contaminants used in this study, for four and ten days increased with increasing concentrations of toxicant.

The decreased in the similiarity of the band following increasing concentrations of toxicant was particularly obvious in the cultures exposed to Cu, Zn, Acidic dye, Basic dye and Malathion for four and ten days.

The intensity of bands in RAPD profiles of the cultures exposed to all chemical contaminants used in this study, for four and ten days increased with increasing concentrations of toxicant up to 10 mg/L toxicant and then decreased at higher concentrations of toxicant except for the cultures exposed to Dichlovos and Malathion for ten days.

The genomic template stability, a qualitative measurement reflecting changes in RAPD pattern shows that the genomic template stability in the cultures exposed to all chemical contaminants used in this study for four and ten days, decreased with increasing concentrations of toxicant except for the cultures exposed to Cd, Zn and Acidic dye for ten days, the genomic template stability in the cultures increased with increasing concentrations of toxicant up to 1 mg/L toxicant and then decreased at higher concentrations of toxicant.

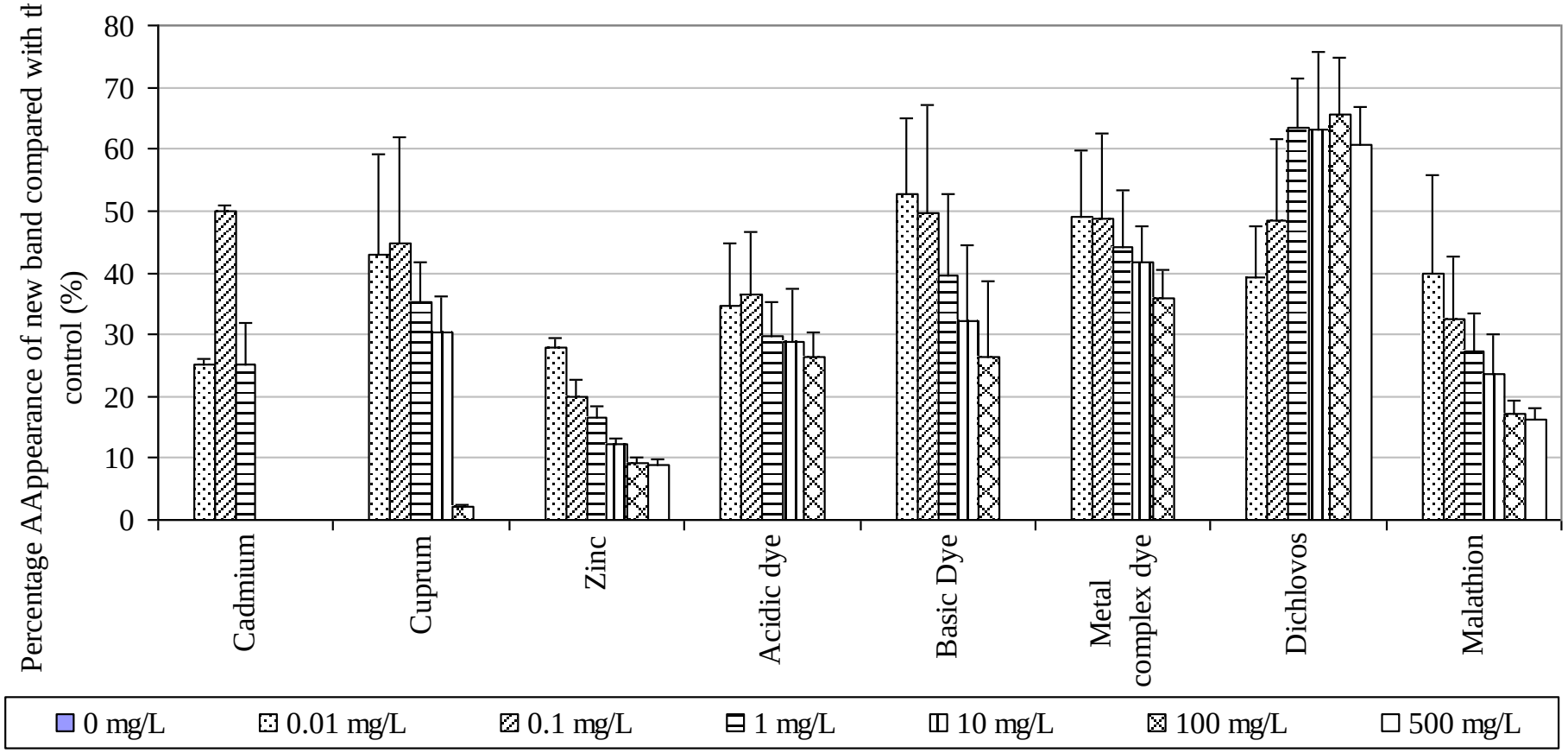


Figure 4.75: Variation of appearance of new bands in comparison to the control (set as 100%) in RAPD profiles of *Ventricaria ventricosa* exposed to different concentrations of selected toxicant for four days

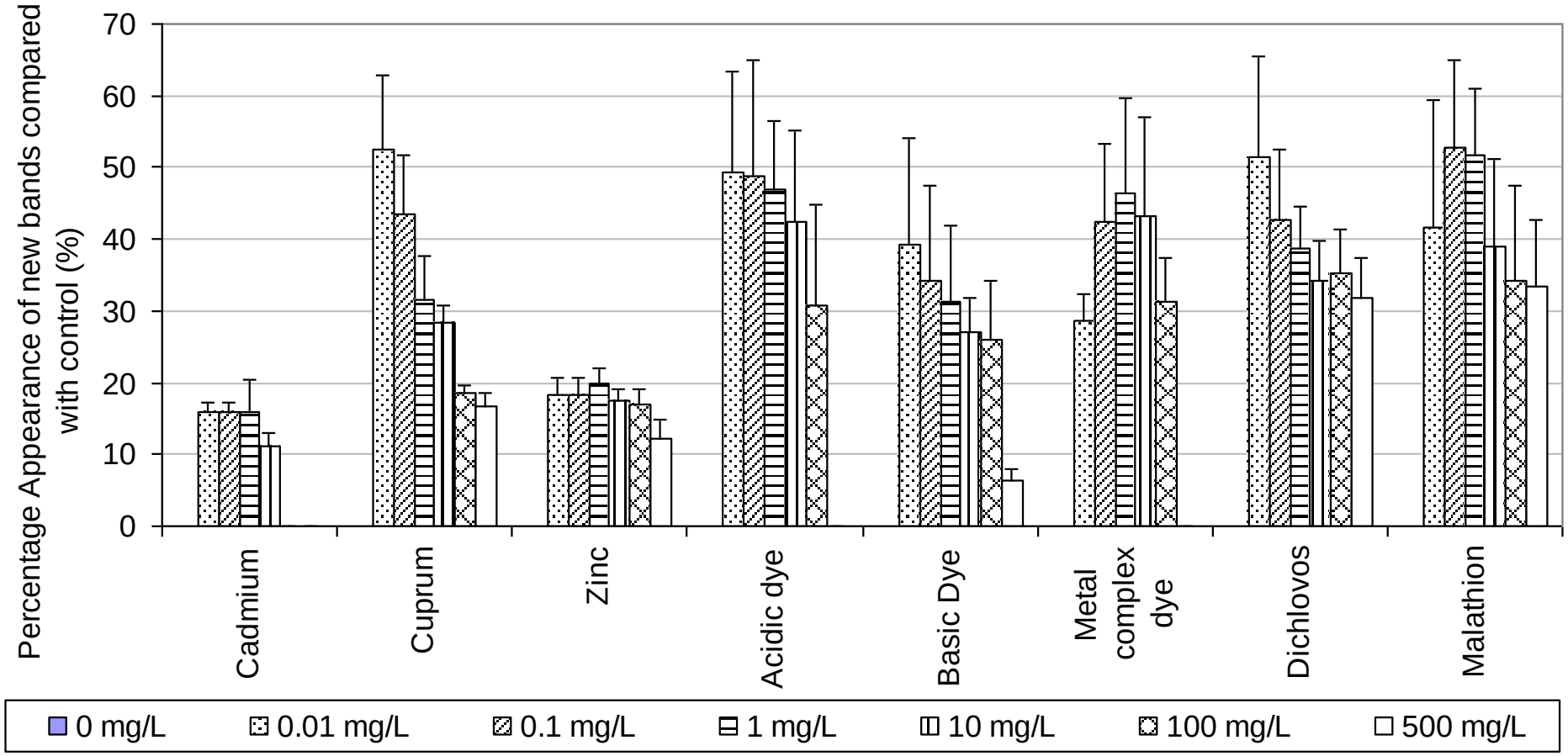


Figure 4.76: Variation of appearance of new bands in comparison to the control (set as 100%) in RAPD profiles of *Ventricaria ventricosa* exposed to different concentrations of selected toxicant for ten days

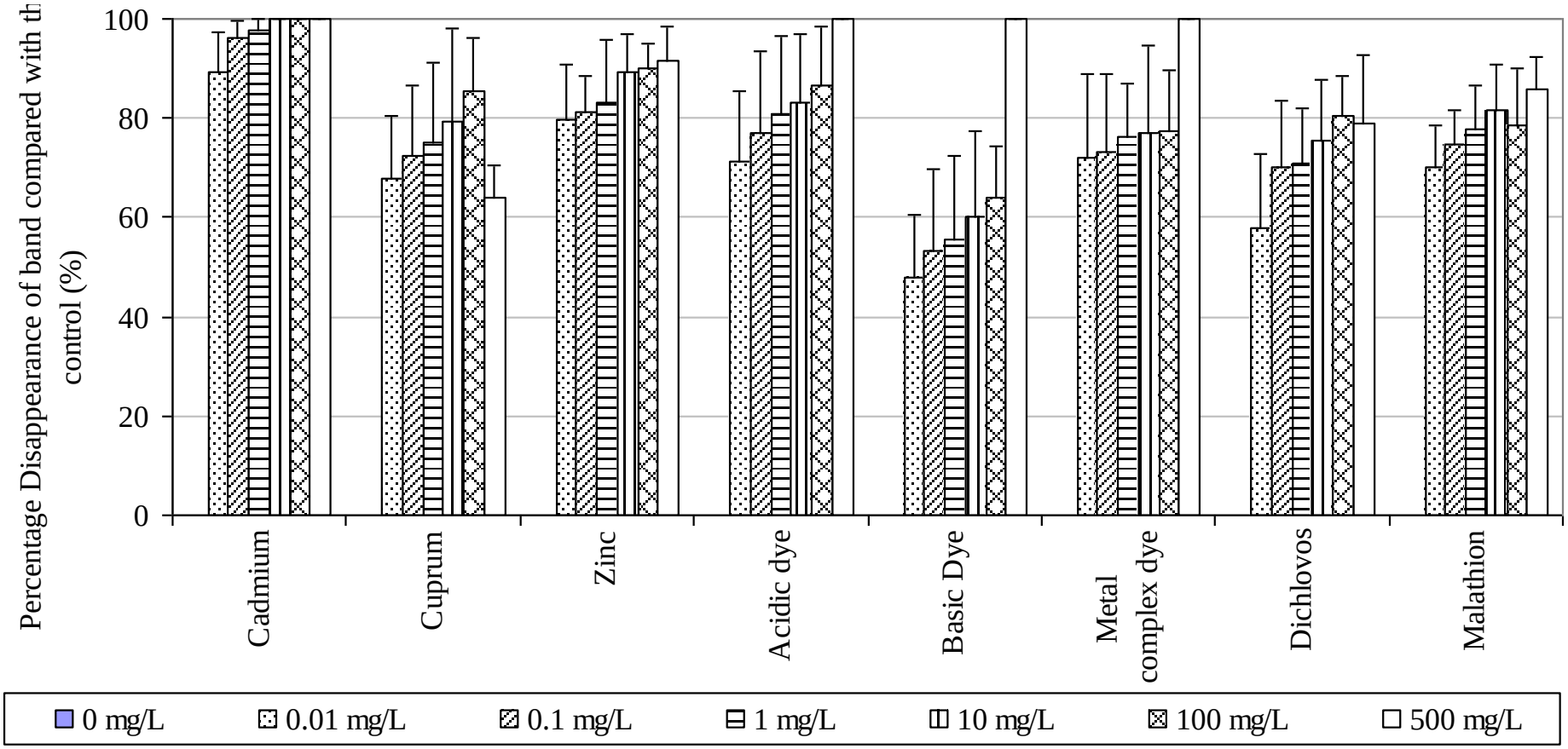


Figure 4.77: Variation of disappearance of bands in comparison to the control (set as 100%) in RAPD profiles of *Ventricaria ventricosa* exposed to different concentrations of selected toxicant for four days

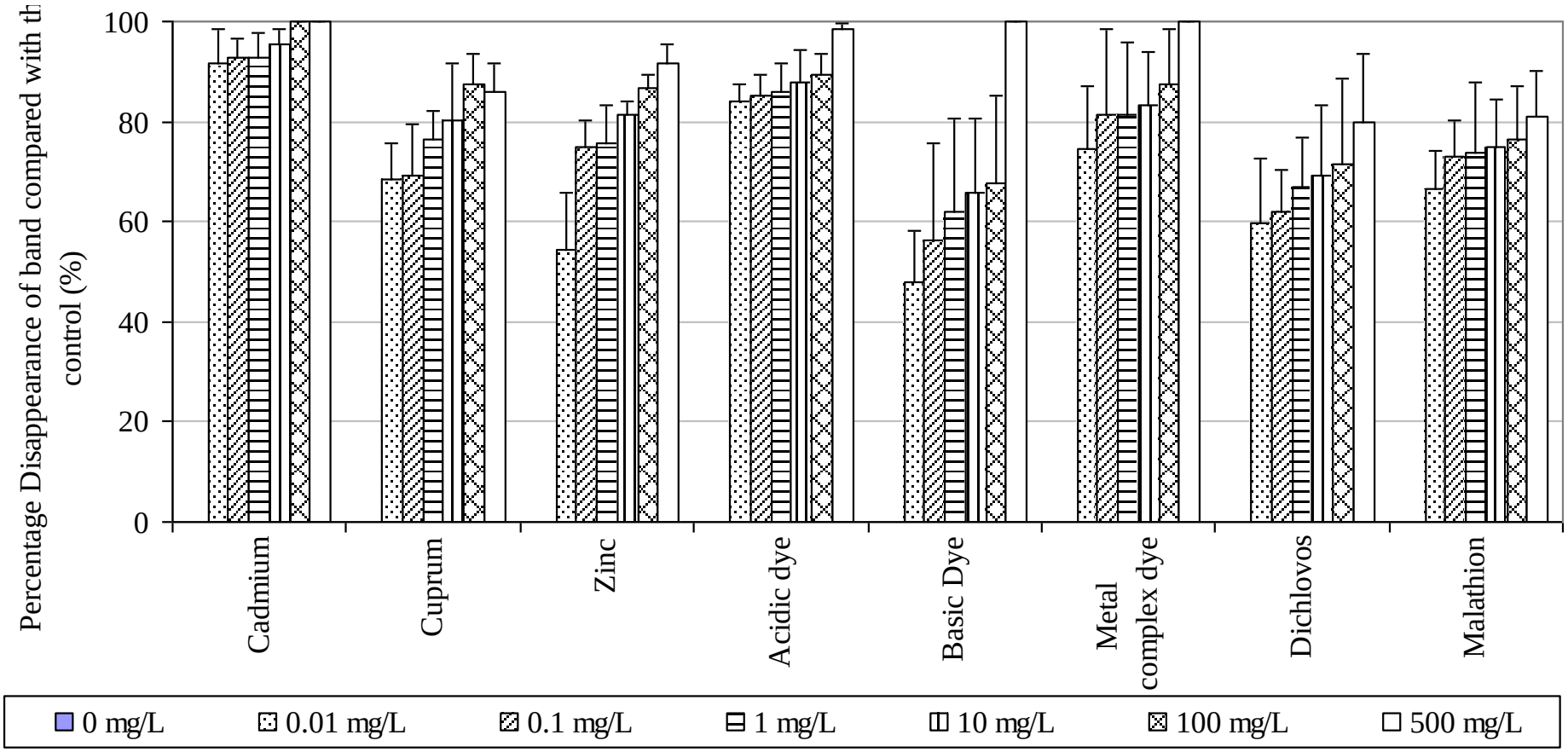


Figure 4.78: Variation of disappearance of bands in comparison to the control (set as 100%) in RAPD profiles of *Ventricaria ventricosa* exposed to different concentrations of selected toxicant for ten days

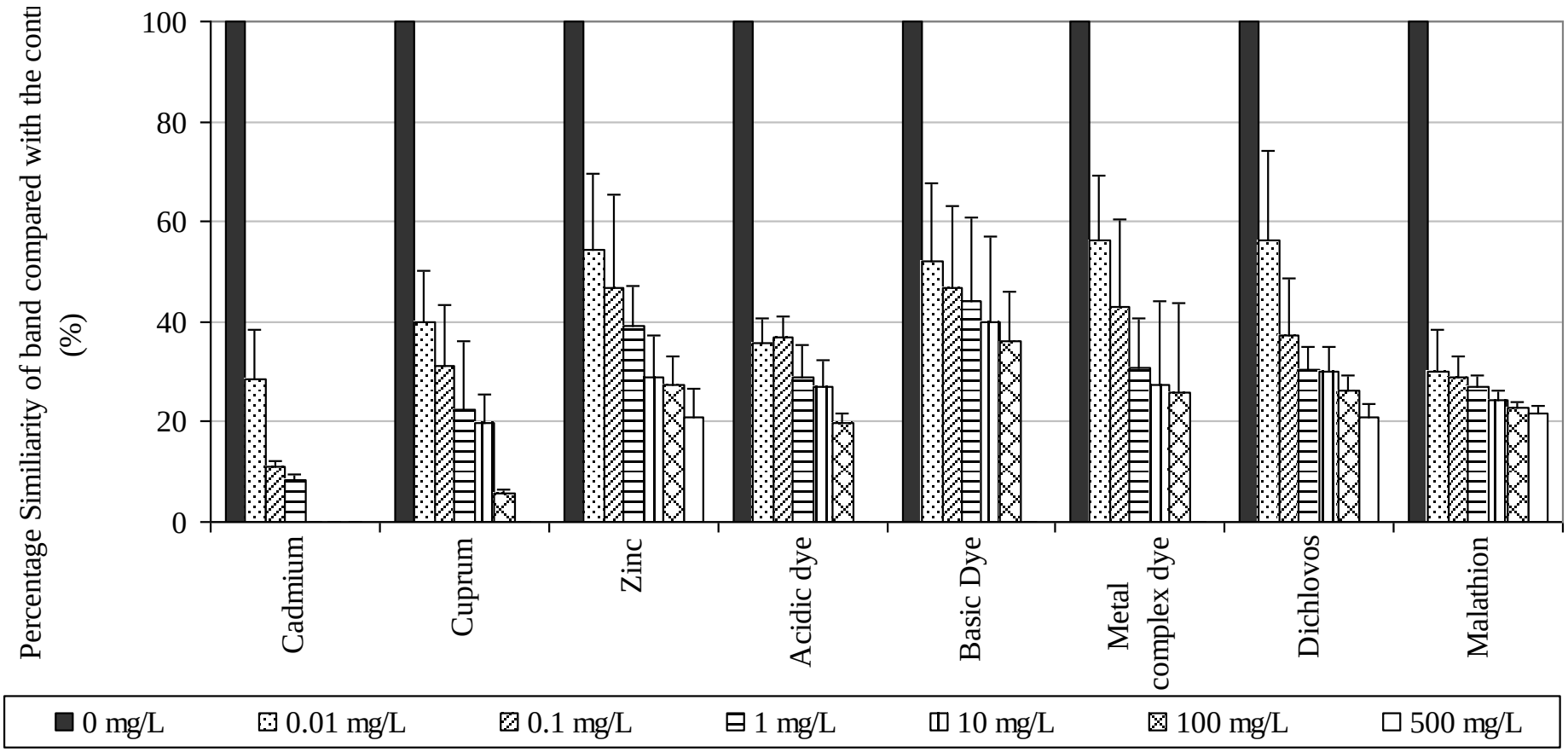


Figure 4.79: Variation of similiarity of bands in comparison to the control (set as 100%) in RAPD profiles of *Ventricaria ventricosa* exposed to different concentrations of selected toxicant for four days

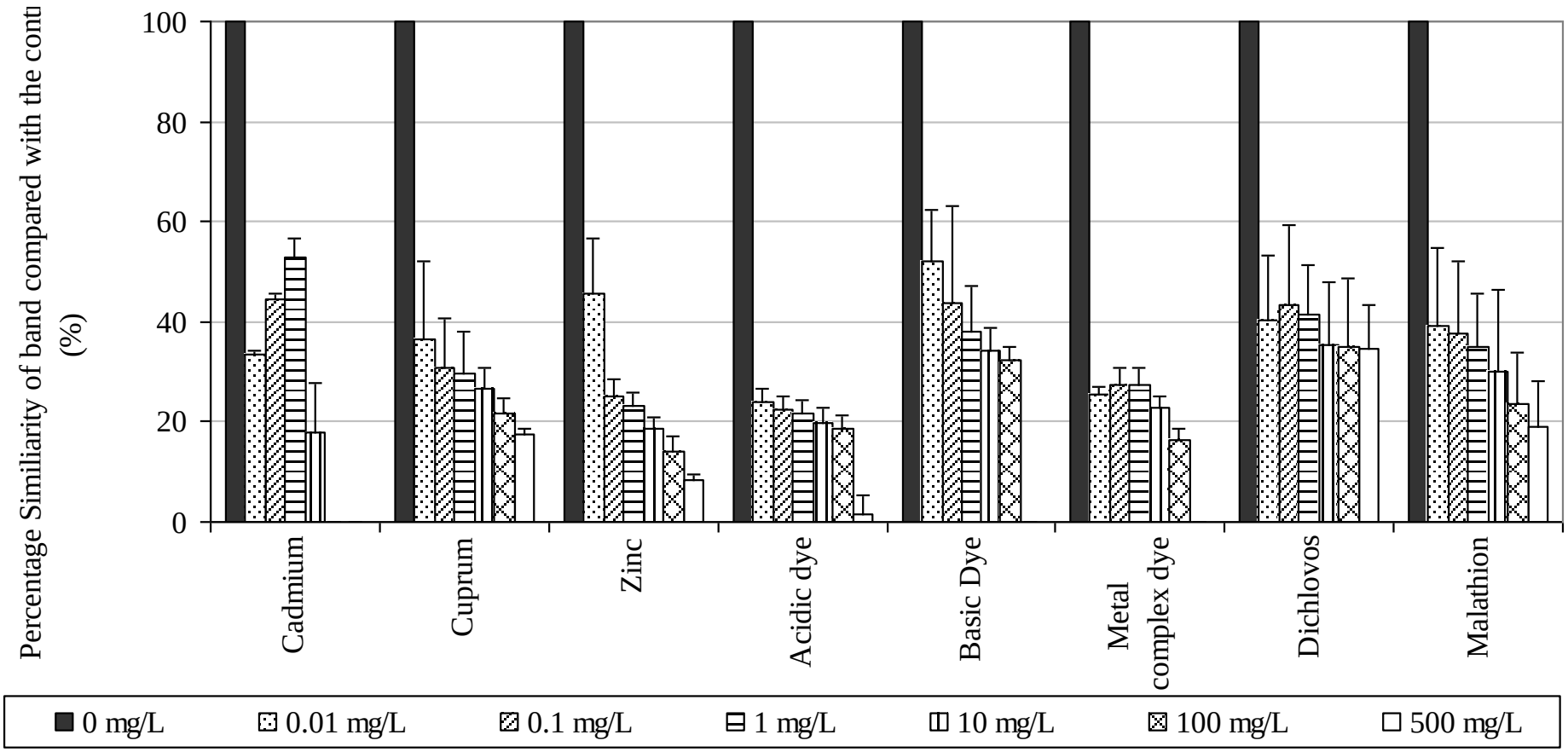


Figure 4.80: Variation of similiarity of bands in comparison to the control (set as 100%) in RAPD profiles of *Ventricaria ventricosa* exposed to different concentrations of selected toxicant for ten days

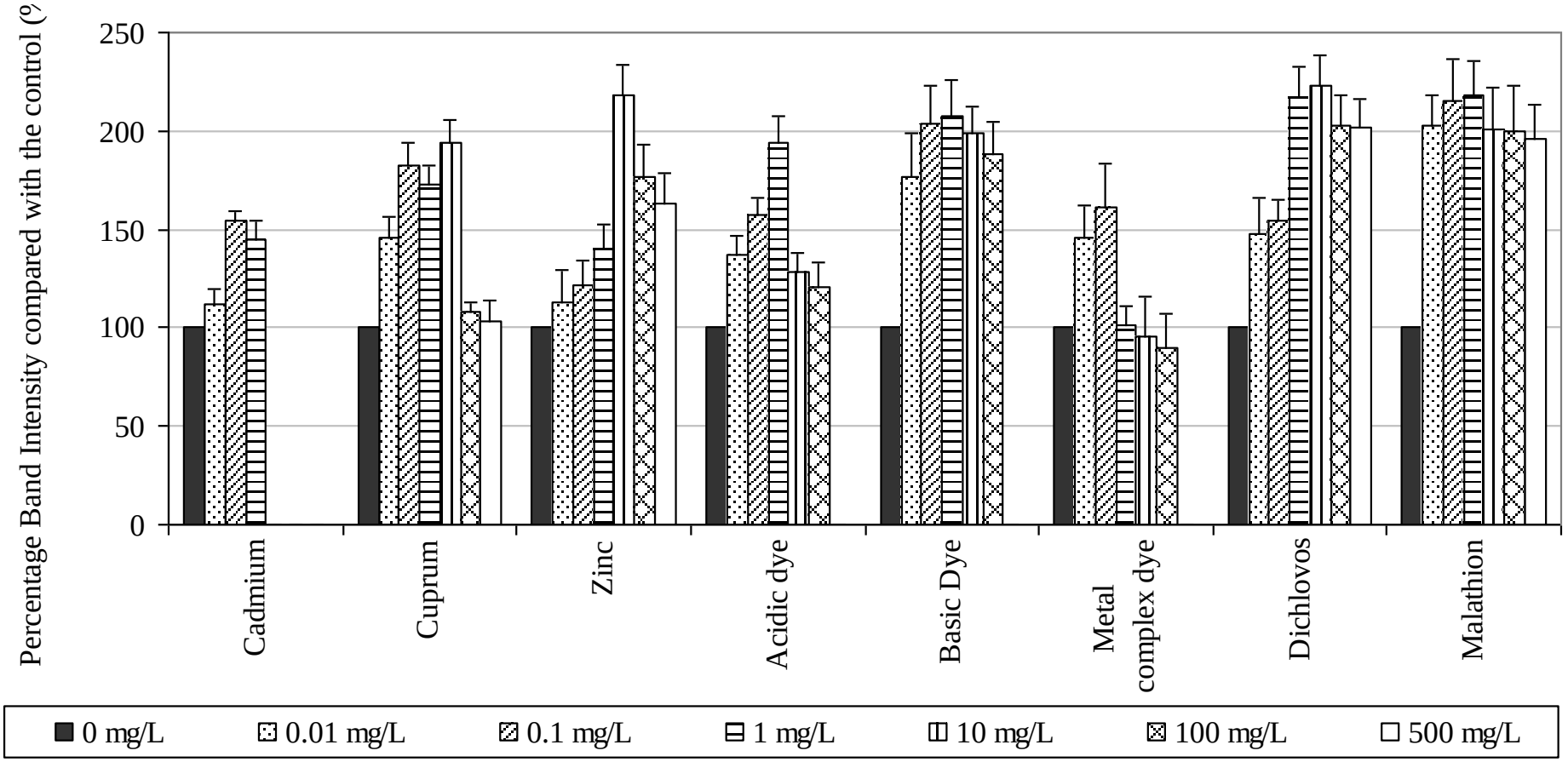


Figure 4.81: Variation of band intensity in comparison to the control (set as 100%) in RAPD profiles of *Ventricaria ventricosa* exposed to different concentrations of selected toxicant for four days

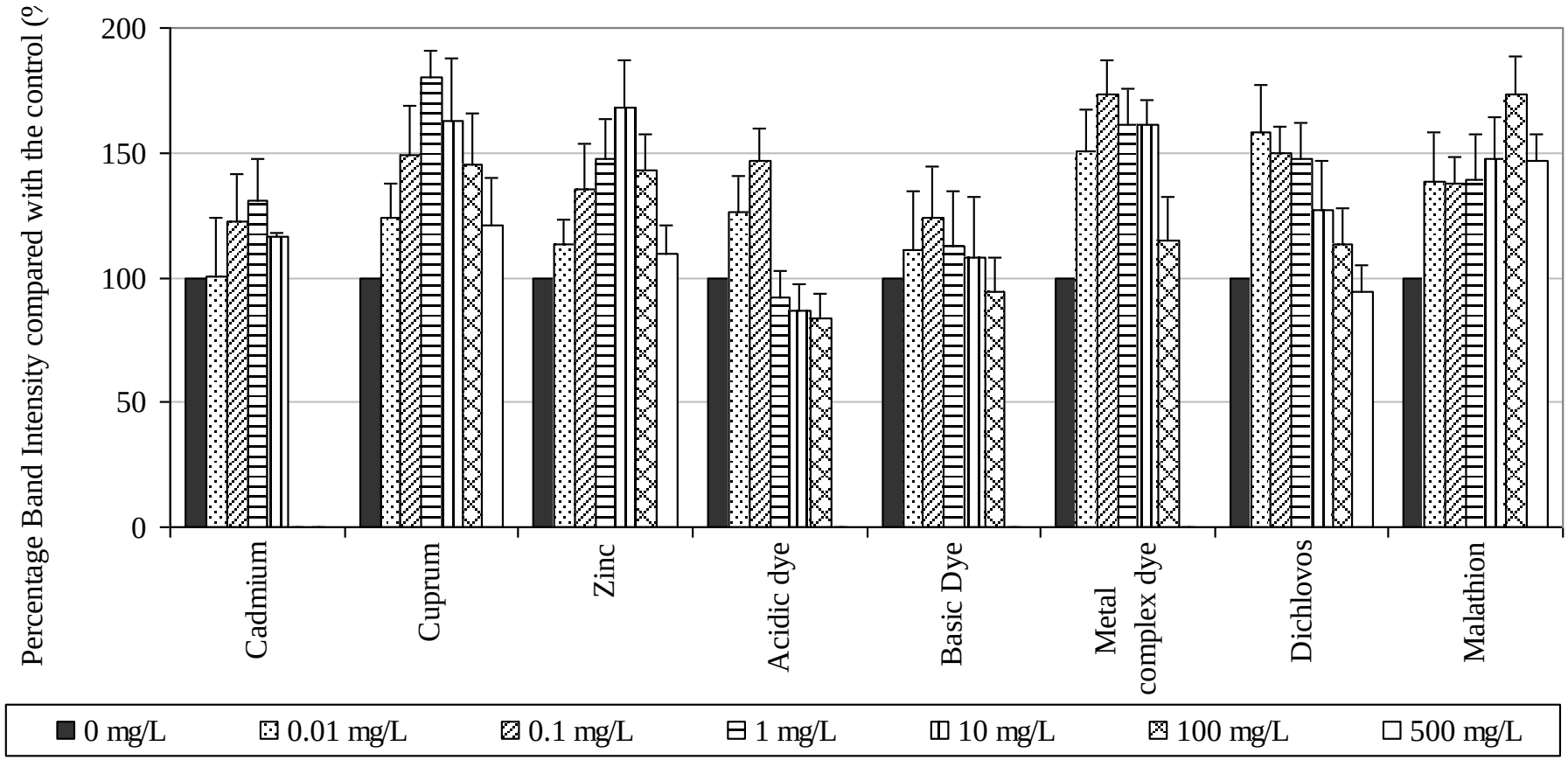


Figure 4.82: Variation of band intensity in comparison to the control (set as 100%) in RAPD profiles of *Ventricaria ventricosa* exposed to different concentrations of selected toxicant for ten days

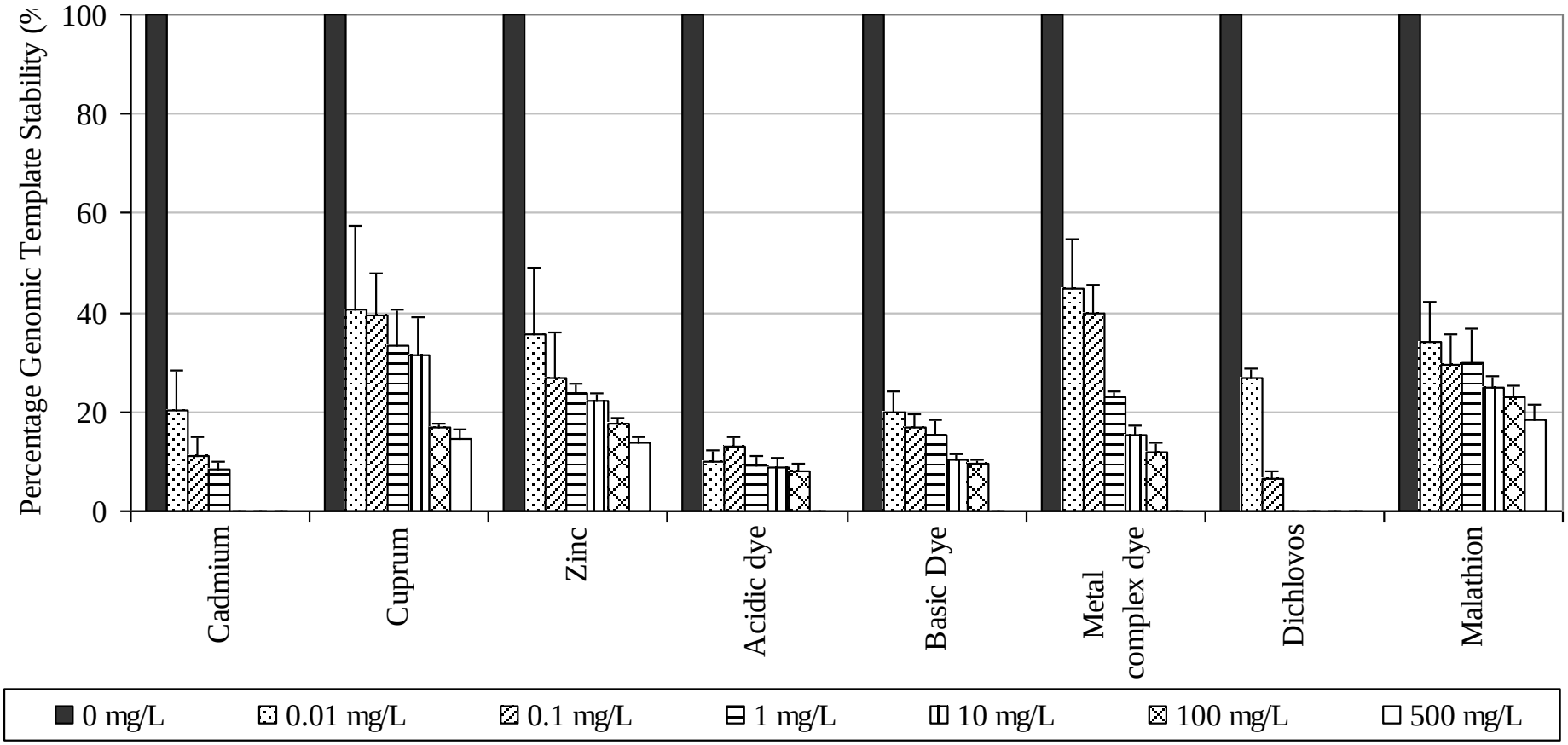


Figure 4.83: Genomic Template Stability of *Ventricaria ventricosa* after being exposed to different concentrations of selected toxicant for four days

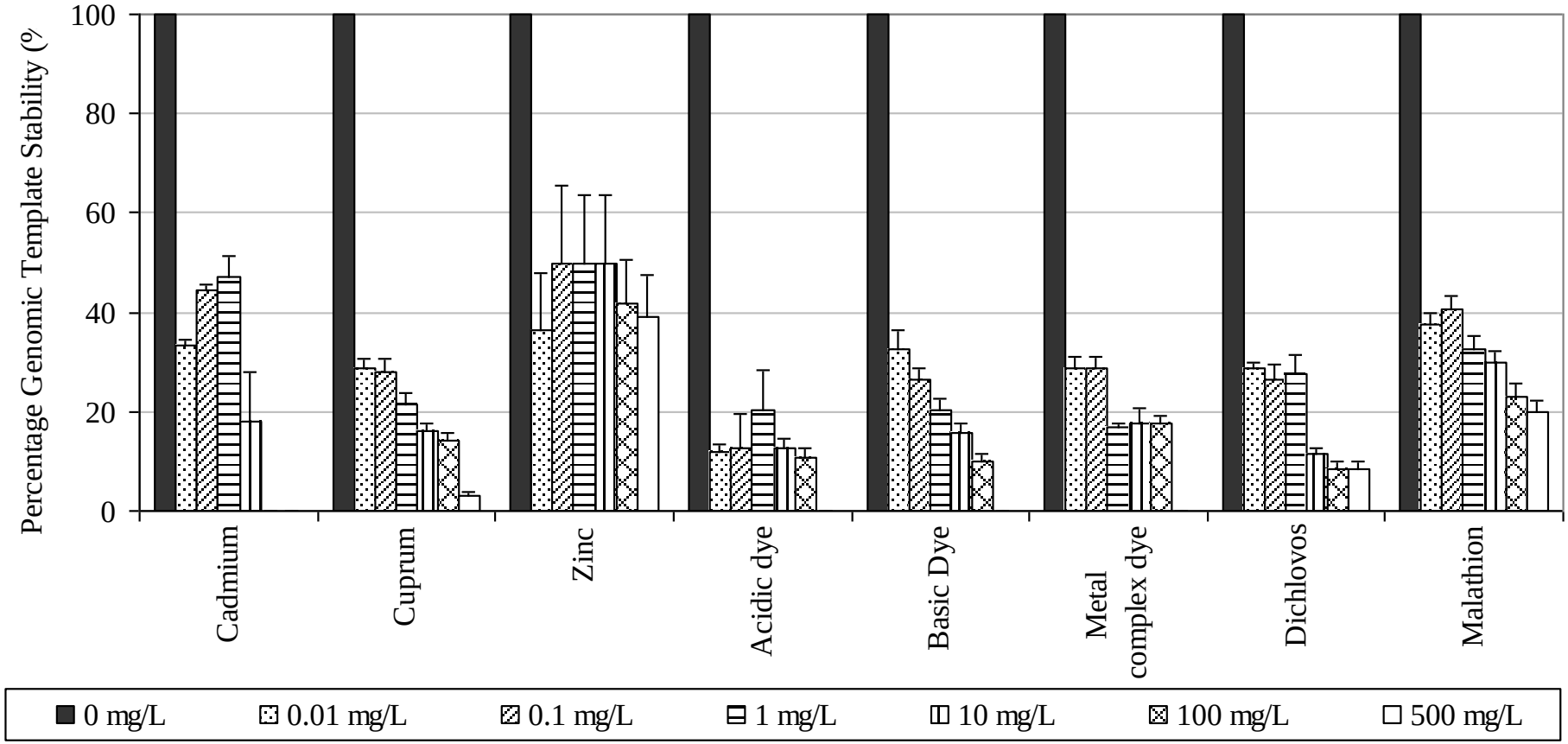


Figure 4.84: Genomic Template Stability of *Ventricaria ventricosa* after being exposed to different concentrations of selected toxicant for ten days

4.4 EFFECT OF SELECTED TOXICANTS ON DNA DAMAGE AND TOXICITY STUDIES: AP-SITE CONTENT

AP-Site (Abasic-site) counting analysis were carried out in *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa* to assess their DNA damage rate based on the quantity of AP- sites (Abasic-site) obtained in the cultures after exposure to two metals in a range of concentrations that represent the most toxic and the least toxic metal to each algae for four and ten days.

In general, the AP-site content in the cultures exposed to toxicant for four and ten days increased with increasing concentrations of toxicant up to 100 mg/L toxicant and then decreased at higher concentrations. Appendix 8, 13, 18 and 23 summarizes the detailed results of the study. The results also show that, all the AP-site content in the cultures exposed to toxicant have higher value compared with the control cultures. Statistical analysis using ANOVA showed there was significant difference ($p<0.05$) for all AP-site value in each concentration of toxicant tested compared with the control culture (Appendix 28, 33, 38 and 43).

4.4.1 AP-Site Content in *Chlorella vulgaris* UMACC 245 After Exposure to Iron and Manganese for Four and Ten Days

Figure 4.85 and 4.86 shows the AP-site value obtained in *Chlorella vulgaris* UMACC 245 cultures after exposure to Iron (Fe) and Manganese (Mn) for four and ten days. The results shows that the cultures grown in Prov50 medium without toxicant (control cultures) for four days, contained 23.678 ± 1.000 AP-site per $1x10^5$bp and in the

ten days cultures, the AP-site value increased nearly twice to 42.523±8.313 AP-site per 1 x 10^5bp.

After exposure to different concentrations of Fe for four and ten days (Fig. 4.85), the AP-site value increased with increasing concentrations of Fe up to 10 mg/L Fe and then decreased at 100 mg/L Fe. Iron was the most toxic metal to *Chlorella vulgaris* UMACC 245 and the results shows that the AP-site value markedly increased from 33.662±1.560 AP-site per 1x10^5bp to 48.034±2.003 AP-site per 1x10^5bp (at 10 mg/L Fe) and then reduced to 28.469±1.522 AP-site per 1x10^5bp in four days cultures ($p<0.05$). In the ten days cultures the AP-site value also increased from 42.891±4.780 AP-site per 1x10^5bp to 48.134±1.805 AP-site per 1x10^5bp (at 0.1 mg/L Fe) and then decreased to 42.791±0.769 AP-site per 1x10^5bp.

Different AP-site pattern were observed in the cultures exposed to Mn for four and ten days (Fig. 4.86). Based on the IC_{50} value results, Mn was the least toxic metal to *Chlorella vulgaris* UMACC 245. In the four days cultures the AP-site content in the cultures fluctuated from 31.233±5.420 AP-site per 1x10^5bp to 31.752±0.704 AP-site per 1x10^5bp between concentrations 0.01 mg/L Mn to 10 mg/L Mn and then slightly increased to 32.003±3.270 AP-site per 1x10^5bp. In spite of that, in the ten days cultures, the AP-site increased from 51.250±4.390 AP-site per 1 x 10^5bp to 53.176±0.630 AP-site per 1x10^5bp (at 10 mg/L Mn) and then reduced to 51.417±0.532 AP-site per 1x 10^5bp at 100 mg/L Mn. Statistical analysis shows that there were no significant difference in AP-site content in four and ten days cultures between concentrations 0.01 mg/L to 10 mg/L Mn ($p>0.05$).

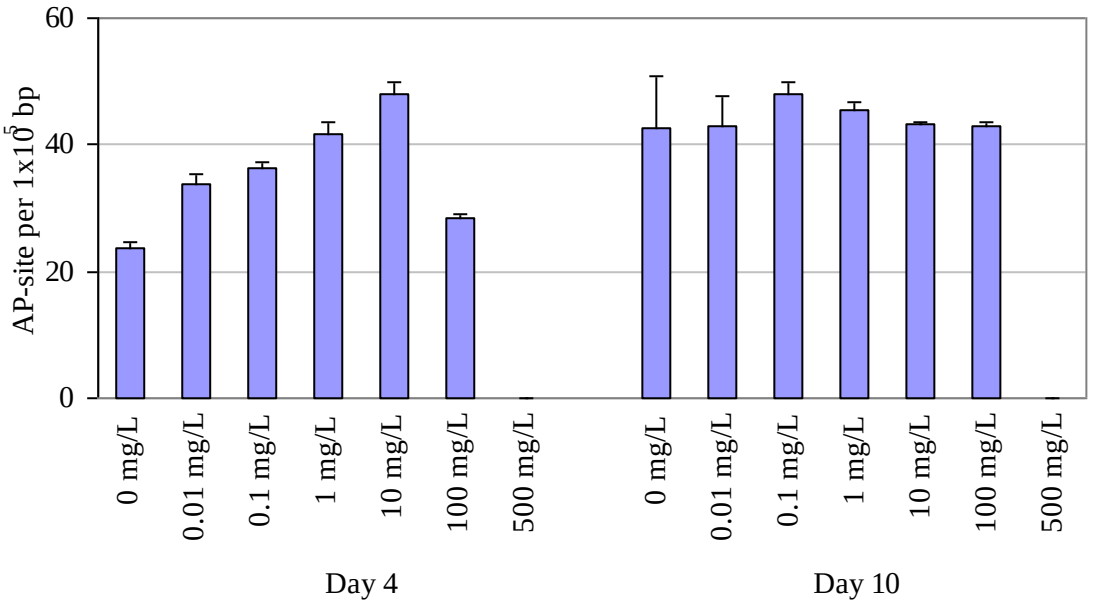


Figure 4.85: AP-site content in *Chlorella vulgaris* UMACC 245 after exposure to different concentrations of Ferum for four and ten days

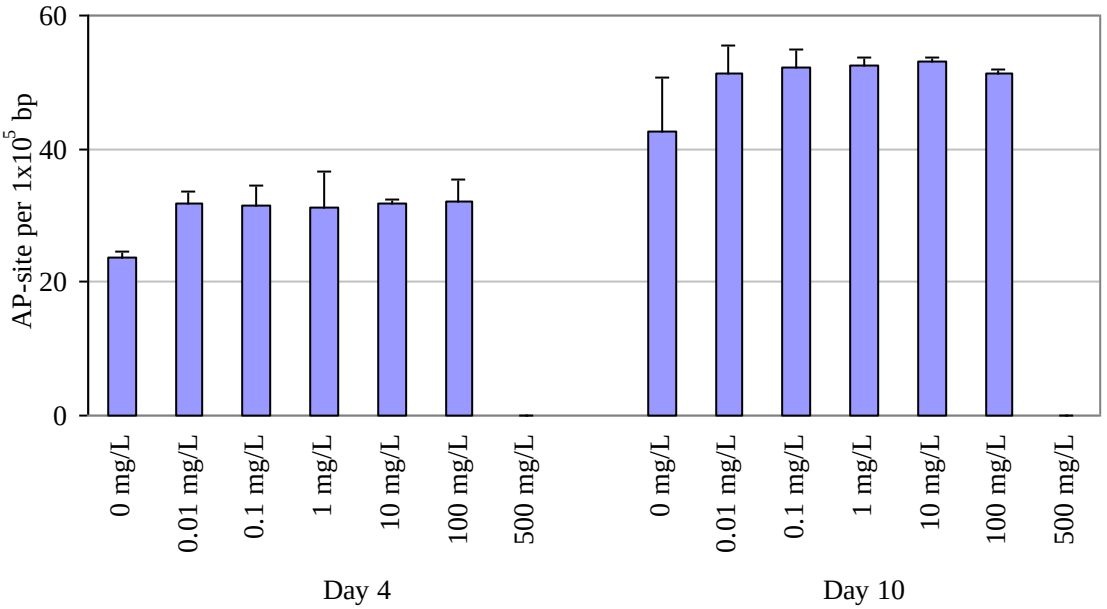


Figure 4.86: AP-site content in *Chlorella vulgaris* UMACC 245 after exposure to different concentrations of Manganase for four and ten days

4.4.2 AP-Site Content in *Tetraselmis tetrahele* UMACC 144 After Exposure to Copper and Chromium for Four and Ten Days

The AP-site value obtained in *Tetraselmis tetrahele* UMACC 144 cultures after exposure to Copper (Cu) and Chromium (Cr) for four and ten days is shown in Figure 4.87 and 4.88. The results show that the cultures grown in Prov50 medium without toxicant (control cultures) for four and ten days, contained 13.126±0.917 AP-site per $1x10^5$bp and 10.421±1.000 AP-site per 1 x 10^5bp respectively.

Copper was the most toxic metal to *Tetraselmis tetrahele* UMACC 144 and the result shows that after exposure to different concentrations of Cu for four days (Fig. 4.88), the AP-site value in the cultures significantly increased from 31.719±4.441 AP-site per $1x10^5$bp to 102.876±0.050 AP-site per $1x10^5$bp ($p<0.05$). However in the ten days cultures, the AP-site content markedly increased from 26.007±1.229 AP-site per $1x10^5$bp to 93.209±2.004 AP-site per $1x10^5$bp (at 1 mg/L Cu) and then reduced to 17.447±4.047 AP-site per $1x10^5$bp ($p<0.05$).

In the cultures exposed to Cr (least toxic metal to *Tetraselmis tetrahele* UMACC 144) for four and ten days, different AP-site content pattern were observed (Fig. 4.87). The AP-site content in the four days cultures significantly decreased with increasing concentrations of Cr where the AP-site content reduced from 25.169±2.698 AP-site per $1x10^5$bp to 4.131±0.362 AP-site per $1x10^5$bp ($p<0.05$). Despite that, in ten days cultures, the AP-site content increased from 18.503±1.053 AP-site per 1 x 10^5bp to 22.573±3.005 AP-site per $1x10^5$bp (at 1 mg/L Cr) and then markedly reduced to 7.832±0.805 AP-site per 1x 10^5bp ($p<0.05$).

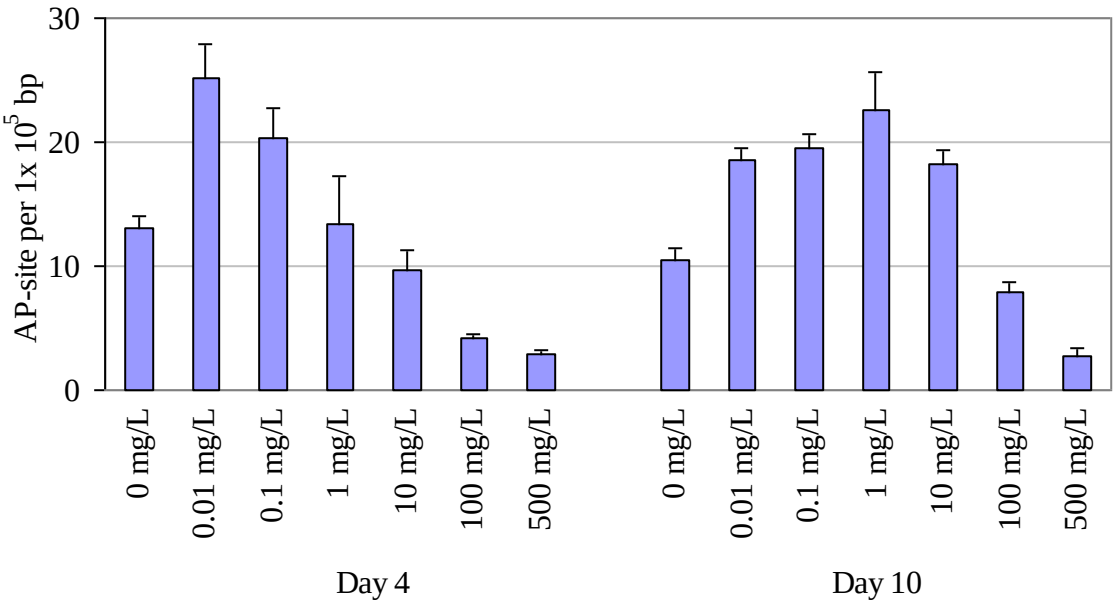


Figure 4.87: AP-site content in *Tetraselmis tetrahele* UMACC 144 after exposure to different concentrations of Chromium for four and ten days

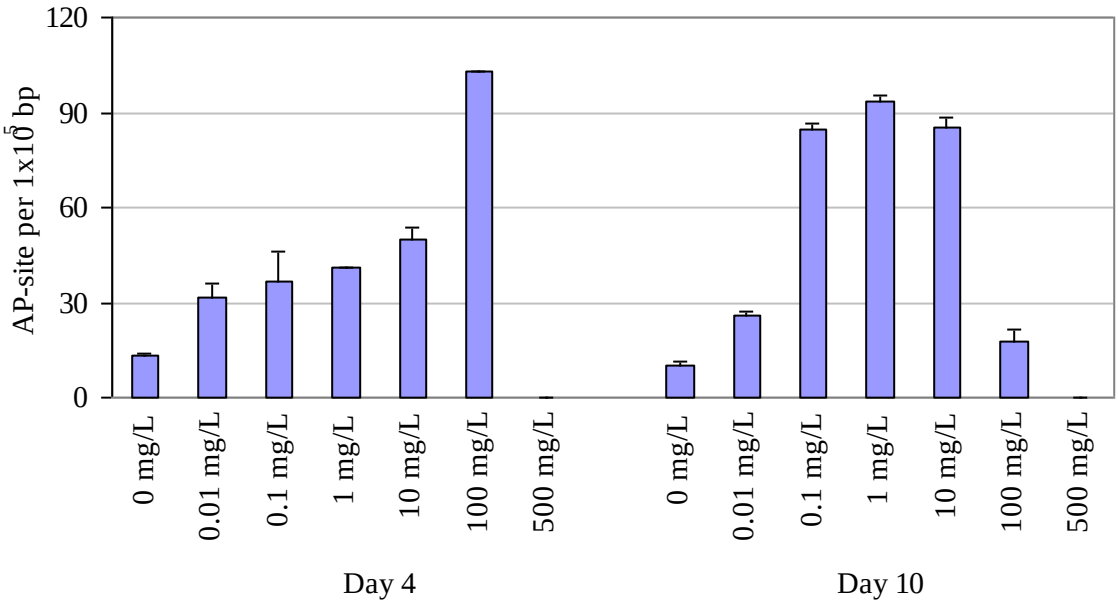


Figure 4.88: AP-site content in *Tetraselmis tetrahele* UMACC 144 after exposure to different concentrations of Copper for four and ten days

4.4.3 AP-Site Content in *Boergesenia forbesii* After Exposure to Copper and Zinc for Four and Ten Days

Figure 4.89 and 4.90 shows the AP-site content in *Boergesenia forbesii* cultures after been exposed to Copper and Zinc for four and ten days. In general, the cultures grown in Prov50 medium without toxicant (control cultures) for four and ten days, contained 132.489±2.863 AP-site per $1x10^5$bp and 158.921±4.591 AP-site per 1 x 10^5bp respectively.

Copper was the most toxic metal to *Boergesenia forbesii* and after exposure to different concentrations of Cu for four days, the AP-site content in the cultures fluctuated between 182.121±3.846 AP-site per 1 x 10^5bp to 189.792±2.004 AP-site per 1 x 10^5bp (at 10 mg/L Cu) and then significantly decreased to 174.516±2.335 AP-site per 1 x 10^5bp. In the ten days cultures, the AP-site content increased from 170.06±7.632 AP-site per 1 x 10^5bp to 184.985±1.889 AP-site per 1 x 10^5bp (at 10 mg/L Cu) and then markedly decreased to 174.131±3.932 AP-site per 1 x 10^5bp (Fig. 4.89). Statistical analysis shows that there were no were no significant difference in AP-site content in four and ten days cultures between concentrations 0.01 mg/L to 10 mg/L Cu ($p>0.05$).

For the cultures exposed to Zn (least toxic metals to *Boergesenia forbesii),* the AP-site content in the four days cultures significantly increased from 138.503±4.768 AP-site per $1x10^5$bp to 182.07±7.789 AP-site per $1x10^5$bp (at 100 mg/L Zn, $p<0.05$). In the cultures exposed to Zn for ten days, the AP-site content markedly increased from 115.085±2.804 AP-site per $1x10^5$bp to 192.137±3.694 AP-site per $1x10^5$bp (at 10 mg/L Zn) and then reduced to 113.578±2.646 AP-site per $1x10^5$bp ($p<0.05$) (Fig. 4.90). The

results also show that, the AP-site content in the ten days cultures were lower than in the ten days cultures.

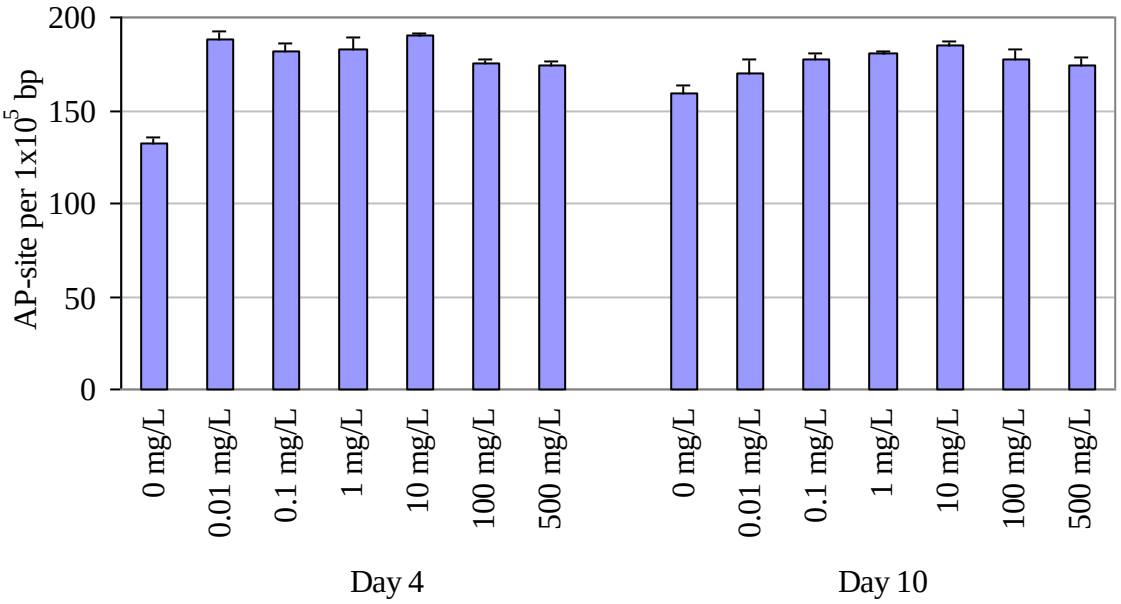


Figure 4.89: AP-site content in *Boergesenia forbesii* after exposure to different concentrations of Copper for four and ten days

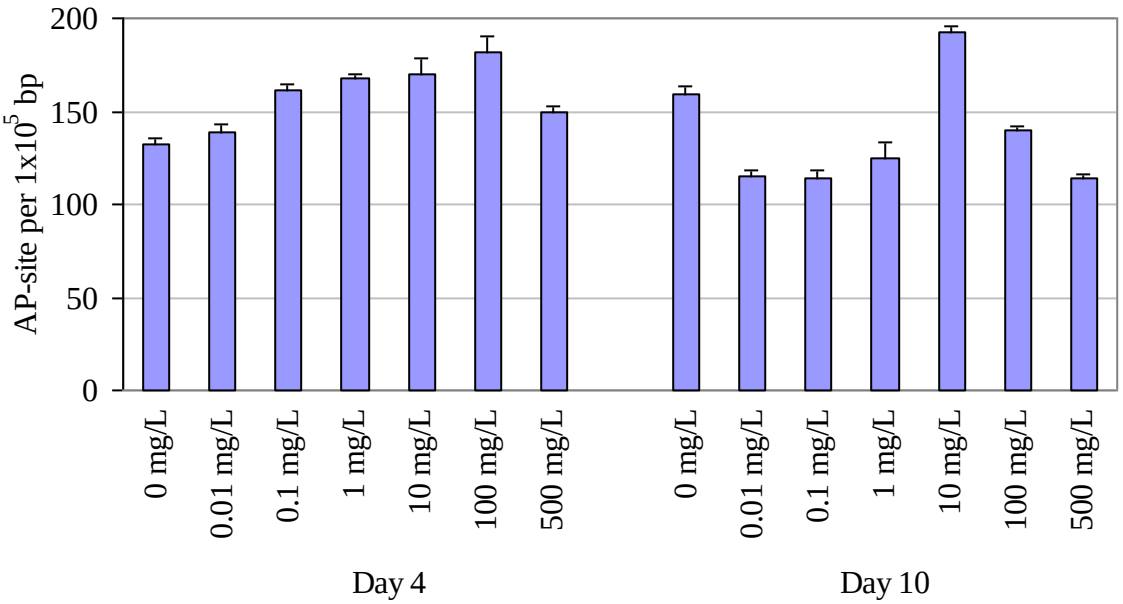


Figure 4.90: AP-site content in *Boergesenia forbesii* after exposure to different concentrations of Zinc for four and ten days

4.4.4 AP-Site Content in *Ventricaria ventricosa* After Exposure to Copper and Zinc for Four and Ten Days

Figure 4.91 and 4.92 shows the AP-site value obtained in *Ventricaria ventricosa* cultures after exposure to Copper and Zinc for four and ten days. The results show that the cultures grown in Prov50 medium without toxicant (control cultures) for four days, contained 3.022±1.000 AP-site per $1x10^5$bp and in the ten days cultures, the AP-site value increased to 4.030±1.000 AP-site per 1 x 10^5bp.

After being exposed to different concentrations of Cu (most toxic metals to *Ventricaria ventricosa*) for four and ten days (Fig. 4.91), the AP-site content in the cultures increased with increasing concentrations of Cu where the AP-site content increased from 3.863±1.693 AP-site per 1 x 10^5bp to 8.302±1.523 AP-site per $1x10^5$bp (at 100 mg/L Cu) in four days cultures. In the ten days cultures, the AP-site value markedly increased from 4.516±1.727 AP-site per $1x10^5$bp to 19.943±2.903 AP-site per 1 x 10^5bp ($p<0.05$).

However, in the cultures exposed to Zn (least toxic metals to *Ventricaria ventricosa*) for four and ten days (Fig. 4.92), different pattern of AP-site content were observed. The AP-site value markedly increased from 6.978±1.143 AP-site per $1x10^5$bp to 8.000±1.293 AP-site per $1x10^5$bp (at 1 mg/L Zn) and then reduced to 2.523±1.522 AP-site per $1x10^5$bp ($p<0.05$) in four days cultures. In the ten days cultures, the AP-site value also increased from 4.707±1.286 AP-site per $1x10^5$bp to 8.771±1.644 AP-site per $1x10^5$bp (at 1 mg/L Zn) and then decreased to 8.538±1.699 AP-site per $1x10^5$bp.

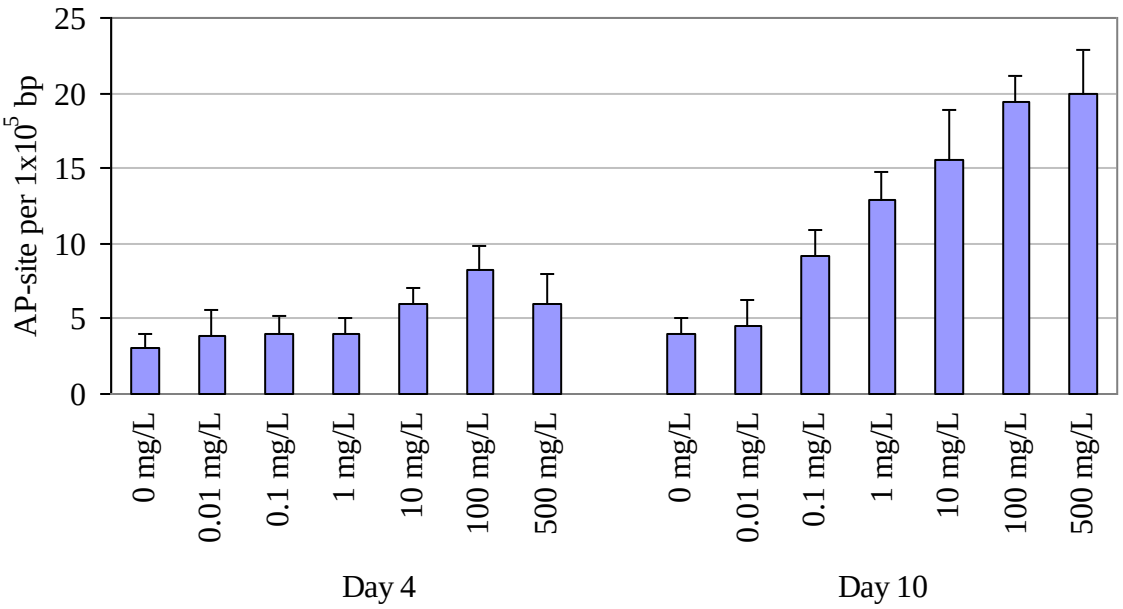


Figure 4.91: AP-site content in *Ventricaria ventricosa* after exposure to different concentrations of Copper for four and ten days

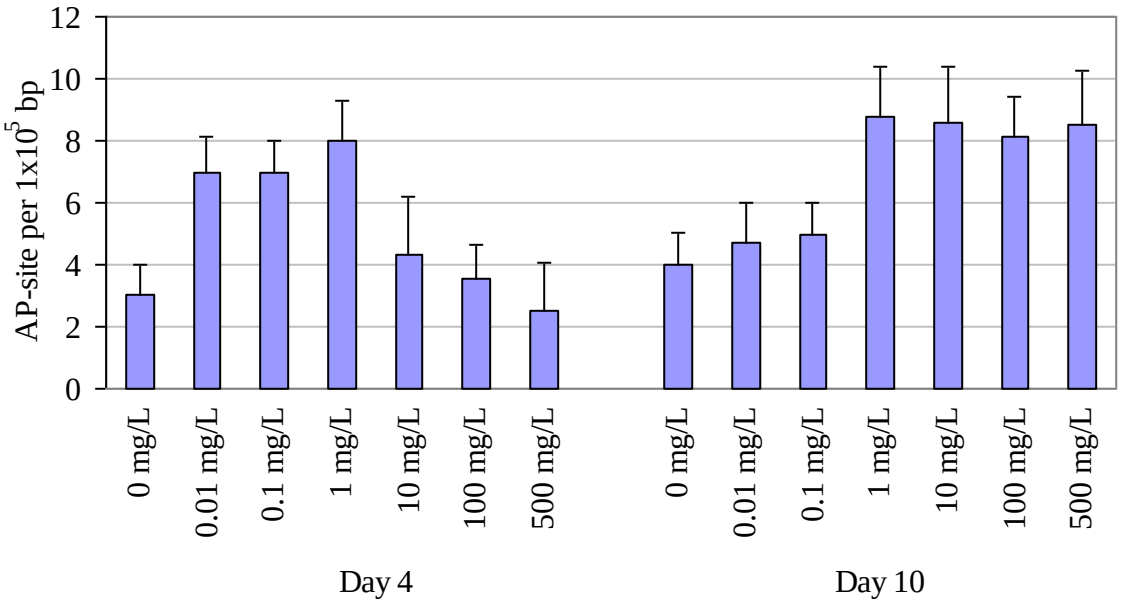


Figure 4.92: AP-site content in *Ventricaria ventricosa* after exposure to different concentrations of Zinc for four and ten days

4.5 SUPEROXIDE DISMUTASE (SOD) ACTIVITY IN ALGAE EXPOSED TO SELECTED TOXICANTS

The effects of selected metals including Cadmium (as reference metal) and two metals (that represent the most toxic and the least toxic metal to each algae), three textile dyes [Supranol Br. Red 3Bur (Acidic dye); Astrazon Red FBL (Basic dye); Lanaset Red 2GA (Metal complex dye)] and two organophosphate pesticides [Malathion; Dichlovos (DDVP)] on Superoxide dismutase (SOD) activity in *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa,*was conducted. Each toxicant at different concentrations (0, 0.01, 0.1, 1, 10, 100 and 500 mg/L) were exposed individually to the algae for four and ten days. SOD activity in the harvested algae was determined and the detailed results of the SOD activity in the algae are shown in Appendix 9, 14, 19 and 24.

4.5.1 *Chlorella vulgaris* UMACC 245: Effects of Selected Toxicants on SOD Activity

Appendix 9 summarise the SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to eight toxicants for four and ten days. The SOD activities in the cultures grown in Prov50 medium without toxicant (control cultures) for four and ten days were 53.858$\pm$1.701 U/mg protein and 50.182$\pm$6.523 U/mg protein respectively.

In general, the SOD activity in the cultures that exposed to selected toxicant for four and ten days contained higher SOD activity compared with the SOD activity in control cultures. Statistical analysis using ANOVA showed there were significant

difference ($p<0.05$) for all SOD activity in each concentration of toxicant tested compared with the control culture (Appendix 29).

4.5.1.1 *Chlorella vulgaris* UMACC 245 exposed to Cadmium: SOD activity

Figure 4.93 show the SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Cadmium (Cd) for four and ten days. The results show that the SOD activity in four days cultures which were 3.79% to 108.79% higher than in control cultures, increased from 55.898±4.658 U/mg protein to 112.452±2.196 U/mg protein. In ten days cultures, the SOD activity also increased from 38.659±0.473 U/mg protein to 77.880±3.392 U/mg protein. Statistical analysis shows that there were no significant difference in SOD activity in four and ten days cultures between concentrations 0.01 mg/L to 1 mg/L Cd ($p<0.05$).

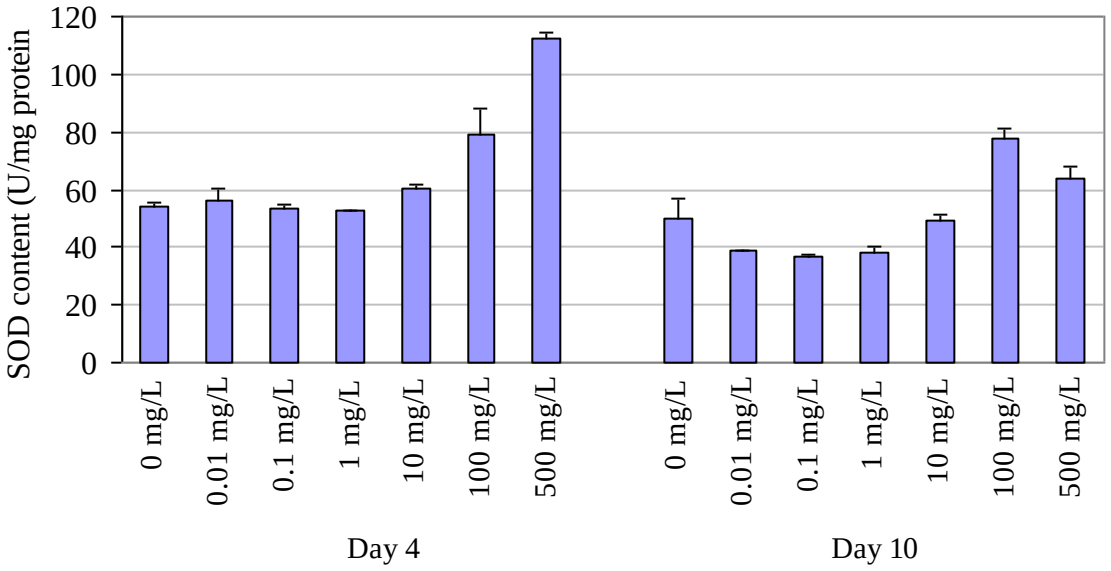


Figure 4.93: SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Cadmium for four and ten days

4.5.1.2 *Chlorella vulgaris* UMACC 245 exposed to Iron: SOD activity

The SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Iron (Fe) for four and ten days is shown in Fig 4.94. Fe was the most toxic metals to *Chlorella vulgaris* UMACC 245. The result shows that the SOD activity in four days cultures significantly increased from 49.952±1.428 U/mg protein to 73.859±5.677 U/mg protein (at 10 mg/L Fe) and then decreased to 45.849±1.723 U/mg protein ($p<0.05$). However in the ten days cultures, the SOD activity markedly decreased from 60.524±1.043 U/mg protein to 2.042±0.218 U/mg protein ($p<0.05$).

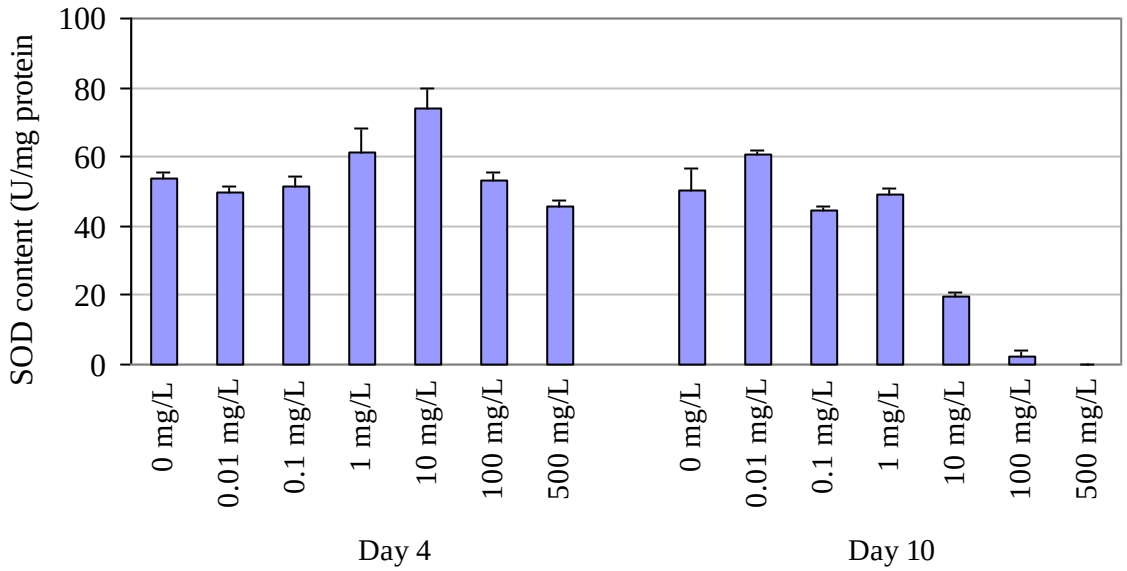


Figure 4.94: SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Iron for four and ten days

4.5.1.3 *Chlorella vulgaris* UMACC 245 exposed to Manganese: SOD activity

After being exposed to Manganese (Mn) which was the least toxic metals to *Chlorella vulgaris* UMACC 245, for four days, the SOD activity in the cultures (Fig 4.95) markedly increased from 51.447±4.558 U/mg protein to 74.032±2.480 U/mg protein (at 10 mg/L Mn) and then reduced to 64.593±3.132 U/mg protein ($p<0.05$). In spite of that, the SOD activity decreased from 52.617±1.877 U/mg protein to 1.295±0.204 U/mg protein in the ten days cultures. There were no significant difference in SOD activity in ten days cultures between concentrations 0.01 mg/L to 0.1 mg/L Mn ($p>0.05$).

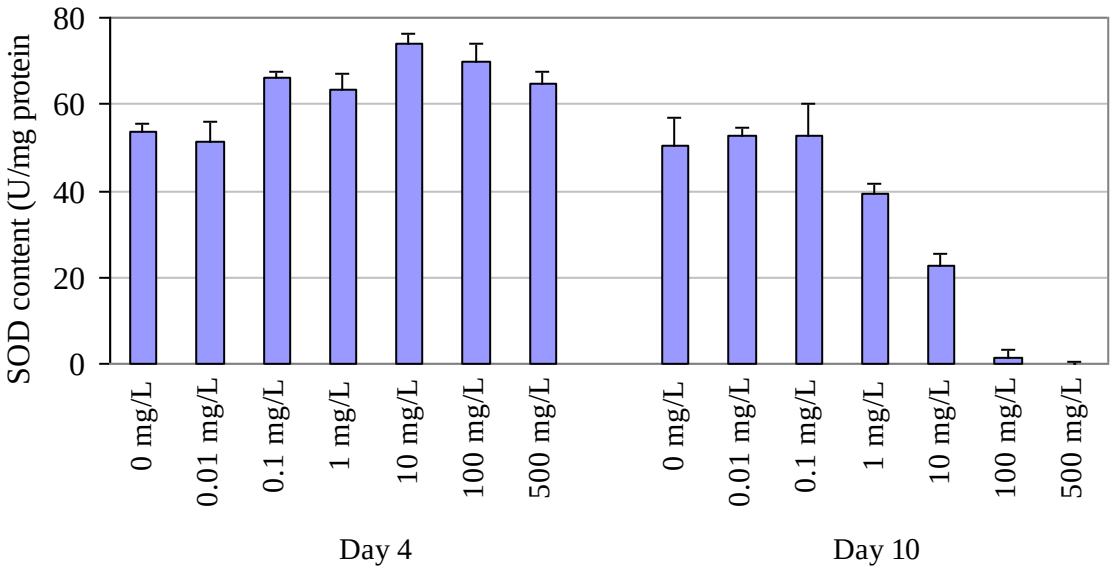


Figure 4.95: SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Manganese for four and ten days

4.5.1.4 *Chlorella vulgaris* UMACC 245 exposed to Acidic dye: SOD activity

Figure 4.96 shows the SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Supranol Br. Red 3Bur (Acidic dye) for four and ten days. The SOD activity in four days cultures significantly increased from 36.928±1.574 U/mg protein to 49.497±4.026 U/mg protein (at 1 mg/L Acidic dye) and then decreased to 64.593±3.132 U/mg protein ($p<0.05$). In ten days cultures, the SOD activity which was 2.64 to 2.83 times higher than in control cultures, also markedly increased from 136.070±1.422 U/mg protein to 142.398±2.854 U/mg protein (at 1 mg/L Acidic dye) and then reduced to 34.996±9.147 U/mg protein ($p<0.05$).

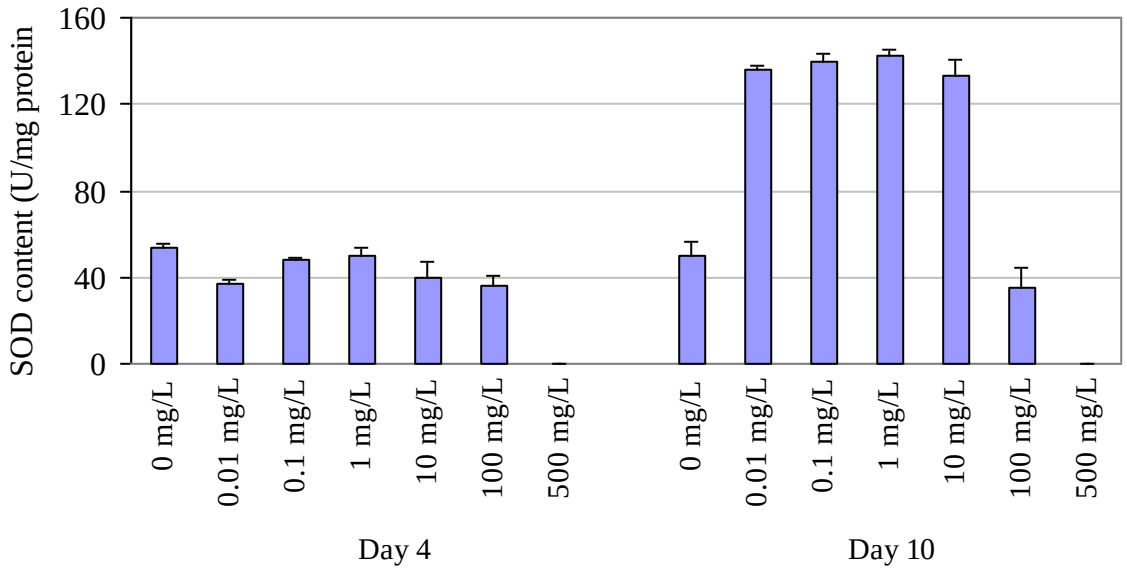


Figure 4.96: SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Acidic dye for four and ten days

4.5.1.5 *Chlorella vulgaris* UMACC 245 exposed to Basic dye: SOD activity

The SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Astrazon Red FBL (Basic dye) for four and ten days is shown in Fig 4.97. The result shows that the SOD activity in four days cultures markedly increased from 30.222±0.904 U/mg protein to 41.514±8.953 U/mg protein (at 10 mg/L Basic dye) and then decreased to 31.033±4.690 U/mg protein ($p<0.05$). In the ten days cultures, the SOD activity which was 1.06 to 3.66 times higher than in control cultures, also significantly increased from 107.009±1.207 U/mg protein to 184.084±5.090 U/mg protein (at 10 mg/L Basic dye) and then reduced to 53.206±1.138 U/mg protein ($p<0.05$).

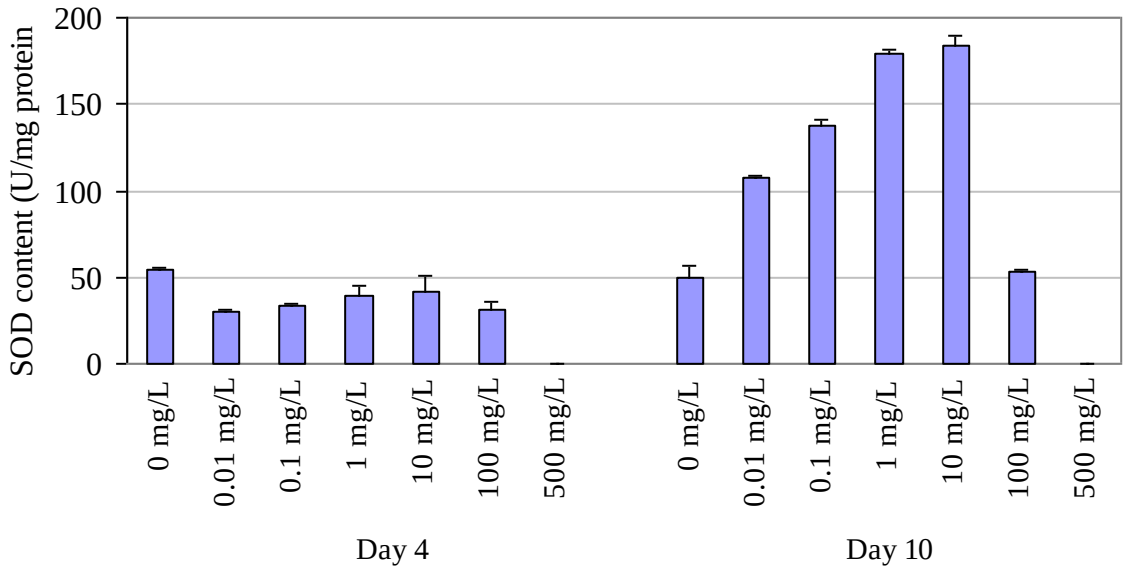


Figure 4.97: SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Basic dye for four and ten days

4.5.1.6 *Chlorella vulgaris* UMACC 245 after exposed to Metal complex dye: SOD activity

After being exposed to Lanaset Red 2GA (Metal complex dye) for four days, the SOD activity in *Chlorella vulgaris* UMACC 245 (Fig 4.98) significantly increased from 34.610±2.821 U/mg protein to 63.135±2.300 U/mg protein (at 10 mg/L Metal complex dye) and then decreased to 25.140±9.500 U/mg protein ($p<0.05$). The SOD activity markedly increased from 109.397±1.119 U/mg protein to 202.986±4.880 U/mg protein (at 1 mg/L Metal complex dye) and reduced to 105.928±3.594 U/mg protein ($p<0.05$). The results also shown that the SOD activities in ten days cultures were 2.11 to 4.04 times higher than in control cultures.

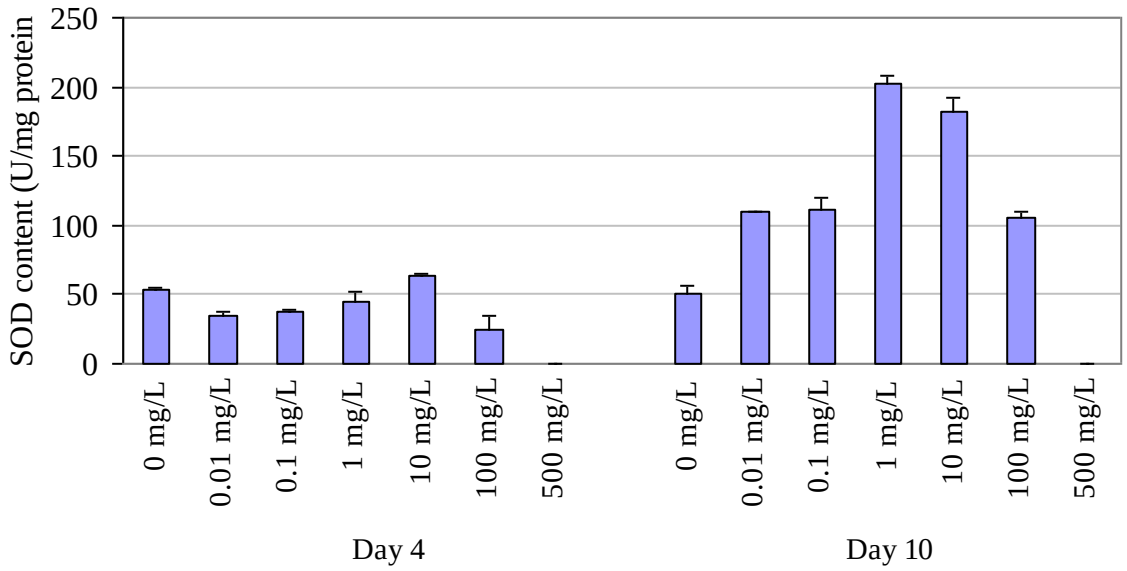


Figure 4.98: SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Metal complex dye for four and ten days

4.5.1.7 *Chlorella vulgaris* UMACC 245 after exposed to Dichlovos: SOD activity

Figure 4.99 shows the SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Dichlovos for four and ten days. The SOD activity in four days cultures markedly increased from 33.283±0.930 U/mg protein to 50.823±0.916 U/mg protein (at 10 mg/L Dichlovos) and then reduced to 23.355±1.034 U/mg protein ($p<0.05$). In ten days cultures, the SOD activity also significantly increased from 45.988±1.515 U/mg protein to 66.170±3.320 U/mg protein (at 0.1 mg/L Dichlovos) and then decreased to 40.326±0.950 U/mg protein ($p<0.05$).

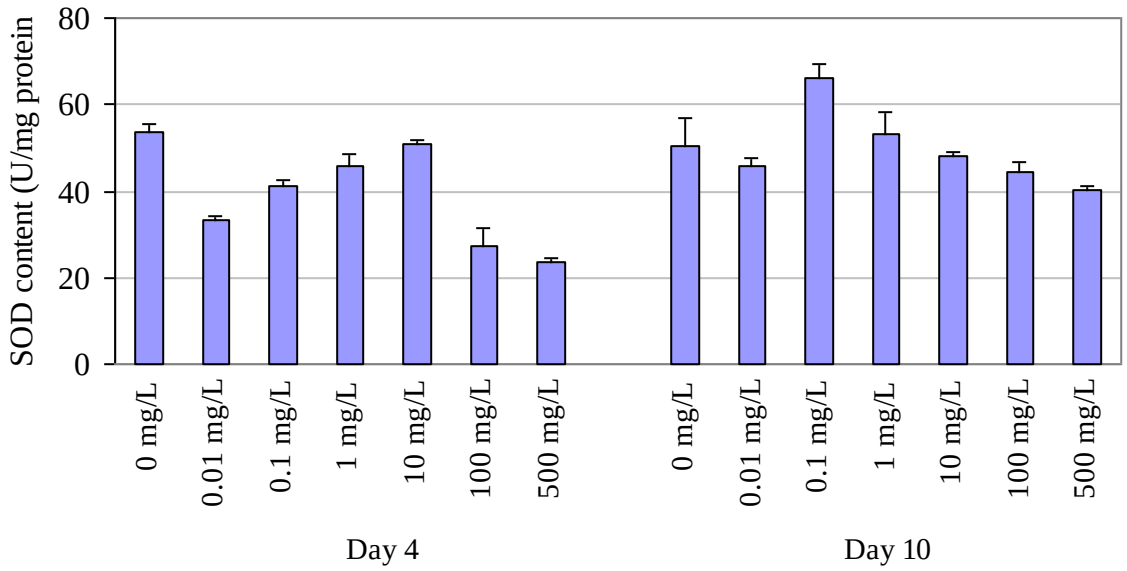


Figure 4.99: SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Dichlovos for four and ten days

4.5.1.8 *Chlorella vulgaris* UMACC 245 after exposed to Malathion: SOD activity

The SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Malathion for four and ten days is shown in Fig 4.100. The result shows that the SOD activity in four days cultures significantly increased from 41.756±6.543 U/mg protein to 55.237±4.249 U/mg protein (at 1 mg/L Malathion) and then reduced to 19.480±1.202 U/mg protein ($p<0.05$). In the ten days cultures, the SOD activity also markedly increased from 76.170±2.068 U/mg protein to 81.481±1.734 U/mg protein (at 0.1 mg/L Malathion) and then decreased to 26.577±2.216 U/mg protein ($p<0.05$).

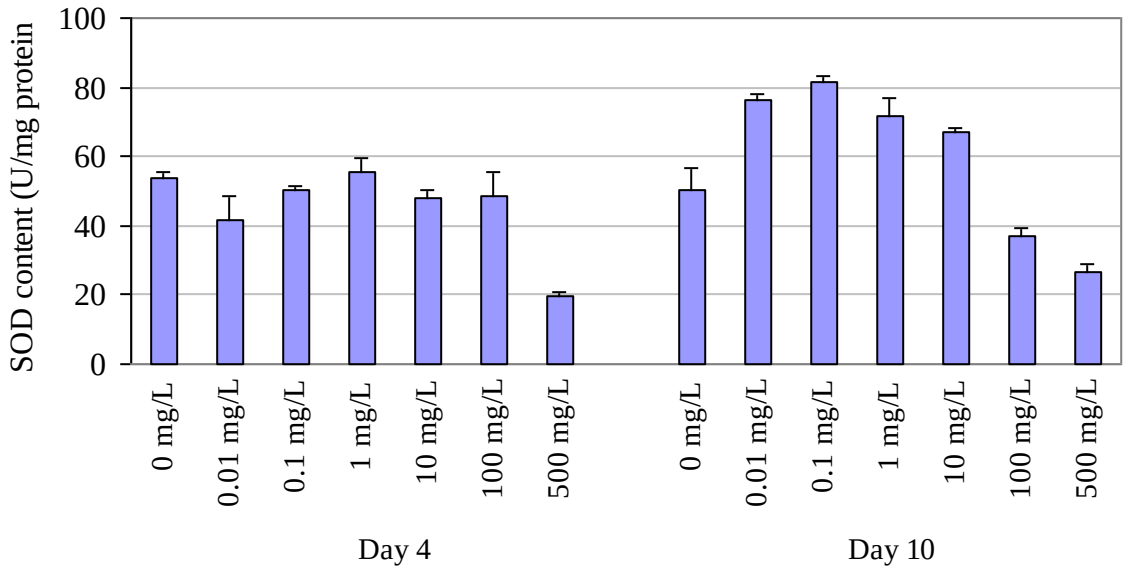


Figure 4.100: SOD activity in *Chlorella vulgaris* UMACC 245 after exposure to Malathion for four and ten days

4.5.2 *Tetraselmis tetrahele* UMACC 144: Effects of Selected Toxicants on SOD Activity

Appendix 14 summarizes the Superoxide dismutase (SOD) activity in *Tetraselmis tetrahele* UMACC 144 after exposure to selected toxicant for four and ten days. The SOD activities in the cultures grown in Prov50 medium without toxicants (control culture) for four and ten days were 45.763±1.000 U/mg protein and 36.192±0.913 U/mg protein respectively.

In general, the SOD activity in the cultures that exposed to selected toxicant for four and ten days contained higher SOD activity compared with the SOD activity in control cultures. Statistical analysis using ANOVA showed there were significant difference ($p<0.05$) for all SOD activity in each concentration of toxicant tested compared with the control culture (Appendix 34).

4.5.2.1 *Tetraselmis tetrahele* UMACC 144 exposed to Cadmium: SOD activity

Figure 4.101, shows the SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Cadmium (Cd) for four and ten days. The result shows that the SOD activity in four days cultures which were 1.43 to 2.13 times higher than in control cultures; increased from 65.453±4.502 U/mg protein to 97.910±0.929 U/mg protein. In ten days cultures, the SOD activity also increased from 40.675±1.367 U/mg protein to 68.729±1.575 U/mg protein. Statistical analysis shows that there were no significant difference in SOD activity in four days cultures between concentrations 0.01 mg/L to 10 mg/L Cd ($p<0.05$).

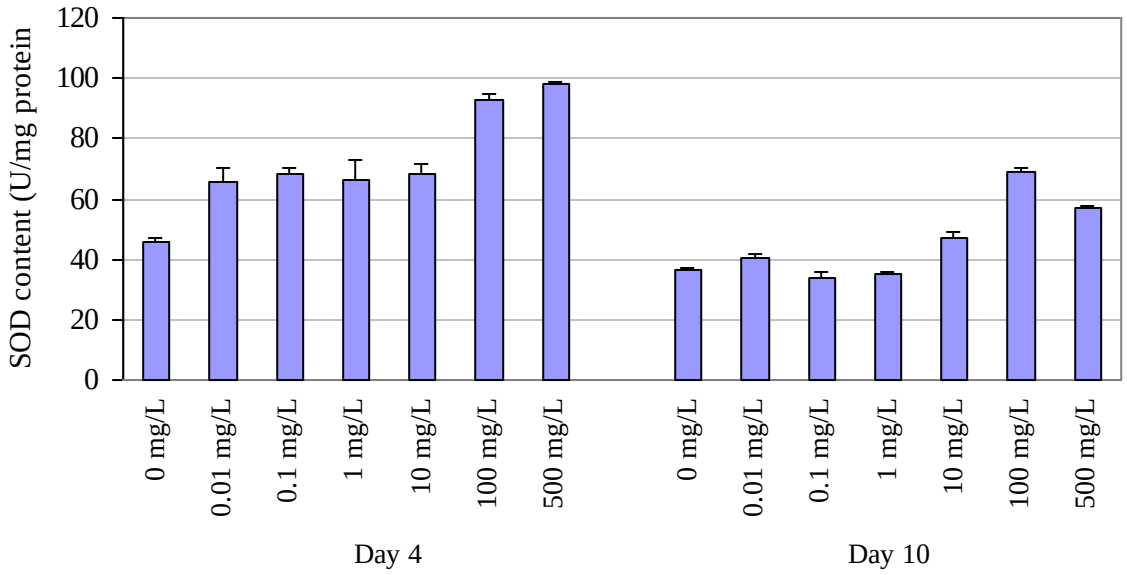


Figure 4.101: SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Cadmium for four and ten days

4.5.2.2 *Tetraselmis tetrahele* UMACC 144 exposed to Copper : SOD activity

The SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Copper (Cu) for four and ten days is shown in Fig 4.102. Cu was the most toxic metals to *Tetraselmis tetrahele* UMACC 144. The result shows that the SOD activity in four days cultures increased from 51.048$\pm$1.781 U/mg protein to 84.336$\pm$3.149 U/mg protein and there were no significant difference in SOD activity in four days cultures between concentrations 0.01 mg/L to 1 mg/L Cu ($p<0.05$). However in the ten days cultures, the SOD activity markedly increased from 31.582$\pm$1.780 U/mg protein to 39.593$\pm$1.366 U/mg protein (at 1 mg/L Cu) and then decreased to 4.602$\pm$0.402 U/mg protein ($p<0.05$).

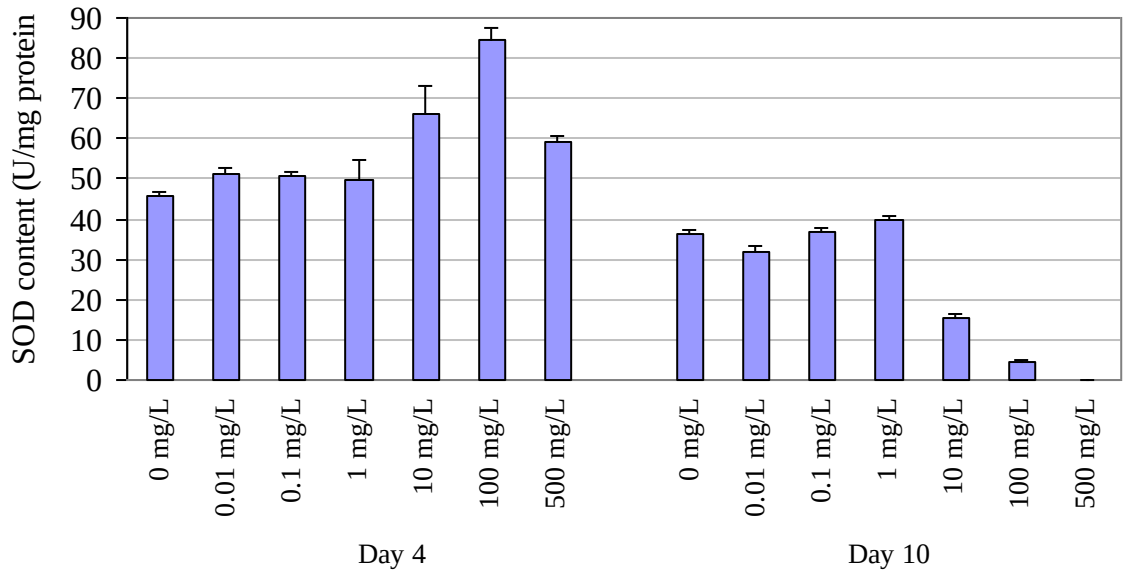


Figure 4.102: SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Cuprum for four and ten days

4.5.2.3 *Tetraselmis tetrahele* UMACC 144 exposed to Chromium: SOD activity

After being exposed to Chromuim (Cr) which was the least toxic metals to *Tetraselmis tetrahele* UMACC 144 for four days, the SOD activity in *Tetraselmis tetrahele* UMACC 144 (Fig 4.103) markedly increased from 34.721±1.801 U/mg protein to 52.785±4.567 U/mg protein (at 0.1 mg/L Cr) and then reduced to 24.047±1.880 U/mg protein ($p<0.05$). In the ten days cultures, the SOD activity also increased from 43.315±2.669 U/mg protein to 45.681±1.714 U/mg protein (at 0.1 mg/L Cr) and then reduced to 2.962±0.219 U/mg protein ($p<0.05$).

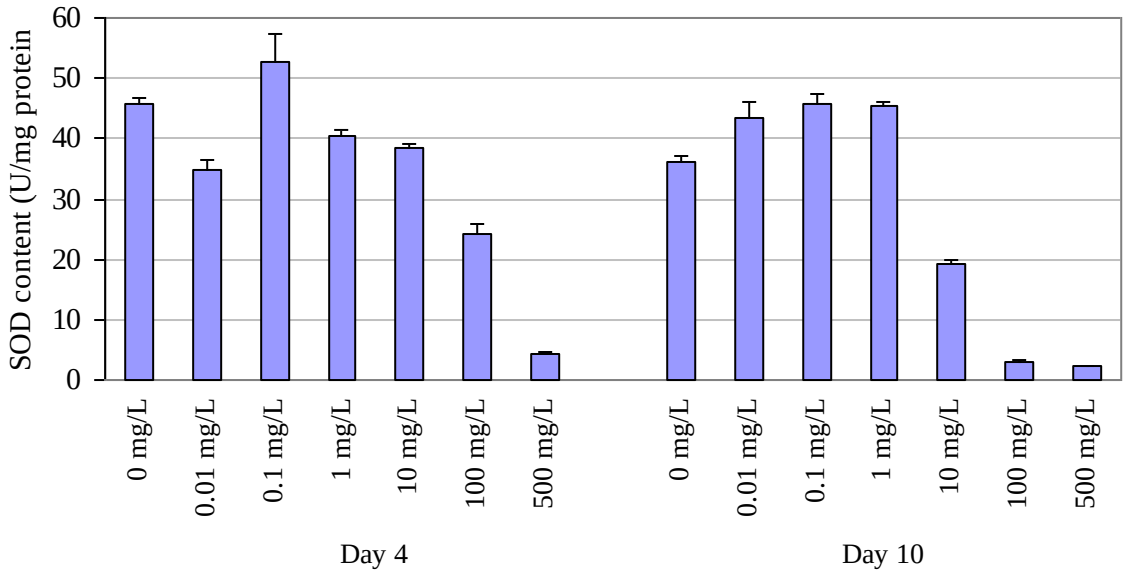


Figure 4.103: SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Chromium for four and ten days

4.5.2.4 *Tetraselmis tetrahele* UMACC 144 exposed to Acidic dye: SOD activity

Figure 4.104 shows the SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Supranol Br. Red 3Bur (Acidic dye) for four and ten days. The SOD activity in four days cultures increased from 38.596±1.249 U/mg protein to 51.013±4.093 U/mg protein and there were no significant difference in SOD activity in four days cultures between concentrations 0.01 mg/L to 1 mg/L Acidic dye ($p<0.05$). In the ten days cultures, the SOD activity which was 2.62 to 4.80 times higher than in control cultures, also significantly increased from 116.074±0.574 U/mg protein to 173.886±3.256 U/mg protein (at 1 mg/L Acidic dye) and then reduced to 94.932±2.170 U/mg protein ($p<0.05$).

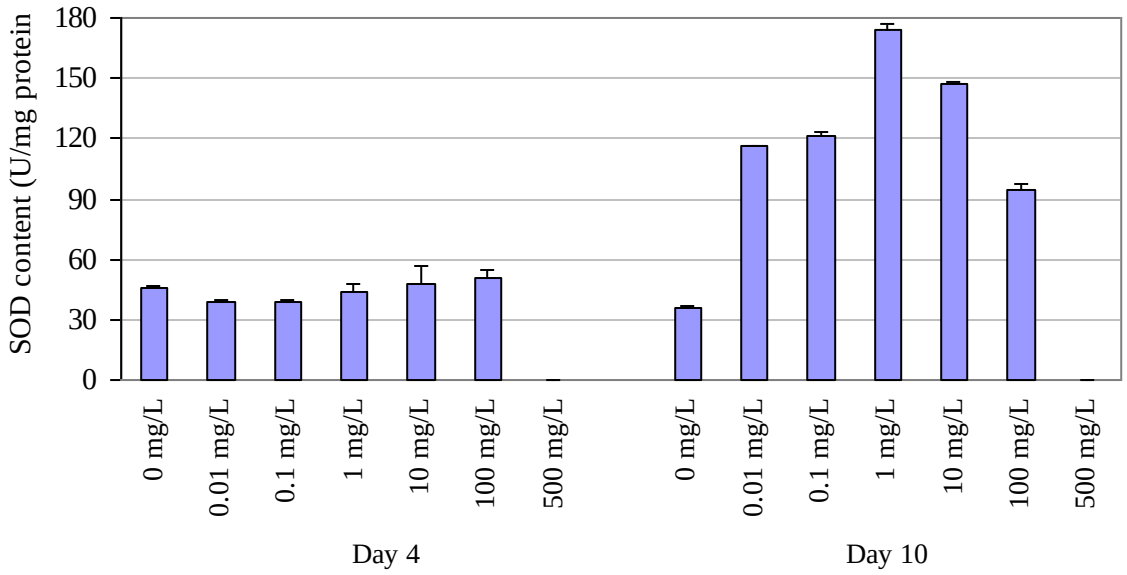


Figure 4.104: SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Supranol Br. Red 3Bur (Acidic dye) for four and ten days

4.5.2.5 *Tetraselmis tetrahele* UMACC 144 exposed to Basic dye: SOD activity

The SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Astrazon Red FBL (Basic dye) for four and ten days is shown in Fig 4.105. The result shows that the SOD activity in four days cultures which was 1.13 to 1.26 times higher than in control cultures, increased from 52.041±3.239 U/mg protein to 58.073±1.027 U/mg protein and statistical analysis shows that there were no significant difference in SOD activity in four days cultures between concentrations 0.01 mg/L to 10 mg/L Basic dye ($p<0.05$). In contrast, in the ten days cultures, the SOD activity which was 3.54 to 4.85 times higher than in control cultures, significantly decreased from 175.614±1.655 U/mg protein to 128.263±3.689 U/mg protein ($p<0.05$).

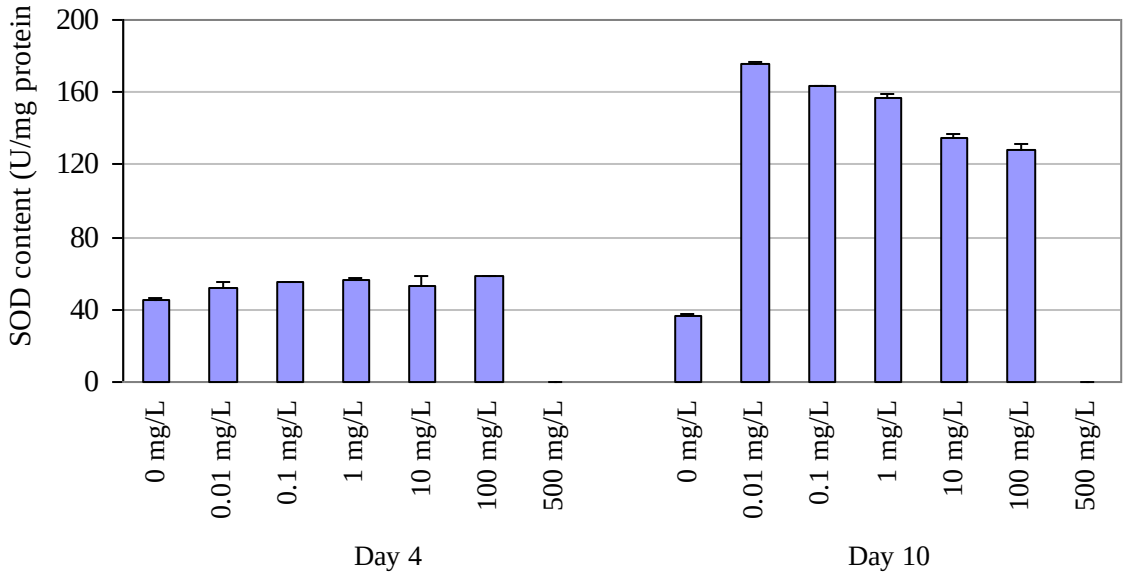


Figure 4.105: SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Astrazon Red FBL (Basic dye) for four and ten days

4.5.2.6 *Tetraselmis tetrahele* UMACC 144 exposed to Metal complex dye: SOD activity

After being exposed to Lanaset Red 2GA (Metal complex dye) for four days, the SOD activity in *Tetraselmis tetrahele* UMACC 144 (Fig 4.106) which was 1.07 to 1.40 times higher than in control cultures, significantly decreased from 64.318±0.705 U/mg protein to 33.000±0.732 ($p<0.05$). In the ten days cultures, the SOD activity which was 2.02 to 4.08 times higher than in control cultures, markedly increased from 73.390±0.645 U/mg protein to 147.689±0.730 U/mg protein (at 10 mg/L Metal complex dye) and then reduced to 79.808±0.545 U/mg protein ($p<0.05$).

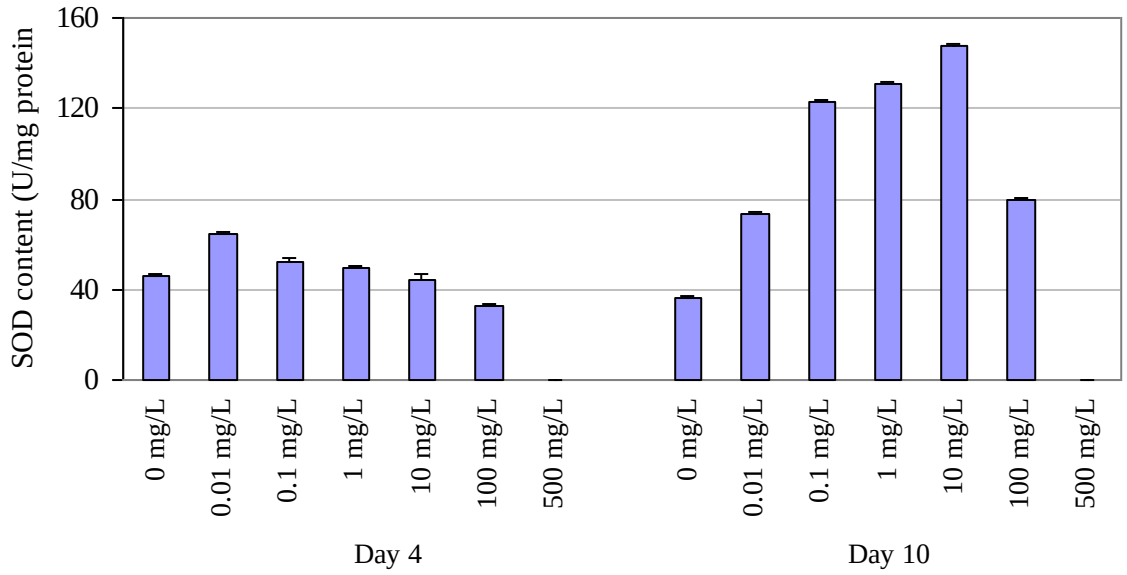


Figure 4.106: SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Lanaset Red 2GA (Metal complex dye) for four and ten days

4.5.2.7 *Tetraselmis tetrahele* UMACC 144 exposed to Dichlovos: SOD activity

Figure 4.107 shows the SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Dichlovos for four and ten days. The SOD activity in four days cultures markedly increased from 36.065$\pm$0.705 U/mg protein to 47.945$\pm$0.851 U/mg protein (at 1 mg/L Dichlovos) and then decreased to 30.915$\pm$5.211 U/mg protein ($p<0.05$). In ten days cultures, the SOD activity also significantly increased from 31.982$\pm$0.829 U/mg protein to 46.588$\pm$0.402 U/mg protein (at 0.1 mg/L Dichlovos) and then reduced to 24.230$\pm$3.414 U/mg protein ($p<0.05$).

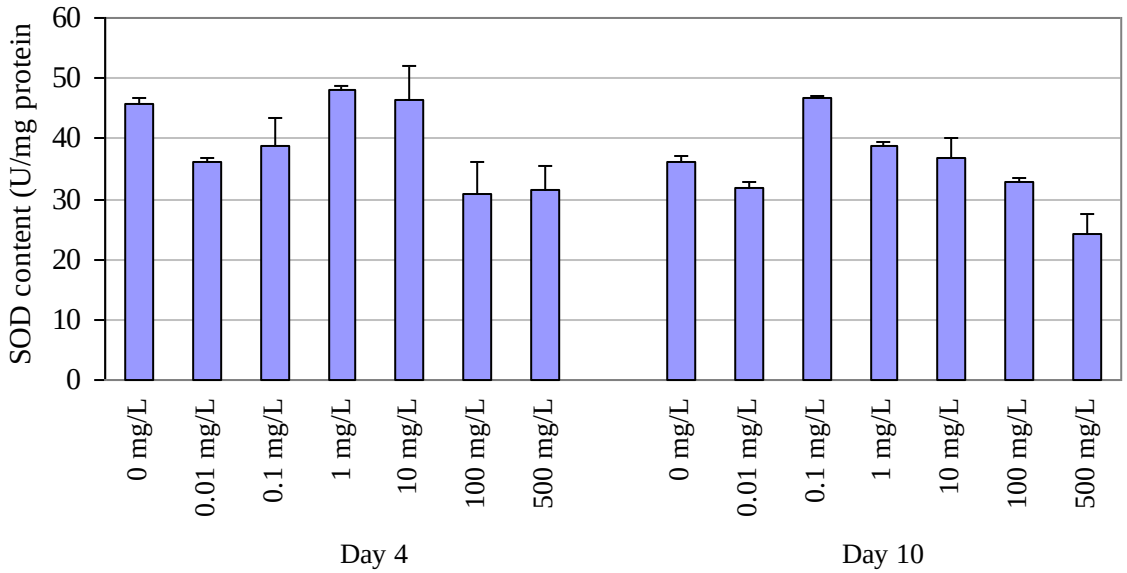


Figure 4.107: SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Dichlovos for four and ten days

4.5.2.8 *Tetraselmis tetrahele* UMACC 144 exposed to Malathion: SOD activity

The SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Malathion for four and ten days is shown in Fig 4.108. The result shows that the SOD activity in four days cultures significantly increased from 32.959$\pm$0.938 U/mg protein to 48.427$\pm$1.048 U/mg protein (at 1 mg/L Malathion) and then decreased to 21.449$\pm$1.862 U/mg protein ($p<0.05$). In the ten days cultures, the SOD activity also markedly increased from 29.044$\pm$1.270 U/mg protein to 35.958$\pm$1.108 U/mg protein (at 1 mg/L Malathion) and then reduced to 1.198$\pm$0.600 U/mg protein ($p<0.05$).

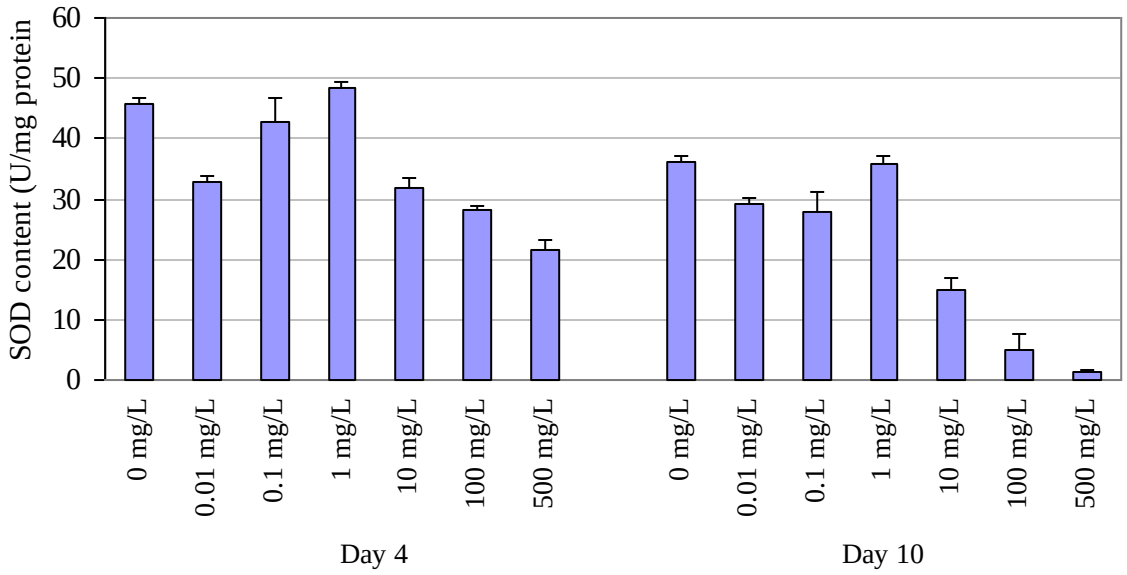


Figure 4.108: SOD activity in *Tetraselmis tetrahele* UMACC 144 after exposure to Malathion for four and ten days

4.5.3 *Boergesenia forbesii*: Effects of Selected Toxicants on SOD Activity

Appendix 19 summarizes the Superoxide dismutase (SOD) activity in *Boergesenia forbesii* after exposure to selected toxicant for four and ten days. The SOD activities in the cultures grown in Prov50 medium without toxicant (control cultures) for four and ten days were 61.393$\pm$1.000 U/mg protein and 70.423$\pm$1.222 U/mg protein respectively.

In general, the SOD activity in the cultures that exposed to selected toxicant for four and ten days contained higher SOD activity compared with the SOD activity in control cultures. Statistical analysis using ANOVA showed there were significant difference ($p<0.05$) for all SOD activity in each concentration of toxicant tested compared with the control culture (Appendix 31).

4.5.3.1 *Boergesenia forbesii* exposed to Cadmium: SOD activity

Figure 4.109 shows the SOD activity in *Boergesenia forbesii* after exposure to Cadmium (Cd) for four and ten days. The result shows that the SOD activity in four days cultures which were 1.02 to 1.72 times higher than in control cultures, fluctuated from 61.023±5.317 U/mg protein to 75.891±6.865 U/mg protein between concentration 0.01 mg/L Cd to 10 mg/L Cd and at 100 mg/L Cd, the SOD activity significantly increased to 105.385±4.237 U/mg protein and then reduced to 81.769±5.111 U/mg protein ($p<0.05$). Statistical analysis shows that there were no significant difference in SOD activity in four days cultures between concentrations 0.1 mg/L to 10 mg/L Cd ($p<0.05$). In the ten days cultures, the SOD activity also markedly increased from 60.954±2.223 U/mg protein to 96.059±0.674 U/mg protein (at 1 mg/L Cd) and decreased to 52.726±1.251 U/mg protein.

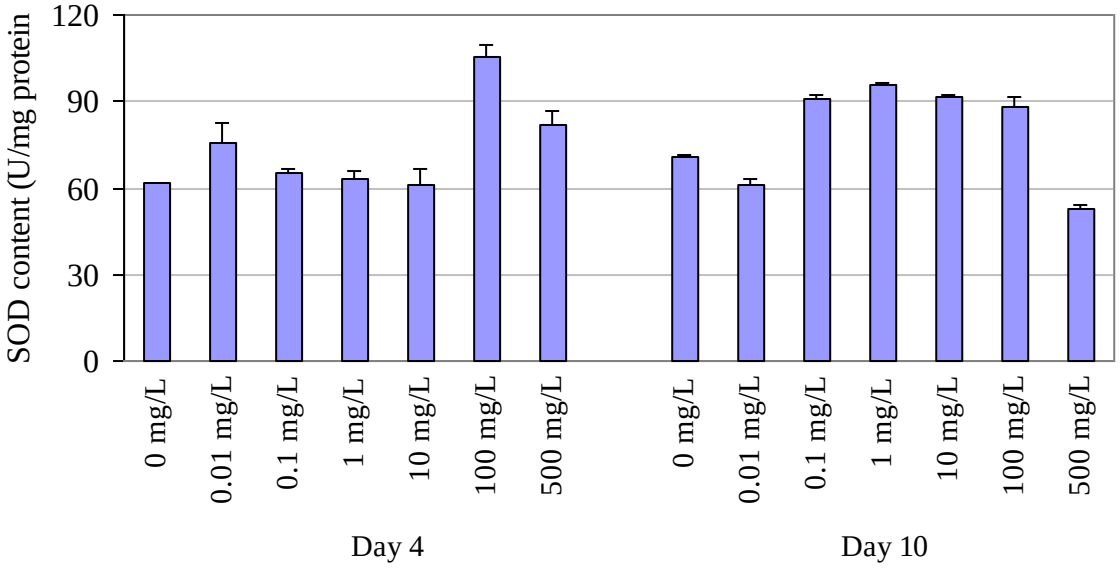


Figure 4.109: SOD activity in *Boergesenia forbesii* after exposure to Cadmium for four and ten days

4.5.3.2 *Boergesenia forbesii* exposed to Copper: SOD activity

The SOD activity in *Boergesenia forbesii* after exposure to Copper (Cu) for four and ten days is shown in Fig 4.110. Cu was the most toxic metals to *Boergesenia forbesii*. The result shows that the SOD activity in four days cultures significantly increased from 47.537±1.462 U/mg protein to 158.709±2.753 U/mg protein ($p<0.05$) and the SOD activity in four days cultures also 1.84 to 2.58 times higher than in control cultures between concentrations 10 mg/L Cu to 500 mg/L Cu. In the ten days cultures, the SOD activity which was 1.04 to 1.40 times higher than in control cultures also markedly increased from 73.300±1.208 U/mg protein to 99.254±0.725 U/mg protein ($p<0.05$).

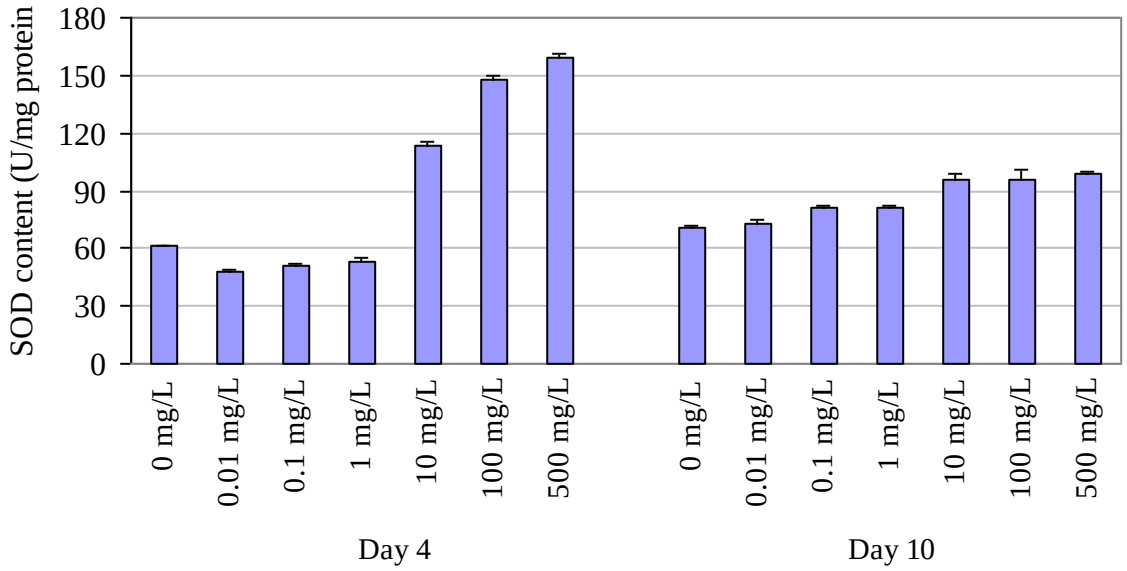


Figure 4.110: SOD activity in *Boergesenia forbesii* after exposure to Copper for four and ten days

4.5.3.3 *Boergesenia forbesii* exposed to Zinc: (SOD) activity

After being exposed to Zinc (Zn) which was the least toxic metals to *Boergesenia forbesii* for four days, the SOD activity in *Boergesenia forbesii* (Fig 4.111) markedly decreased from 102.640±1.674 U/mg protein to 9.833±1.131 U/mg protein ($p<0.05$). In contrast, the SOD activity in ten days cultures, significantly increased from 59.290±5.330 U/mg protein to 102.962±1.093 U/mg protein ($p<0.05$).

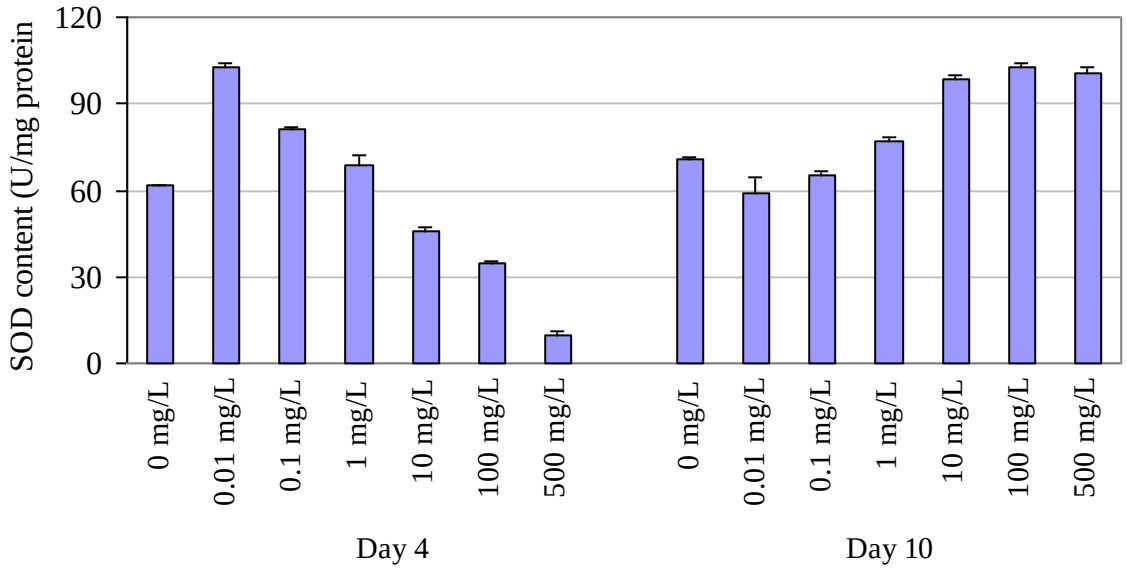


Figure 4.111: SOD activity in *Boergesenia forbesii* after exposure to Zinc for four and ten days

4.5.3.4 *Boergesenia forbesii* exposed to Acidic dye: SOD activity

Figure 4.112 shows the SOD activity in *Boergesenia forbesii* after exposure to Supranol Br. Red 3Bur (Acidic dye) for four and ten days. The SOD activity in four days cultures which was 1.20 to 1.93 times higher than in control cultures significantly increased from 110.516±1.719 U/mg protein to 119.073±5.017 U/mg protein (at 0.1 mg/L Acidic dye) and then decreased to 74.087±087 U/mg protein ($p<0.05$). However,

in ten days cultures, the SOD activity was markedly decreased from 89.268±1.229 U/mg protein to 46.546±0.706 U/mg protein ($p<0.05$).

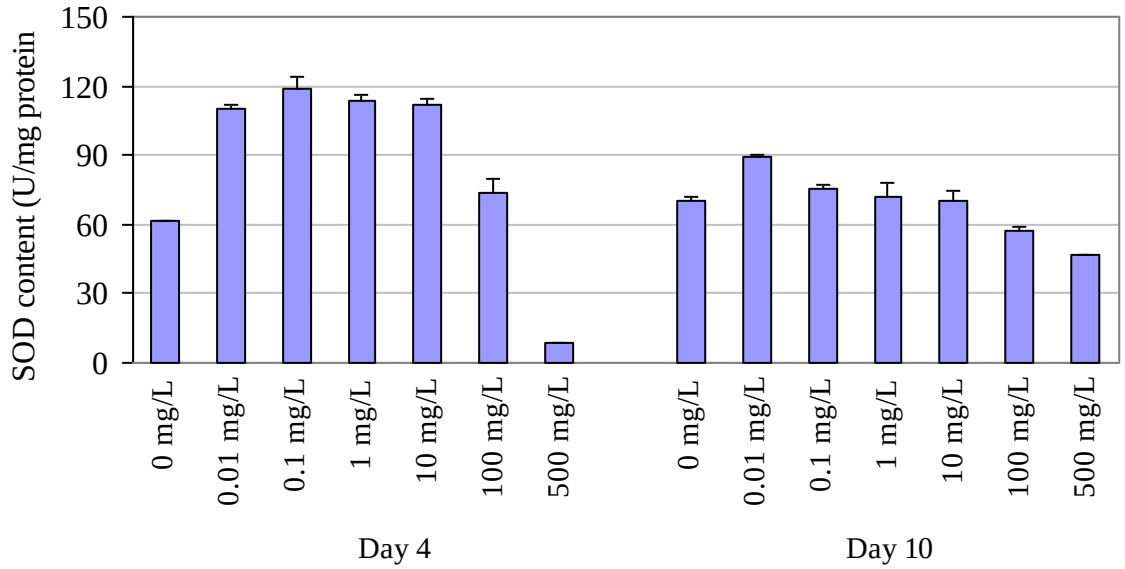


Figure 4.112: SOD activity in *Boergesenia forbesii* after exposure to Supranol Br. Red 3Bur (Acidic dye) for four and ten days

4.5.3.5 *Boergesenia forbesii* exposure to Basic dye: SOD activity

The SOD activity in *Boergesenia forbesii* after exposure to Astrazon Red FBL (Basic dye) for four and ten days is shown in Fig 4.113. The result shows that the SOD activity in four days cultures which was 1.78 to 2.43 times higher than in control cultures markedly decreased from 149.774±5.637 U/mg protein to 109.543±5.926 U/mg protein ($p<0.05$). In the ten days cultures, the SOD activity which was 1.18 to 1.40 times higher than in control cultures, also significantly increased from 92.850±0.954 U/mg protein to 98.729±2.075 U/mg protein (at 10 mg/L Basic dye) and then reduced to 83.365±1.692 U/mg protein ($p<0.05$).

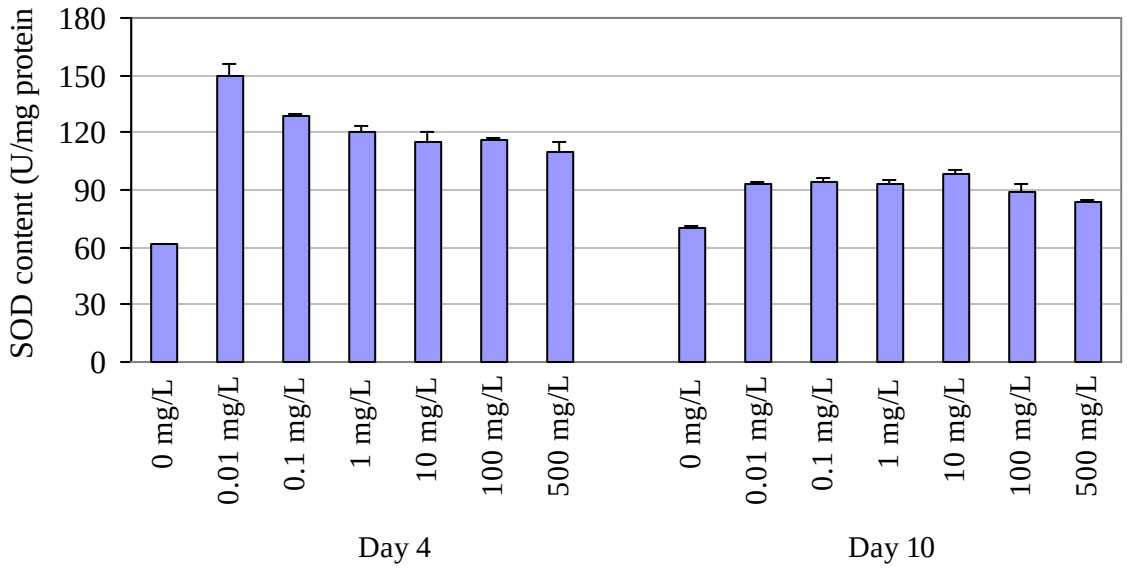


Figure 4.113: SOD activity in *Boergesenia forbesii* after exposure to Astrazon Red FBL (Basic dye) for four and ten days

4.5.3.6 *Boergesenia forbesii* exposed to Metal complex dye: SOD activity

After being exposed to Lanaset Red 2GA (Metal complex dye) for four days, the SOD activity in *Boergesenia forbesii* (Fig 4.114) which was 1.41 to 2.91 times higher than in control cultures significantly decreased from 178.688±0.990 U/mg protein to 30.251±1.155 U/mg protein. The SOD activity markedly increased from 82.738±4.867 U/mg protein to 93.907±5.145 U/mg protein (at 10 mg/L Metal complex dye) and then reduced to 57.355±6.434 U/mg protein ($p<0.05$). The results also shown that the SOD activities in ten days cultures were 1.17 to 1.33 times higher than in control cultures.

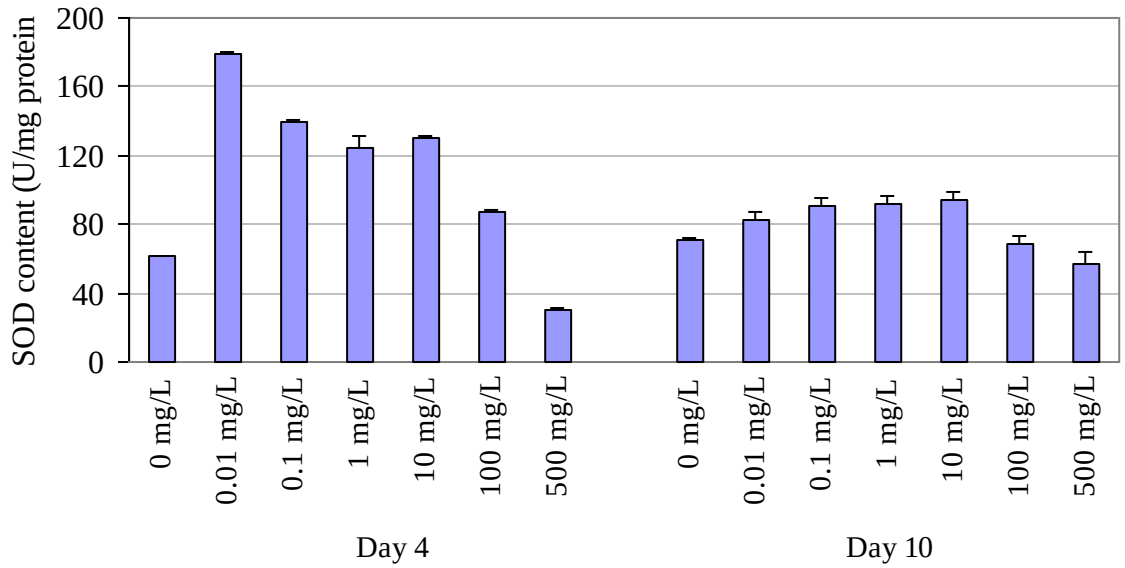


Figure 4.114: SOD activity in *Boergesenia forbesii* after exposure to Lanaset Red 2GA (Metal complex dye) for four and ten days

4.5.3.7 *Boergesenia forbesii* exposed to Dichlovos: SOD activity

Figure 4.115 shows the SOD activity in *Boergesenia forbesii* after exposure to Dichlovos for four and ten days. The SOD activity in four days cultures which was 1.02 to 2.17 times higher than in control cultures markedly decreased from 133.667$\pm$6.800 U/mg protein to 45.615$\pm$3.745 U/mg protein ($p<0.05$). However, in ten days cultures, the SOD activity fluctuated from 87.529$\pm$2.439 U/mg protein to 89.685$\pm$0.814 U/mg protein between concentrations 0.01 mg/L to 100 mg/L Dichlovos and then decreased to 63.414$\pm$03.285 U/mg protein at 500 mg/L Dichlovos. Statistical analysis shows that there were no significant difference in SOD activity in ten days cultures between concentrations 0.01 mg/L to 100 mg/L Dichlovos ($p>0.05$).

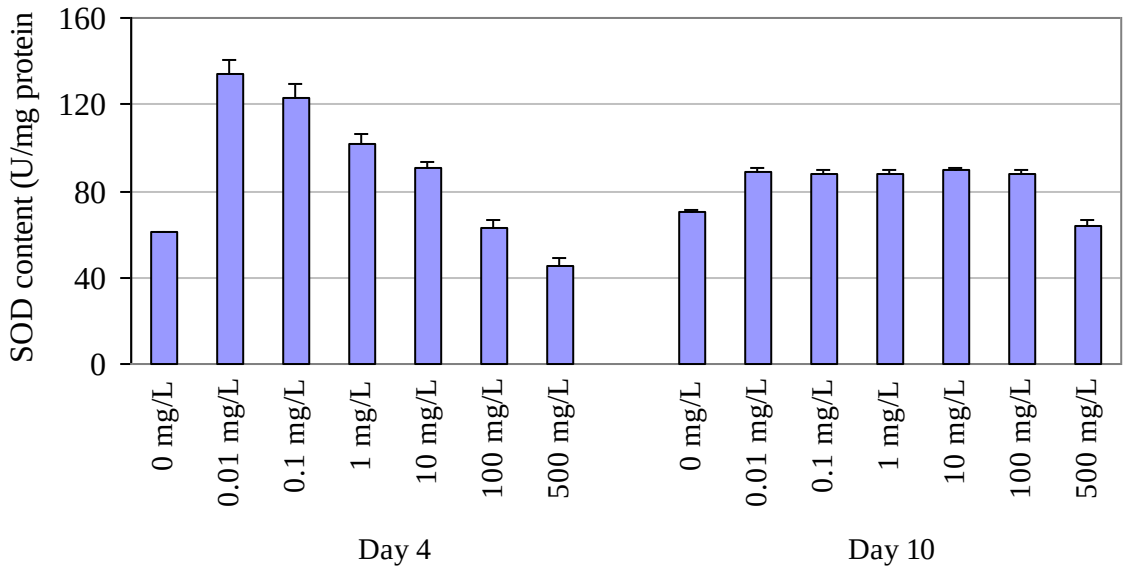


Figure 4.115: SOD activity in *Boergesenia forbesii* after exposure to Dichlovos for four and ten days

4.5.3.8 *Boergesenia forbesii* exposed to Malathion: SOD activity

The SOD activity in *Boergesenia forbesii* after exposure to Malathion for four and ten days is shown in Fig 4.116. The result shows that the SOD activity in four days cultures which was 1.04 to 1.99 times higher than in control cultures significantly increased from 97.977±2.055 U/mg protein to 122.663±5.071 U/mg protein (at 10 mg/L Malathion) and then reduced to 64.131±4.068 U/mg protein ($p<0.05$). In the ten days cultures, the SOD activity was highest at 0.01 mg/L Malathion (76.159±1.579 U/mg protein) and then start to fluctuated from 63.478±6.475 U/mg protein to 66.709±2.587 U/mg protein between concentrations 0.1 mg/L to 100mg/L Malathion and significantly decreased to 51.628±1.689 U/mg protein at 500mg/L Malathion ($p<0.05$). Statistical analysis shows that there were no significant difference in SOD activity in ten days cultures between concentrations 0.1 mg/L to 100 mg/L Malathion ($p>0.05$).

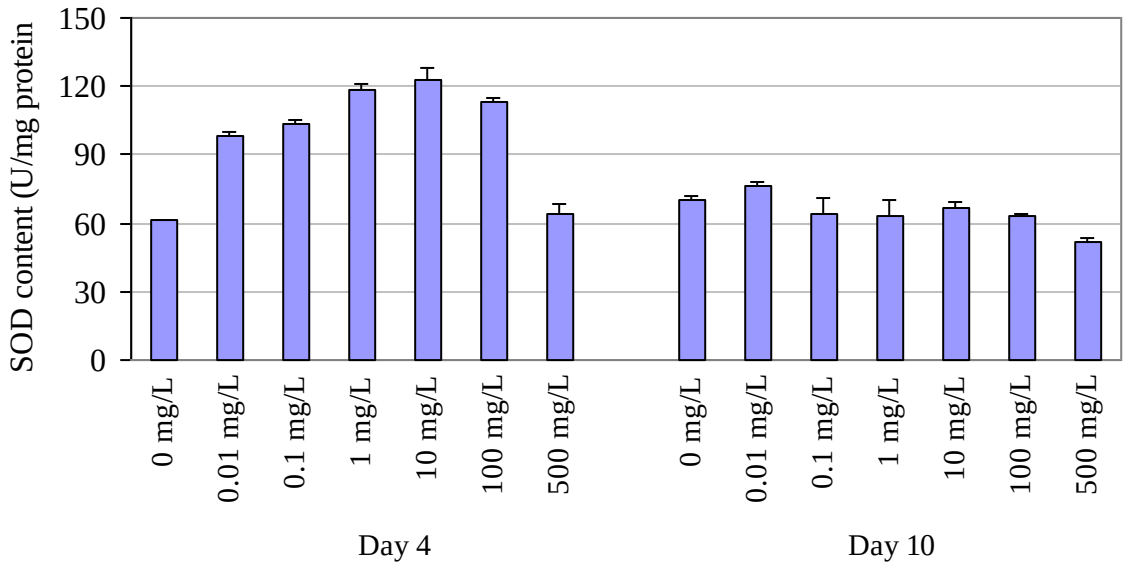


Figure 4.116: SOD activity in *Boergesenia forbesii* after exposure to Malathion for four and ten days

4.5.4 *Ventricaria ventricosa*: Effects of Selected Toxicants on SOD Activity

Appendix 24 summarizes the Superoxide dismutase (SOD) activity in *Ventricaria ventricosa* after exposure to selected toxicant for four and ten days. The SOD activities in the cultures grown in Prov50 medium without toxicant (control cultures) for four and ten days were 5.698±1.000 U/mg protein and 10.368±1.500 U/mg protein respectively.

In general, the cultures exposed to selected toxicant for four and ten days contained higher SOD activity compared with the SOD activity in control cultures. Statistical analysis using ANOVA showed there were significant difference ($p<0.05$) for all SOD activity in each concentration of toxicant tested compared with the control culture (Appendix 44).

4.5.4.1 *Ventricaria ventricosa* exposed to Cadmium: SOD activity

Figure 2.117 shows the SOD activity in *Ventricaria ventricosa* after exposure to Cadmium (Cd) for four and ten days. The result shows that the SOD activity in four days cultures which were 6.93 to 11.08 times higher than in control cultures, significantly decreased from 63.136±7.104 U/mg protein to 39.491±5.725 U/mg protein. In ten days cultures, the SOD activity which were 1.27 to 3.07 times higher than in control cultures, increased from 13.241±1.073 U/mg protein to 31.849±1.430 U/mg protein.

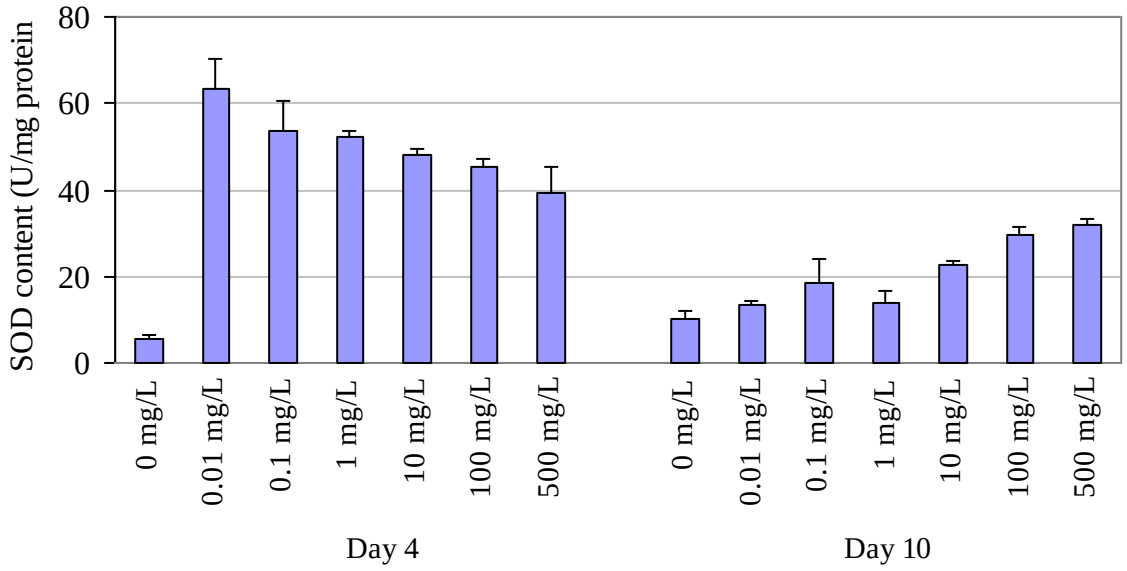


Figure 4.117: SOD activity in *Ventricaria ventricosa* after exposure to Cadmium for four and ten days

4.5.4.2 *Ventricaria ventricosa* exposed to Copper: SOD activity

The SOD activity in *Ventricaria ventricosa* after exposure to Copper (Cu) for four and ten days is shown in Fig 4.118. Cu was the most toxic metals to *Ventricaria ventricosa*. The result shows that the SOD activity in four days cultures which were 5.10 to 20.26 times higher than in control cultures, significantly increased from 29.069±1.000

U/mg protein to 115.484±1.844 U/mg protein ($p<0.05$). In the ten days cultures, the SOD activity which were 1.39 to 9.09 times higher than in control cultures, also markedly increased from 9.873±1.911 U/mg protein to 94.270±5.684 U/mg protein ($p<0.05$).

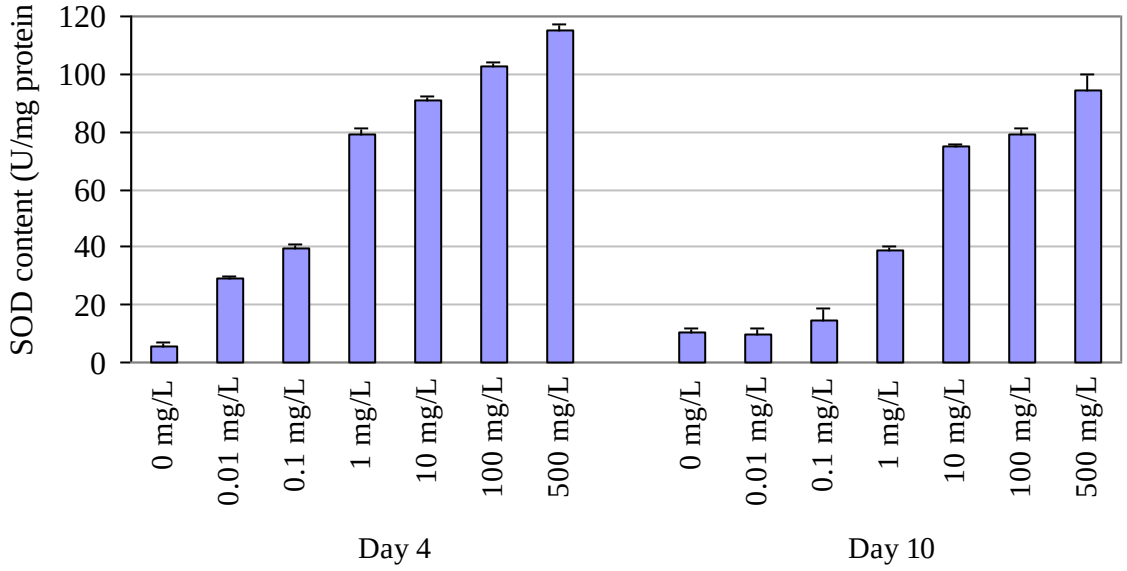


Figure 4.118: SOD activity in *Ventricaria ventricosa* after exposure to Copper for four and ten days

4.5.4.3 *Ventricaria ventricosa* exposed to Zinc: SOD activity

After being exposed to Zinc (Zn) for four days, the SOD activity in *Ventricaria ventricosa* (Fig 4.119) which were 9.08 to 15.53 times higher than in control cultures, markedly decreased from 88.504±3.142 U/mg protein to 51.757±2.900 U/mg protein ($p<0.05$). In the ten days cultures, the SOD activity which were 1.44 to 4.23 times higher than in control cultures, also significantly decreased from 43.954±1.926 U/mg protein to 15.012±2.466 U/mg protein ($p<0.05$).

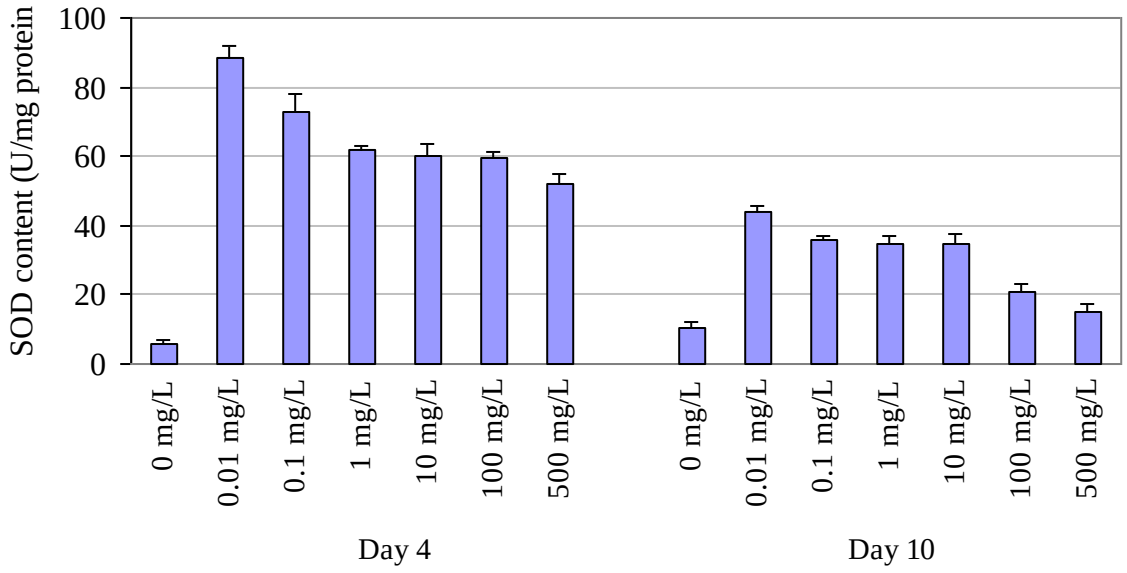


Figure 4.119: SOD activity in *Ventricaria ventricosa* after exposure to Zinc for four and ten days

4.5.4.4 *Ventricaria ventricosa* exposed to Acidic dye: SOD activity

Figure 4.120 shows the SOD activity in *Ventricaria ventricosa* after exposure to Supranol Br. Red 3Bur (Acidic dye) for four and ten days. The SOD activity in four days cultures which were 4.56 to 6.79 times higher than in control cultures, significantly increased from 30.321$\pm$1.239 U/mg protein to 38.710$\pm$4.348 U/mg protein (at 0.1 mg/L Acidic dye) and then decreased to 26.002$\pm$1.310 U/mg protein ($p<0.05$). In ten days cultures, the SOD activity which was 1.56 to 3.68 times higher than in control cultures, also markedly increased from 35.041$\pm$4.915 U/mg protein to 38.247$\pm$3.950 U/mg protein (at 0.1 mg/L Acidic dye) and then reduced to 16.273$\pm$1.798 U/mg protein ($p<0.05$).

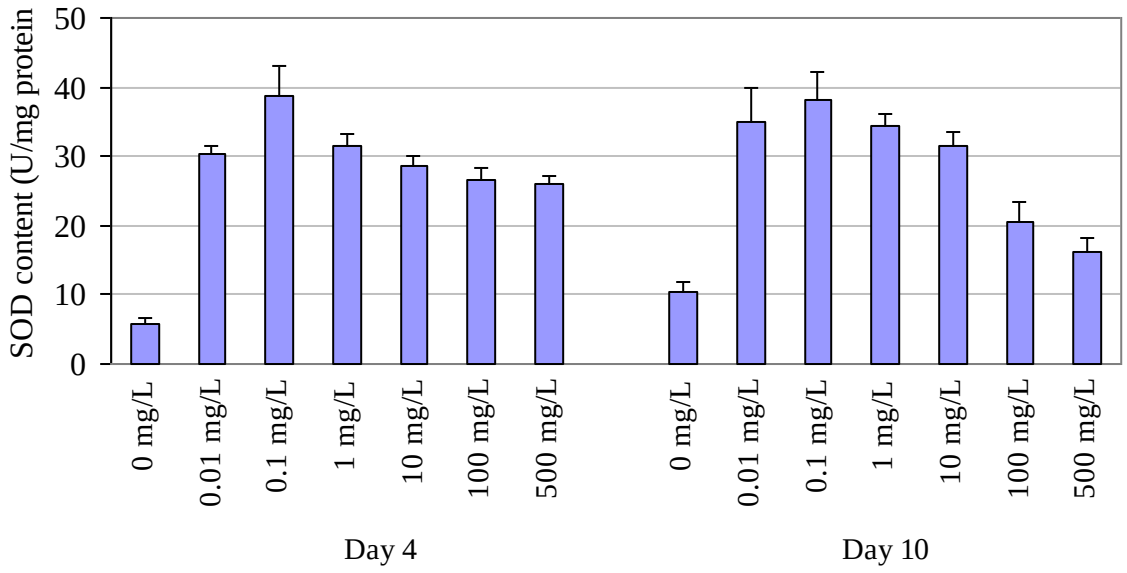


Figure 4.120: SOD activity in *Ventricaria ventricosa* after exposure to Supranol Br. Red 3Bur (Acidic dye) for four and ten days

4.5.4.5 *Ventricaria ventricosa* exposed to Basic dye: SOD activity

The SOD activity in *Ventricaria ventricosa* after exposure to Astrazon Red FBL (Basic dye) for four and ten days is shown in Fig 4.121. The result shows that the SOD activity in four days cultures which was 5.20 to 10.17 times higher than in control cultures, markedly increased from 46.575±0.970 U/mg protein to 57.995±3.949 U/mg protein (at 1 mg/L Basic dye) and then decreased to 29.637±1.404 U/mg protein ($p<0.05$). In the ten days cultures, the SOD activity which was 1.55 to 2.92 times higher than in control cultures, also significantly increased from 22.425±1.867 U/mg protein to 30.316±2.254 U/mg protein (at 0.1 mg/L Basic dye) and then reduced to 16.157±4.915 U/mg protein ($p<0.05$).

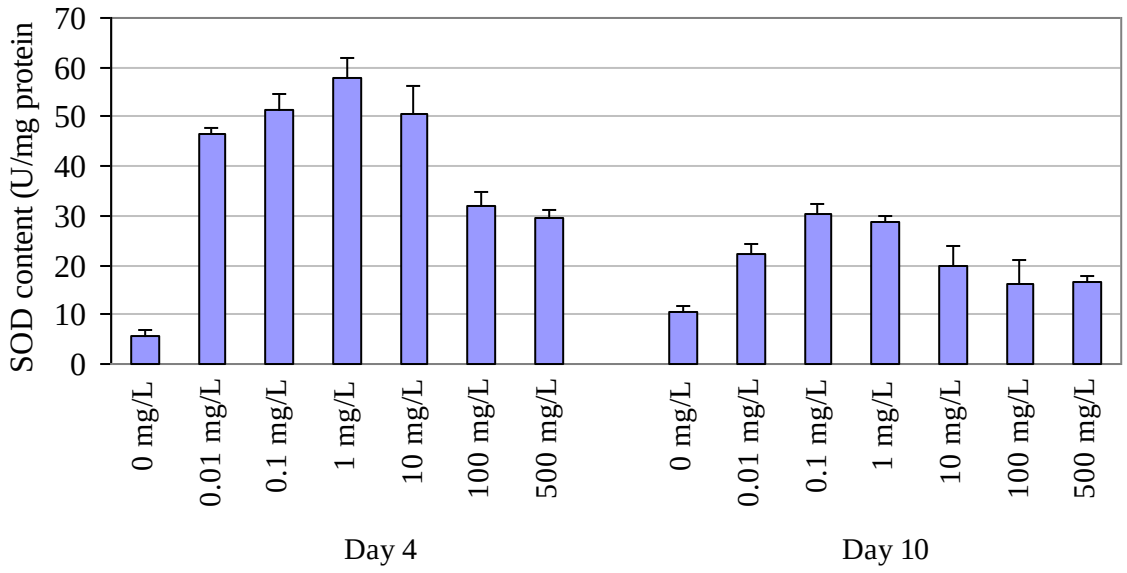


Figure 4.121: SOD activity in *Ventricaria ventricosa* after exposure to Astrazon Red FBL (Basic dye) for four and ten days

4.5.4.6 *Ventricaria ventricosa* exposure to Metal complex dye: SOD activity

After being exposed to Lanaset Red 2GA (Metal complex dye) for four days, the SOD activity in *Ventricaria ventricosa* (Fig 4.122) which was 2.66 to 8.24 times higher than in control cultures, significantly increased from 41.538±2.797 U/mg protein to 46.984±1.739 U/mg protein (at 0.1 mg/L Metal complex dye) and then decreased to 15.181±3.043 U/mg protein ($p<0.05$). The SOD activity markedly increased from 20.624±7.113 U/mg protein to 31.023±1.090 U/mg protein (at 0.1 mg/L Metal complex dye) and reduced to 5.285±0.234 U/mg protein ($p<0.05$). The results also shown that the SOD activity in ten days cultures were 1.48 to 2.99 times higher than in control cultures.

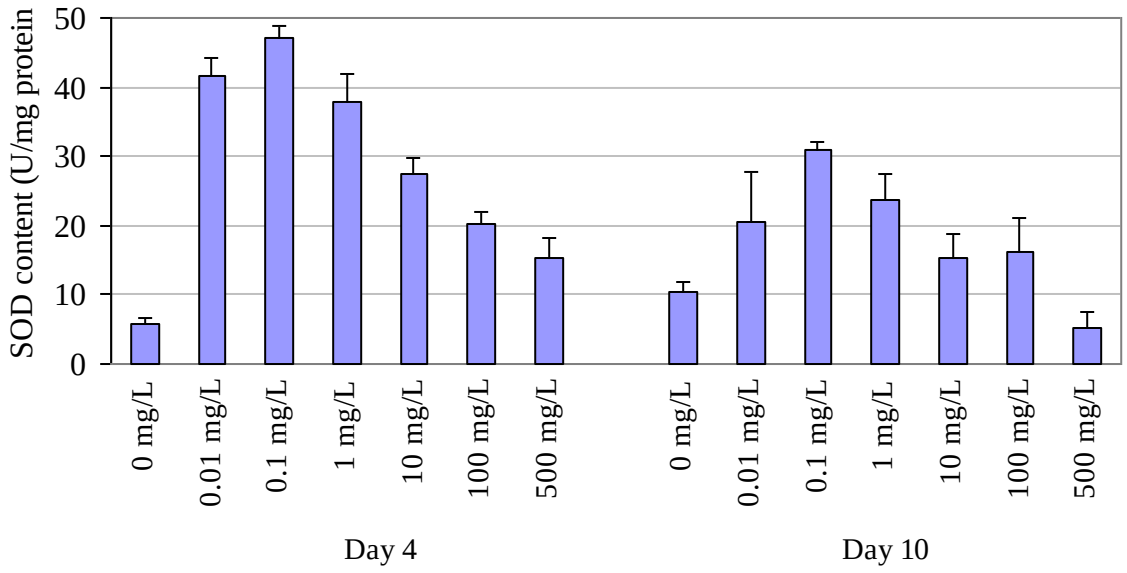


Figure 4.122: SOD activity in *Ventricaria ventricosa* after exposure to Lanaset Red 2GA (Metal complex dye) for four and ten days

4.5.4.7 *Ventricaria ventricosa* exposed to Dichlovos: SOD activity

Figure 4.123 shows the SOD activity in *Ventricaria ventricosa* after exposure to Dichlovos for four and ten days. The SOD activity in four days cultures which was 4.59 to 5.97 times higher than in control cultures, markedly decreased from 34.044±2.848 U/mg protein to 26.192±4.630 U/mg protein ($p<0.05$). In spite of that, in the ten days cultures, the SOD activity which was 1.35 to 2.22 times higher than in control cultures, significantly increased from 18.350±2.848 U/mg protein to 23.061±2.852 U/mg protein (at 10 mg/L Dichlovos) and then decreased to 14.062±0.842 U/mg protein ($p<0.05$).

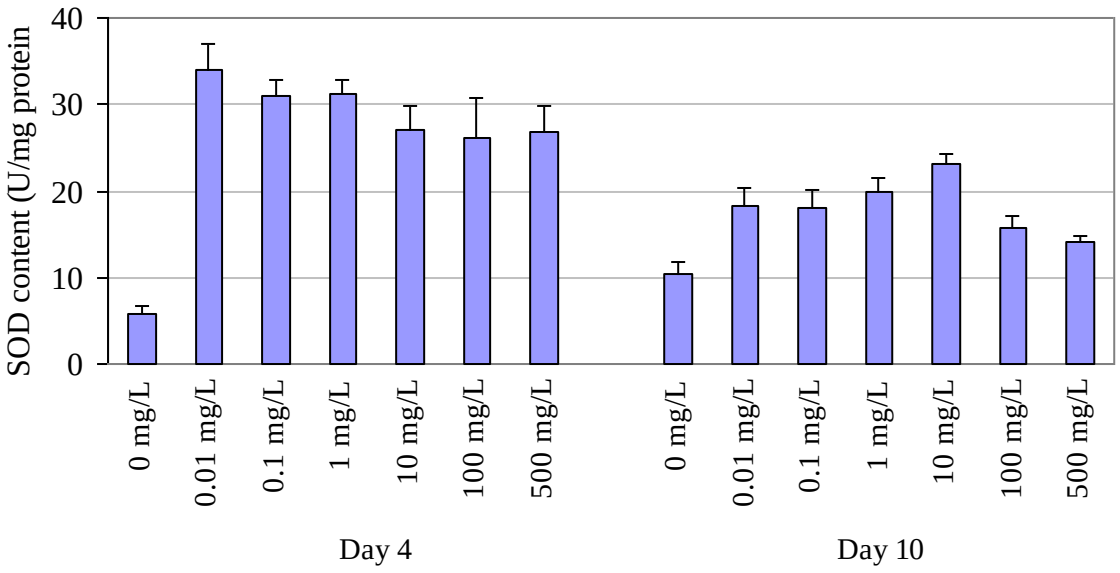


Figure 4.123: SOD activity in *Ventricaria ventricosa* after exposure to Dichlovos for four and ten days

4.5.4.8 *Ventricaria ventricosa* exposed to Malathion: SOD activity

The SOD activity in *Ventricaria ventricosa* after exposure to Malathion for four and ten days is shown in Fig 4.124. The result shows that the SOD activity in four days cultures which was 3.61 to 7.63 times higher than in control cultures, significantly decreased from 43.530$\pm$3.260 U/mg protein to 20.573$\pm$5.680 U/mg protein ($p<0.05$). However, in the ten days cultures, the SOD activity which was 1.26 to 2.78 times higher than in control cultures, markedly increased from 23.322$\pm$4.195 U/mg protein to 28.914$\pm$3.755 U/mg protein (at 10 mg/L Malathion) and then decreased to 13.150$\pm$1.718 U/mg protein ($p<0.05$).

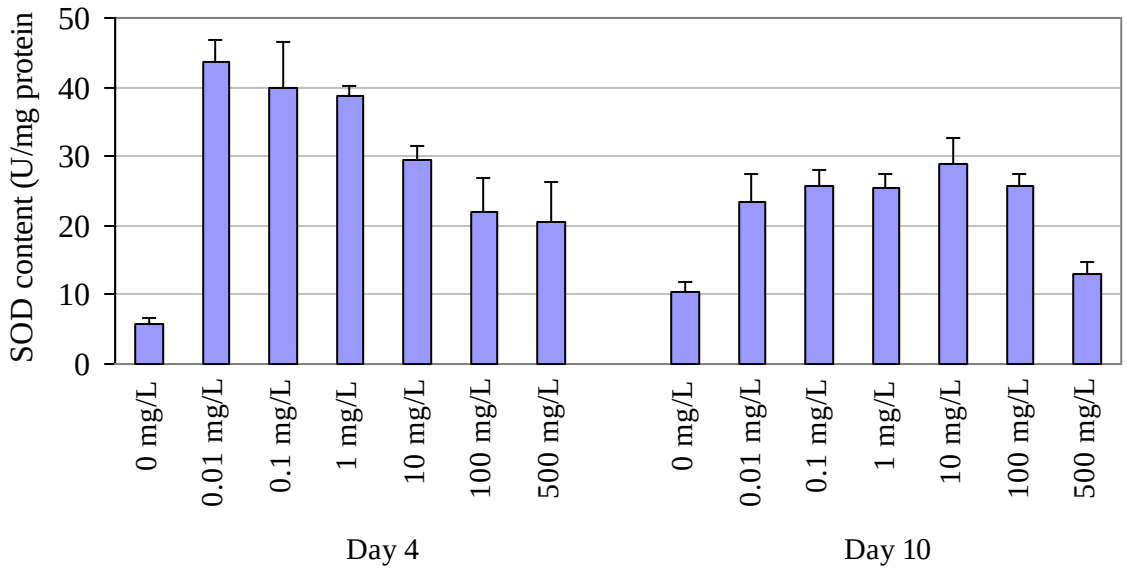


Figure 4.124: SOD activity in *Ventricaria ventricosa* after exposure to Malathion for four and ten days

5.0 DISCUSSION

5.1 WATER QUALITY AND NUTRIENT CONTENT AT COLLECTION SITE OF TEST ORGANISMS (MACROALGAE)

The quality of the seawater collected at the sample collection site of the macroalgae used in this study had the ammoniacal-nitrogen, orthophosphate, cadmium and zinc contents similar to the Class 2 (NH_3-N: 70µg/L; PO_4^3: 75µg/L; Cd: 3µg/L; Zn: 50µg/L) of the Marine Water Quality Criteria and Standard (MWQCS) (Appendix 2). For the nitrate and cuprum, their contents exceeded the Class 3 (NO_3-N: 1000µg/L; Cu: 10µg/L) of the MWQCS. The other nutrients like Ca, Cl, Na were high while K, SiO2 were low. The metal like Fe, were low while Co and Mn were undetectabale.

The C:N:P ratio in the seawater is one of the parameters to measure the ability of seawater to support algae growth. C, N and P (CNP) are the macronutrients for the growth of microalgae and macroalgae. The C:N:P ratio of the seawater averaged 307:8:1 indicating that the seawater was phosphate limited, as the optimum C:N:P ratio for algae growth is 58:8:1 (Phang, 2001). In general, seawater is usually deficient in phosphate (PO_4) but not nitrogen (NH_3-N) as reported in some studies conducted in Malaysia (DOE, 2010).

Carbon, hydrogen, oxygen, nitrogen, phosphorus, potassium, calcium, magnesium and sulfur and trace elements are essential macronutrients to plant growth. In sea water, carbon occurs in the form of bicarbonate and carbonate ions, as carbonic acid

and molecular CO_2. All these forms are in equilibrium with each other and with the hydrogen ions present (Hurd, *et al.,* 2009; Boney, 1969; Barsanti and Gualtieri, 2006).

Nitrates, ammonium compounds and some organic forms of nitrogen are utilised by algae for growth. Nitrogen is a constituent of amino acids, proteins, coenzymes, nucleic acids and chlorophyll. Nitrogen has a great affect on plant growth and a deficiency or excess markedly affects plant growth. Nitrogen–deficiency in algae can be observed as a marked increase in lipid content and photosynthesis is reduced but not completely stopped (Boney, 1969; Barsanti and Gualtieri, 2006).

Phosphorus plays an important role in photosynthesis. Phosphorus is a constituent of ATP, nucleic acids, phospholipids and certain coenzymes. It is very important in the plants' energy transfer system and the deficiency can slow the growth. Phosphorus toxicity may occur, interfering with the normal function of other elements such as iron, manganese and zinc. A ratio of nitrogen : phosphorus of 16.3 : 1 has been given for phytoplankton which will ensure satisfactory plant growth, compared with 20 : 1 in the sea (Boney, 1969; Barsanti and Gualtieri, 2006).

Iron, chlorine, manganese, boron, zinc, copper and molybdenum are micronutrients essential for the normal algal growth. Their availability in the sea decreases due to precipitation as insoluble oxides or hydroxides and absorption on suspended detritus (organic and inorganic). Copper acts as an electron carrier and as a constituent of certain proteins and enzymes. Iron is required for the synthesis of chlorophyll and is an essential part of the cytochromes which serve as electron carriers in photosynthesis and respiration. Iron deficiency can cause the loss of chlorophyll and

decrease in the photosynthesis rate, together with slow down in growth rate. Manganese activates some of the enzymes involved in fatty acids synthesis, DNA and RNA formation and the enzyme isocitrate dehydrogenase in the Krebs cycle. It is involved in production of oxygen from water in photosynthesis and may be involved in chlorophyll synthesis (Boney, 1969; Barsanti and Gualtieri, 2006).

The analysis of the seawater from the collection site of the macroalgae used in this study, namely *Boergesenia forbesii* and *Ventricaria ventricosa*, show that the study site was not very polluted except for higher presence of nitrate and copper.

5.2 SCREENING OF MICROALGAE FOR THE STUDIES

Photosynthetic organisms like algae (microalgae and seaweed) have been used as biodetectors to monitor environmental conditions and aquatic organism health in the marine environment due to their sensitivity to changes in water quality (Volterra and Conti, 2000; Sanchez-Rodrıguez, *et al*., 2001; Barreiro, *et al*., 2002; Conti and Cecchetti, 2003; Conti, *et al*., 2007). They respond to low dissolved oxygen level, high nutrient levels, toxic contaminants, poor food quality or abundance and predation. Because of their natural and wide-spread occurence along seashores worldwide, algae may be useful for a time-intergrated picture of the ecosystem response to exposure to toxic compounds (Torres, *et al*., 2008).

Algae species which are sensitive to toxic compounds can be useful as indicators for the toxicants and may be used as the test organism in toxicity test for use in the formulation of water quality criteria and standards. Tolerant species can be used for long term biomonitoring, as they can bioaccumulate high level of toxic compounds without being damaged or killed.

In this study, of the 25 microalgae species and two macroalgae exposed for four days to $CdCl_2$, *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa* were able to tolerate up to 100 mg/L $CdCl_2$ (90% viability). These results present a useful reference point for further detailed investigation on evaluation of toxic compounds on these algae. Two microalgae, *Chlorella vulgaris* UMACC 245 and *Tetraselmis tetrahele* UMACC 144 and two macroalgae, *Boergesenia forbesii* and *Ventricaria ventricosa* were selected for detailed investigation, for the following reasons:

(i) of the 25 microalgae, *Chlorella vulgaris* UMACC 245 and *Tetraselmis tetrahele* UMACC 144 were most tolerant to $CdCl_2$ which is one of the most toxic compounds to algae. This makes them useful for chemical contaminant toxicity testing (ii) They are unicellular (iii) the cells are large (diameter: 10-16µm) (iv) They grow fast and easily [growth rate for Chlorella: μ=0.12-0.28day^{-1}; Tetraselmis: μ=0.25-0.42day^{-1} (unpublish data)] (v) They have relatively short life-cycle (vi) They are easy to handle in the laboratory and vii) distinct morphological changes occur in these algae under 12:12 h light:dark or continuous illumination observed. They have stable morphological characters which therefore would not interfere with their application in bioassays or toxicity tests. This is important to make sure that morphological characteristics do not change under different cultural conditions.

Chlorella sp. and *Tetraselmis* sp. are among the most widely distributed green microalgae, since these genera are found in marine environments in the world. Acording to previous report, *Chlorella* sp. [carbohydrate (1-20%), protein (9-45%) and lipid (17-32%) content] and *Tetraselmis* sp. [carbohydrate (14-20%), protein (15-45%) and lipid (15-23%) content] (Lourenco, *et al.*, 1997; unpublish data) have good biochemical composition and their biomass have economic value as organic fertilizer, animal fodder, fish feed and can be used for wastewater remediation, carbon capture and sequesterian, and also can provide chemical and unique compounds for use as biofuel, nutraceuticals, pharmaceuticals, food ingredients and health food (Edwards, 2010).

5.3 EFFECTS OF SELECTED TOXICANTS ON GROWTH OF THE ALGAE

Photoautotrophs, like algae, when exposed to toxicants, can generate reactive oxygen species (ROS), the primary cytotoxic compounds of oxidative stress which are mainly byproducts from the electron transport chains in chloroplasts (Asada, 1999), mitochondria and the plasma membrane (cytochrome *b* mediated electron transfer) (Elstner, 1987; Liu, *et al*., 2007). In general, the chloroplasts contain a highly organized thylakoid membrane system and provide all structural properties such as chlorophyll, carotenoid, protein and enzymes molecules, for optimal light harvesting (Allen and Forsberg, 2001; Qian, *et al*., 2008).

In photosynthetic organisms such as algae, parameters reflecting the status of the photosynthetic machinery such as chlorophyll and carotenoid content are often used as an indicator of algae physiological status (Miller-Moreya and Van Dolah, 2004). For the present study, growth rate (based on Chl *a*), IC_{50} value and pigment content (carotenoid)

obtained in the algae exposed to different concentrations (0, 0.01, 0.1,1,10,100 and 500 mg/L) of toxicants such as metals, textile dye and organophosphate pesticides for four days (short term) and ten days (long term) was used as indicator to assess the effects of these toxicants on the growth of the algae.

5.3.1 Growth Rate of Algae (Based on Chl *a* Content)

The growth rate of a microalgae and macroalgae is a measure of the increase in biomass over time and it is determined from the exponential phase. Growth rate is one of the important way of expressing the relative ecological success of a species or strain in adapting to its natural environment or the experimental environment imposed upon it (Barsanti and Gualtieri, 2006). In the present study, growth rate (measured based on the Chl *a* value) are used to assess the toxicity effect of selected toxicants on growth of the algae, after being exposed for short and long term duration.

5.3.1.1 Growth rate trends in the study

For the present study, the growth rate of the *Chlorella* (1.254 day^{-1}), *Tetraselmis* (1.614day^{-1}) and *Boergesenia* (1.126day^{-1}) grown for four days in the Prov50 Medium without toxicant (control cultures) were higher than in the cultures exposed to all toxicants used in this study (Cd, Co, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion). Similar results were also obtained in the *Chlorella* (0.673day^{-1}) and *Boergesenia* (0.463day^{-1}) grown for ten days in the Prov50 Medium without toxicant (control cultures). The low value of growth rate in the cultures exposed to these toxicants in comparison to the control cultures shows that after being

exposed to these toxicant for short and long term duration, the growth rate of the algae are adversely affected.

However, for the *Ventricaria* cultures exposed to Cd, Co, Cr, Fe, Mn, Zn, Acidic dye, Metal complex dye, Dichlovos and Malathion for four days, the growth rate value obtained in these cultures were higher than in the control cultures ($0.912 day^{-1}$) between concentration 0.01 mg/L to10 mg/L toxicant. The same trends were also observed in the ten days (long term exposure) cultures of *Tetraselmis* (exposed to Cd, Co, Cu, Fe, Mn, Zn and Metal complex dye) and *Ventricaria* cultures (exposed to Co, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion) where the growth rate value obtained in these cultures were higher than in the control cultures (*Tetraselmis*: 0.759 day^{-1}; *Ventricaria*: 0.371 day^{-1}) at low concentration of toxicant (0.01 mg/L-10 mg/L toxicant).

The higher growth rate values in the cultures exposed to these toxicants than in the control cultures indicate that addition of metals like Cd, Co, Cr, Fe, Mn and Zn, which are the important micronutrients for the *Tetraselmis* and *Ventricaria* at lower concentrations of metals (0.01 mg/L – 10 mg/L metals), can enhance the growth rate of the algae. According to Barsanti and Gualtieri, (2006) this condition also indicate that the 'bioavailablity' of these micronutrients in the culture medium is less than the optimal micronutrient requirement for *Tetraselmis* and *Ventricaria* growth.

In this study, growth of the algae is expressed as the growth rate (based on Chl *a* content measurement). There were different growth trends observed in the algae exposed to selected toxicants for four days (short term exposure) and ten days (short term

exposure) as summarized in Table 5.1. Four different growth trends were obtained in this study:

(i) The growth of the algae decreased with increasing concentrations of toxicants, after the algae being exposed to toxicant for both incubation periods (four days and ten days). This condition shows that these toxicants could inhibit and give adverse effects on the growth of the algae following increasing concentrations of toxicant, after being exposed for short term (four days) and long terms (ten days) durations.

(ii) The growth of the algae increased with increasing concentration of toxicant up to 1 mg/L toxicant and then decreased at higher concentrations of toxicant, after exposure to toxicants for short term (four days) and long term (ten days) durations. This condition occurred because, at the three lowest concentrations of toxicants (0.01 mg/L to 1 mg/L toxicants) used in this study, some of the chemicals contained in these toxicants (metal, textiles dye, organophosphate pesticides) may be used as additional nutrients to enhance the growth of the algae. However at the higher concentrations of toxicants (10 mg/L to 500 mg/L toxicants), these chemicals will inhibit the growth of the algae.

(iii) The growth of the algae decreased with increasing concentrations of toxicant in the four days cultures and in the ten days cultures the growth of the algae increased with increasing concentrations of toxicant up to 0.1 mg/L toxicant and then reduced at higher concentrations of toxicant. This is because of, after being introduced and exposed to toxicant for short term (four days) durations, the algae were stressed by the new condition, therefore, the photosynthesis capacity in the algae will be reduced, in turn decreased the growth rate of the algae. However after long term (ten days) exposure to

Table 5.1: Growth rate trend in the algae cultures exposed to selected toxicants

Growth rate trend (Chl *a*)			Treatment conditions
	Day four cultures	Day ten cultures	
I	Decreased with increasing concentrations of toxicant	Decreased with increasing concentrations of toxicant	● *Chlorella* exposed to Cd, Cu, Acidic dye, Metal complex dye & Malathion ● *Tetraselmis* exposed to Mn & Metal complex dye ● *Boergesenia* exposed to Fe, Basic dye & Malathion ● *Ventricaria* exposed to Cd, Co, Fe, Mn
II	Increased with increasing concentrations of toxicant up to 1 mg/L toxicant and then decreased at higher concentrations of toxicant	Increased with increasing concentrations of toxicant up to 1 mg/L toxicant and then decreased at higher concentrations of toxicant	● *Chlorella* exposed to Cr & Co ● *Tetraselmis* exposed to Cd, Cr, Fe, Cu, Zn, Acidic dye & Basic dye ● *Boergesenia* exposed to Cr, Mn, Zn, Acidic dye & Metal complex dye ● *Ventricaria* expposed to Cr, Cu, Acidic dye & Dichlovos
III	Decreased with increasing concentrations of toxicant	Increased with increasing concentrations of toxicant up to 1 mg/L toxicant and then decreased at higher concentrations of toxicant	● *Chlorella* exposed to Fe, Zn, Basic dye & Dichlovos ● *Tetraselmis* exposed to Dichlovos & Malathion ● *Boergesenia* exposed to Cd, Co & Cu ● *Ventricaria* exposed to Basic dye & Metal complex dye
IV	Increased with increasing concentrations of toxicant up to 1 mg/L toxicant and then decreased at higher concentrations of toxicant	Decreased with increasing concentrations of toxicant	● *Chlorella* exposed to Mn ● *Tetraselmis* exposed to Co ● *Boergesenia* exposed to Dichlovos ● *Ventricaria* exposed to Zn & Malathion

this toxicant, the algae start to adapt to that conditions where at the lower concentrations of toxicants (0.01 mg/L to 0.1mg/L), the metals like Cd, Co, Cr, Cu, Fe, Mn and Zn, which are important micronutrients for the algae can enhance the growth of the algae. For the Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion, the molecule structure of these toxicants may be degraded after long term exposure to the algae. The degraded by-products may be less toxic to the algae and may induce the growth of the algae at lower concentration of toxicant. Despite that, at higher concentration of toxicants (1mg/L to 500 mg/L toxicants), the algae may not be able to tolerate these toxicants anymore and finally died after long term exposure.

(iv) The growth of algae increased with increasing concentrations of toxicant up to 0.1 mg/L toxicant and then decreased at higher concentrations of toxicant in the four days cultures and in the ten days cultures the growth of algae decreased following increasing concentrations of toxicant. This condition occurred because, after being introduced and exposed to the toxicants for short term duration (four days), the algae can still tolerate the new conditions where at the lower concentrations of toxicant, the chemicals in the toxicant (metal, textiles dye, organophosphate pesticides) can be used as additional nutrients to enhance the growth of the algae. However at the higher concentrations of toxicants, these toxicants will inhibit the growth of the algae. In spite of that, after exposure for long term duration to these toxicants, the algae cannot tolerate the conditions further, photosynthesis in algae become inhibited and finally will lead to the death of the algae.

Our results which show decreased growth rate with increasing concentration of toxicants was similarly reported by Labra, *et al.,* (2007) who showed a reduction in growth rate (based on cell number) in *Pseudokirchneriella subcapitata* following increasing concentration of toxicant, after exposure to 1.0 ppm, 2.5 ppm, 5.0 ppm and 7.5 ppm chromium, ($Cr_2K_2O_7$) for 24h, 48h 72h. They also found that the growth rate after 72h calculated for the control (0.53±0.01) was statistically higher than those estimated for cell treated with 1.0, 2.5, 5.0 and 7.5 ppm potassium dichromate (0.46± 0.02; 0.32±0.01; 0.25±0.01 and -0.02±0.04, respectively).

Reduction in growth with increasing concentrations of toxicants also has been demonstrated by Bajguz, (2010), who exposed *Chlorella vulgaris* to Cd, Cu, Pb (10^{-6}M to 10^{-4}M) for 24 hr and growth is expressed as cell numbers. Similar results shown by Geoffroy, *et al*., (2002), who exposed *Scenedesmus obliquus* to two types of herbicides, Oxyfluorfen (10 to 40µ/L) and diuron (5 to 30µ/L) for 24 hrs. Singh, *et al*., (2004) also reported that the growth of *Chlorella vulgaris* was decreased after being exposed to copper (1 to 4 µg/ml) and used chlorophyll fluorescence method to evaluate the physiological state of the algae.

The growth trend showing increase of growth rate following increasing concentrations of toxicants, at lower concentration of toxicant obtained in this study and then decrease at high concentrations of toxicants, also has been reported by Hassler, *et al*., (2005) who exposed *Chlorella kesslerii*, to different concentration of Zn ($1.6x10^{-11}$M, $1.7x10^{-9}$M and $1.6x10^{-6}$M Zn^{2+}) in growth medium for 72h and 200hr (3 to 8.3 days). Their results showed, the growth rate increased up to $1.7x10^{-9}$M Zn and decreased at higher concentration ($1.6x10^{-6}$M Zn^{2+}). They also found that *C. kesslerii* was quite

tolerant to high concentration of Zn^{2+}, since no major effects on growth were seen for $1.6x10^{-11}Zn^{2+}$.

The elevated value of algae growth following increasing concentration of toxicants at three lowest concentration (0.01 to 1 mg/L) used in this study may also indicate that the algae have developed an adaptation mechanism in order to reduce the toxicity of these toxicants in the cell. Adaptation mechanism can be critical to the toxic effects of metal and organisms have a variety of ways to adapt to toxic metal exposure. Typically adaptation is acquired after the first few exposures and can be long lasting or transient after exposure ceases (Klaassen, 2008). Adaptation can be (i) at a level of uptake (ii) or excretion (iii) or with some metal (iv) through long-term storage in toxicological inert form (v) sequestration of toxic metals (vi) Metal exposure can also induce a cascade of molecular/genetic responses that may in turn, reduce toxicity, such as with metal-induced oxidative stress responses (Valko, *et al*., 2006). One example of adaptation is: Cadmium exposure causes the overexpression of metallothionein which will sequester cadmium and reduce its toxicity as an adaptive mechanism (Klaassen and Liu, 1998).

For the present study, the results also show that the algae exposed to metal and organophosphate pesticides can enhanced the growth and tolerance to toxicant up to 1 mg/L toxicant. However in the algae exposed to textile dyes, the algae can only tolerate up to 0.1 mg/L toxicant. This is because metals such as Cu, Fe, Mn, Zn which are important trace minerals for algae growth are used to maintain the growth of the algae. The chemicals contained in the organophosphate pesticides which also includes important micronutrients such as phosphate and nitrogen or any other metal component

that are released as single metal after degradation. So, at low concentrations of toxicant (0.01 to 1 mg/L metals or organophosphate pesticides), these chemicals will enhanced the growth of algae.

In addition, baseline nutrient levels in the seawater from the habitat, also influence the growth trend of the algae. Based on the metal content in the seawater collected at the site of algae collection, these algae usually grow in medium containing metals such as Cu (0.14 mg/L), Fe (0.04 mg/L) and Zn (0.04 mg/L) which are higher than the lowest concentration levels tested in this study. Therefore, at this low concentrations of metal, the growth of algae were not yet inhibited. However at higher concentrations, algal growth is inhibited, these chemicals induced oxidative stress in the algae cell, thereby adversely affecting the photosynthesis rate in the algae.

Despite that, for the cultures that were exposed to textile dyes, the algae can only tolerate up to 0.1 mg/L textile dye which was lower than in the algae cultures exposed to metal or organophosphate pesticides. This indicates that the coloured compound in the textile dye can reduce the light penetration in the growth medium, that can be absorb by the algae during the photosynthesis process. According to Barsanti and Gualtieri (2006) and Soeder and Stengel, (1974), light plays an important role in the photosynthesis process where the relationship between irradiance and rates of photosynthesis and photoautotrophic growth show a rectangular hyperbolic function with a inhibition of growth occurring at supersaturating irradiance. However for the present study, it is suggested that, the growth rate of the algae exposed to the textile dye was lower than in the cultures that were exposeds to metals because the photosynthesis rate (which

influence the growth rate value) of the algae were reduced due to the reduction of irradiance available to the algae.

5.3.1.2 Growth rate sensitivity and tolerance in the study

To evaluate the sensitivity and tolerance of the algae to the toxicants, we ranked the growth rate (based on the highest value obtained in each group of treatment between concentration 0.01 mg/L to 500 mg/L toxicant), to assess the effects of these toxicants on growth rate of the algae. Table 5.2 summarizes the growth rate sensitivity and tolerance in the algae cultures exposed to selected toxicants.

In this study, we observed that, the algae cultures exposed to the most toxic toxicant for each type of algae: *Chlorella* (Fe, Metal complex dye and Dichlovos most toxic to *Chlorella*), *Tetraselmis* (Metal complex dye and Dichlovos most toxic to *Tetraselmis*), *Boergesenia* (Cu and Malathion most toxic to *Boergesenia*) and *Ventricaria* (Basic dye most toxic to *Ventricaria*), contained the lowest growth rate value in comparison to other toxicants tested in this study.

The same results were also observed in the *Chlorella* (Metal complex dye most toxic to *Chlorella*) and *Boergesenia* (Metal complex dye most toxic to *Boergesenia*), after being exposed for ten days (long term exposure). This condition shows that, these toxicants give significantly adverse effect on the growth rate of the algae, following increasing concentration of toxicant after short term and long term exposure.

Table 5.2: Growth rate sensitivity and tolerance in the algae cultures exposed to selected toxicants

Algae cultures	Exposure duration	Growth rate (highest value obtained in the cultures)
Chlorella vulgaris UMACC245	Four days	control (1.254 day^{-1}) Fe (0.874 day^{-1})< Zn (0.874 day^{-1})< Basic dye (0.874 day^{-1}) < Metal complex dye (0.874 day^{-1}) < Cr (0.973 day^{-1}) < Co (0.978 day^{-1}) < Mn (0.978 day^{-1}) < Cd (1.002 day^{-1}) < Cu (1.032 day^{-1}) < Dichlovos (1.044 day^{-1}) < Malathion (1.308 day^{-1}) <Acidic dye (1.131 day^{-1}).
	Ten days	control (0.673 day^{-1}) Dichlovos (0.545 day^{-1})< Malathion (0.555 day^{-1}) < Basic dye (0.561 day^{-1}) < Metal complex dye (0.561 day^{-1}) < Co (0.571 day^{-1}) < Cr (0.588 day^{-1}) < Mn (0.588 day^{-1}) < Acidic dye (0.594 day^{-1}) < Cd (0.595 day^{-1}) < Fe (0.602 day^{-1}) < Zn (0.610 day^{-1}) < Cu (0.625 day^{-1}).
Tetraselmis tetrahele UMACC 144	Four days	control (1.614 day^{-1}) Dichlovos (0.973 day^{-1})< Malathion (1.086 day^{-1}) < Metal complex dye (1.364 day^{-1}) < Basic dye (1.402 day^{-1}) < Acidic dye (1.443 day^{-1}) < Mn (1.489 day^{-1}) < Zn (1.506 day^{-1}) < Fe (1.528 day^{-1}) < Cu (1.545 day^{-1}) < Cd (1.562 day^{-1}) < Co (1.570 day^{-1}) < Cr (1.570 day^{-1}).
	Ten days	control (0.759 day^{-1}) Malathion (0.571 day^{-1})< Dichlovos (0.581day^{-1}) < Basic dye (0.743 day^{-1}) < Acidic dye (0.747 day^{-1}) < Cr (0.752 day^{-1}) < Co (0.773 day^{-1}) < Cu (0.781 day^{-1}) < Metal complex dye (0.785 day^{-1}) < Mn (0.788 day^{-1}) < Cd (0.803 day^{-1}) < Zn (0.808 day^{-1}) < Fe (0.826 day^{-1}).
Boergesenia forbesii	Four days	control (1.126 day^{-1}) Mn (0.945 day^{-1})< Co (0.948 day^{-1}) < Cu (0.956 day^{-1}) < Malathion (0.961 day^{-1}) < Metal complex dye (0.992 day^{-1}) < Basic dye (0.998 day^{-1}) < Cd (1.014 day^{-1}) < Fe (1.046 day^{-1}) < Acidic dye (1.047 day^{-1}) < Dichlovos (1.047 day^{-1}) < Cr (1.060 day^{-1}) < Zn (1.125 day^{-1}).
	Ten days	control (0.463 day^{-1}) Malathion (0.223 day^{-1})< Dichlovos (0.3610 day^{-1}) < Fe (0.405 day^{-1}) < Zn (0.410 day^{-1}) < Cr (0.419 day^{-1}) < Co (0.431 day^{-1}) < Mn (0.434 day^{-1}) < Acidic dye (0.436 day^{-1}) < Metal complex dye (0.436 day^{-1}) < Cu (0.438 day^{-1}) < Cd (0.439 day^{-1}) < Basic dye (0.442 day^{-1}).
Ventricaria ventricosa	Four days	control (0.912 day^{-1}) Basic dye (0.445 day^{-1})< Cu (0.894 day^{-1}) < Malathion (0.914 day^{-1}) < Mn (0.916 day^{-1}) < Zn (0.920 day^{-1}) < Acidic dye (0.926 day^{-1}) < Cd (0.938 day^{-1}) < Metal complex dye (0.966 day^{-1}) < Co (0.969 day^{-1}) < Dichlovos (0.975 day^{-1}) < Fe (1.068 day^{-1}) < Cr (1.077 day^{-1}).
	Ten days	control (0.371 day^{-1}) Cd (0.365 day^{-1})< Zn (0.372 day^{-1}) < Dichlovos (0.389 day^{-1}) < Acidic dye (0.400 day^{-1}) < Metal complex dye (0.401 day^{-1}) < Cr (0.404 day^{-1}) < Cu (0.404 day^{-1}) < Malathion (0.406 day^{-1}) < Fe (0.421 day^{-1}) < Mn (0.421 day^{-1}) < Co (0.422 day^{-1}) < Basic dye (0.430 day^{-1}).

Chloroplasts have been reported as a primary target for metal toxicity in *Chlorella* species (Wong, *et al*.,1994). Heavy metals inhibit photosynthesis by disrupting the electron transport chain, and/or replacing the magnesium atom (Mg^{2+}) of chlorophyll molecules (Kupper, *et al.*,1996; Kupper, *et al.*,1998), thus damaging the photosystems and the antenna complex. In addition, metal like copper can affect the synthesis of the D1 protein that assembles chlorophyll molecules in the reaction center (Patsikka, *et al*.,1998).

This data suggests that toxicant used in this study, even from the lowest tested concentration, are highly toxic to the algae, and that this algal strain is a sensitive organism suitable for monitoring chemical contaminats such as metals, textile dye and organophosphate pesticides in marine water.

For present study, we also found that exposure of organophosphate pesticides for long term duration to the algae can also reduced the growth rate of the algae. As shown in our results, the *Chlorella, Tetraselmis* and *Boergesenia* cultures exposed to Dichlovos and Malathion for ten days produced the lowest growth rate in comparison to other toxicants tested which indicate that Dichlovos and Malathion are highly toxic to these algae. This condition may be caused by several factors such as excess nutrients, light limitation and absorption capacity of the algae.

In general, organophosphate pesticides contained phosphorus compounds. Phosphorus play a significant role in most cellular process, especially those involved in generating and transforming metabolic energy. Therefore deficiencies or excess of phosphorus compounds in the algae cell can disturb the role of phosphorylated

compounds in the conversion of light energy to biological energy in green plants (Stewart, 1974; Barsanti and Gualtieri, 2006).

For this case, we observed that, the colour of the culture medium are quite cloudy due to undissolved organophosphate pesticides in the aqueous medium (especially in the medium containing high concentration of organophosphate pesticides) which indicate that the irradiance that can be absorped by the algae is reduced by the presence of small undissolved particles obtained in the culture medium. Therefore, the photosynthesis rate was reduced due to lower irradiance available to the algae. As reported by Okamoto, *et al.,* (2001), reduction in light harvesting pigments like Chl *a* and carotenoid can result in reduced growth rate of the algae during acute and chronic metal stress, due to reduced photosynthetic capacity. Indeed, photosynthesis is probably the metabolic process most sensitive to environmental stress in plants and algae.

In addition, the absorption capacity also plays an important role in toxicity mechanism. Absorption is the transfer of a chemical from the site of exposure, usually an external or internal body surface, into the systemic circulation. The rate of absorption is related to the concentration of the chemical at the absorbing surface, which depends on the rate of exposure and the dissolution of the chemical. It is also related to the area of the exposed site, the physicochemical properties of the toxicant and etc. Lipid solubility is usually the most important property influencing absorption. In general, lipid-soluble chemicals such as organophosphate pesticides are absorbed more easily than are water-soluble substances. Therefore, the absorption capacity of the organophosphate pesticides into the algae cell is higher which will increase its toxicity level to the algae.

In comparison of growth rate of the algae between short term (4 days) and long term (10 days) exposure, we observed that the growth rate of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* grown for four days in the Prov50 medium without toxicant (control cultures) was 46.33%, 52.97%, 58.88% and 59.32% respectively higher than in the ten days cultures. This condition may be because of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* cultures grown in Prov50 medium without toxicant were able to reach exponential phase during two to four days where the growth rate of the algae was very high due to higher capacity of photosynthesis rate during this period. However, after being grown for ten days, the algae already reach the stationary phase where the growth of the algae will become constant. Therefore the growth rate of the four days cultures will be higher than in the ten days cultures.

The higher growth rate in the algae cultures exposed to the toxicants for four days than in the ten days culture, indicate that, growth rate of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* cultures exposed to toxicant for short term duration (four days), could still can tolerate the presence of the toxicants. However after been exposed for long term (10 days), the tolerance of the algae were exceeded, leading to the death of the algae.

However, for the *Ventricaria* cultures exposed to Basic dye, the growth rate value in ten days cultures was higher than in four days cultures, showing *Ventricaria* can tolerate the toxicity of Basic dye even after long term exposure. This condition may be due to the molecular structure of Basic dye being degraded after long term exposure to the algae. The degraded by-products may less toxic to the algae and may even served as nutrients to enhance the growth of the algae.

Toxicants can produce differential effects on algae species (and even strains within the same species) (Kessler, 1986; Baos, *et al*., 2002; Yan and Pan, 2002). The differential effects maybe due to morphological and structural factors such as size, cell volume and presence or absence of a cell wall and mucilaginous sheet and their chemical composition (Schiariti, *et al*., 2004; Levy, *et al*., 2007), and also their physiological mechanisms for metal detoxification.

Higher value of growth rate obtained in *Tetraselmis* than in *Chlorella* after being exposed to toxicants for short term and long term duration may be due to the differences in cell wall function and composition of each algae species used in this study. These factors allow the algae to tolerate different types and high concentrations of toxicants.

However, for the *Chlorella* cultures exposed to Dichlovos (for four days) and Malathion (for four days and ten day) the growth rate of *Chlorella* cultures was higher than the *Tetraselmis* cultures which indicate that *Chlorella* is more tolerant to Dichlovos and Malathion, than Tetraselmis after being exposed to for four and ten days. Higher tolerance of *Chlorella* to organophosphate pesticides used in this study, in comparison to *Tetraselmis* may be because *Chlorella*, the cosmopolitan microalgae usually found growing in seawater, have already adapted to organophosphate pesticides that are discharged regularly into the marine water system from the agriculture sector. Therefore *Chlorella* may have become more tolerant to the organophosphate pesticides than *Tetraselmis*.

The ability of microalgae to remove metals from their system, is one of the important factors that determine the difference in sensitivity and tolerance of algae species to the toxicants. As demonstrated by Perez-Rama, *et al*., (2010), who used a sorption isotherm model to investigate the Cd biosorption capacity in living cell of the marine microalgae *Tetraselmis suecica*, which have high tolerance to this metal (Perez-Rama, *et al.*, 2006), they found that *Tetraselmis suecica* have the ability to remove metals like Cd which have the maximum sorption capacity estimated to be 40.22 mg Cd/g after 72h using the Langmuir sorption model .

According to Sabatini, *et al*., (2009), who compared the sensitivity of two microalgae (*Scenedesmus vacuolatus* and *Chlorella keslleri*) to copper, the different capacity to accumulate extra- and intracellular copper by *Scenedesmus vacuolatus* and *Chlorella keslleri* indicated that these species have different protection mechanisms against metal exposure. They suggested that the possibility is that the complex polysaccharides of the *Scenedesmus vacuolatus* cell wall were an efficient barrier to avoid metals entering into the cytoplasm, thus resulting in low intracellular metal accumulation. In addition, size and cellular volume, copper internalization capability as well as differences in the chemical composition of cell walls, together with the observed antioxidant response, may explain this tolerance differences.

5.3.2 IC_{50} Value

IC_{50} value (concentrations to inhibit growth by 50%) is one of the important toxicity endpoints use in this study to evaluate the sensitivity of algae to the toxicants after being exposed for short (4 days) and long (ten days) term duration. According to

Levy, *et al.,* (2007), there are several factors affecting algae sensitivity to toxicants: (i) cell size, (ii) cell wall type, (iii) taxonomic grouping (iv) metal-cell partitioning and other factors such as different up-take rates for algal species; relationship toxicant internalization and adsorption to non specific surface binding; detoxification mechanisms include exclusion, internal sequestration and active efflux mechanisms; induction of the antioxidant enzymes like superoxide dismutase and etc.

5.3.2.1 Algae sensitivity to toxicants based on IC_{50} value

For present study, we used IC_{50} value to determine the sensitivity and tolerance level of the algae exposed the toxicants by ranking their IC_{50} value. Table 5.3 summarizes the algae sensitivity to toxicant based on IC_{50} value. The sensitivity of the four marine algae to toxicants are varied with IC_{50} values ranging from 0.0050mg/L to 213.28 mg/L toxicants. It is difficult to compare the sensitivity of these species with literature data due to the different test conditions, cell densities and test media used.

(i) Toxicity of metals used in the study

Metals are nondegradable and can accumulate and concentrate as they move up the food chain (Bajguz, 2010). The toxic effect of metals on algae growth and development is commonly known (Mellado, *et al.*, 2012; Piotrowska-Niczyporuk, *et al.*, 2012; Han, *et al.*, 2008; Ip and Chen, 2005). Inhibition of growth will limited photosynthesis and respiration process in the cell. The most frequently observed symptoms of metal toxicity are inhibited biosynthesis of chlorophyll and carotenoids and reduced phosphorylation. However, some algae are able to grow on sites contaminated with metals. This condition occurred as a result of several mechanisms, which reduce the toxicity of metal ions. These include the immobilisation of metals within cell wall,

Table 5.3: Algae sensitivity to toxicant based on IC_{50} value

Algae cultures	Exposure duration	IC_{50} value
Chlorella vulgaris UMACC245	Four days	Basic dye (0.0064mg/L)= Metal complex dye (0.0064mg/L) < Fe (0.0066mg/L) < Co (0.0067mg/L) < Cr (0.0068mg/L) < Zn (0.0070mg/L) < Cu (0.0076mg/L) < Cd (0.0079mg/L) < Mn (0.0081mg/L) < Dichlovos (0.0088mg/L) < Acidic dye (0.0332mg/L) < Malathion (0.0918mg/L)
	Ten days	Dichlovos (0.0065mg/L) < Malathion (0.0072mg/L) < Basic dye (0.0074mg/L) < Metal complex dye (0.0074mg/L) < Co (0.0076mg/L) < Cr (0.0077mg/L) < Fe (0.0082mg/L) < Mn (0.0087mg/L) < Acidic dye (0.0091mg/L) < Cd (0.0092mg/L) < Cu (0.0973mg/L) < Zn (1.0353mg/L).
Tetraselmis tetrahele UMACC 144	Four days	Dichlovos (0.0054mg/L) < Malathion (0.0057mg/L) < Metal complex dye (0.0079mg/L) < Basic dye (0.0085mg/L) < Acidic dye (0.0094mg/L) < Cu (0.5574mg/L) < Zn (1.6632mg/L) < Fe (2.8923mg/L) < Co (4.8514mg/L) < Mn (5.1250mg/L) < Cd (6.5138mg/L) < Cr (8.288mg/L).
	Ten days	Dichlovos (0.0060mg/L) = Malathion (0.0060mg/L) < Cr (3.1040mg/L) < Cu (5.4390mg/L) < Metal complex dye (5.9060mg/L) <Cd (7.1740mg/L) < Mn (8.8580mg/L) <Co (9.7710mg/L) < Basic dye (41.9660mg/L) <Acidic dye (52.2550mg/L) <Zn (74.3920mg/L) < Fe (97.9680mg/L)
Boergesenia forbesii	Four days	Cu (0.0250mg/L) < Mn (0.0720mg/L) < Co (0.0800mg/L) < Malathion (0.2770mg/L) < Metal complex dye (6.7860mg/L) < Basic dye (6.9320mg/L) < Cr (8.9740mg/L) < Acidic dye (20.4970mg/L) < Fe (23.8000mg/L) < Dichlovos (30.6250mg/L) < Cd (52.9550mg/L) < Zn (68.3700mg/L).
	Ten days	Malathion (0.0050mg/L) < Dichlovos (0.0052mg/L) < Co (4.2900mg/L) < Zn (18.7000mg/L) < Cu (19.8830mg/L) < Fe (25.2340mg/L) < Mn (32.3880mg/L) < Metal complex dye (36.3460mg/L) < Cr (54.0010mg/L) < Cd (54.9170mg/L) < Basic dye (67.3270mg/L) < Acidic dye (126.5730mg/L).
Ventricaria ventricosa	Four days	Basic dye (0.0100mg/L) < Cd (5.9800mg/L) < Cu (6.2100mg/L) < Acidic dye (8.9200mg/L) < Mn (10.4500mg/L) < Co (13.1800mg/L) < Cr (16.1500mg/L) < Dichlovos (16.4300mg/L) < Metal complex dye (26.6800mg/L) < Fe (52.9700mg/L) < Zn (57.4200mg/L) < Malathion (100.0000mg/L).
	Ten days	Zn (8.8100mg/L) < Fe (15.5500mg/L) < Basic dye (20.6200mg/L) < Metal complex dye (27.1100mg/L) < Mn (45.6000mg/L) < Acidic dye (46.1600mg/L) < Co (52.0200mg/L) < Cr (55.9900mg/L) < Cu (56.9900mg/L) < Cd (62.8300mg/L) < Dichlovos (178.1700mg/L) < Malathion (213.2800mg/L).

reduction of metal uptake at plasmamembranes, sequestration of metals in vacuoles and complexation of metal ions within cytoplasm by phytochelatins (Poskuta, *et al*., 1996; Bajguz, 2004; Cobbett and Goldsbrough, 2002).

For the present study, based on the IC_{50} value obtained in all the algae cultures grown for four days in the Prov50 Medium containing selected metal used in this study, Fe was the most toxic metal to *Chlorella* (with IC_{50} value of 0.0066mg/L) and Cu was the most toxic metal to *Tetraselmis, Boergesenia* and *Ventricaria*, with IC_{50} value of 0.5574mg/L, 0.0250mg/L and 6.2069mg/L respectively.

However, Mn was least toxic metal to *Chlorella* (with IC_{50} value of 0.0081mg/L), Cr was least toxic metal to *Tetraselmis* (with IC_{50} value of 8.2880 mg/L), and Zn was least toxic metal to *Boergesenia* and *Ventricaria*, (with IC_{50} value of 68.3696 mg/L and 57.4183 mg/L respectively).

In the algae cultures exposed to the toxicants for ten days, Co was the most toxic metal to *Chlorella* and *Boergesenia* (with IC_{50} value of 0.0076 mg/L and 4.2895mg/L respectively); Cr was the most toxic metal to *Tetraselmis* (with IC_{50} value of 3.1037 mg/L), and Zn was the most toxic metal to *Ventricaria* (with IC_{50} value of 8.8095mg/L).

However, Zn was least toxic metal to *Chlorella* (with IC_{50} value of 1.0353 mg/L), Fe was least toxic metal to *Tetraselmis* (with IC_{50} value of 97.9684 mg/L), and Cd was least toxic metal to *Boergesenia* and *Ventricaria* (with IC_{50} value of 54.9171mg/L and 62.8261mg/L respectively).

Our results shows the *Chlorella* exposed to Fe have the lowest growth rate in comparison to other toxicants tested in this study, indicating that *Chlorella* are most sensitive to Fe. Fe is an essential metal for heme enzymes, metalloflavoprotein enzymes and mitochondrial enzymes. Fe metabolism is regulated by a complex series of events that maintain homeostasis, mainly involving absorption, storage and excretion. The excess Fe may produce oxidative stress via the Fenton reaction. As reported by previous researchers, Fe has been widely used as toxicant induced oxidative stress studies in the algae (Johnstone, *et al.*, 2006; Ip and Chen, 2005; Estevez, *et al.*, 2001).

In this study we also observed that Cu was the most toxic metal to *Tetraselmis*, *Boergesenia* and *Ventricaria*. Cu is essential component of several metalloenzymes, including cytochrome c oxidase, dopamine ß-hydroxylase, superoxide dismutase and lysyl oxidase. Cu is also a cofactor for important enzymes. Cu absorption may also be dependent on the protein metallothinien since excess intake of either Cu or Zn inteferes with the absorption of the other (Klaassen, 2008). Previous studies have demonstrated that Cu was toxic to the green algae such as *Chlorella* (Bajguz, 2010; Sabatini, *et al.*, 2009; Xia and Tian, 2009; Singh, *et al*., 2004), *Scenedesmus* (Sabatini, *et al*., 2009; Tripathi, *et al*., 2006; Tripathi and Gaur, 2004), *Ulva* (Mellado, 2012), *Tetraselmis* (Debelius, *et al*., 2008) and *Nannochloropsis* (Debelius, *et al*., 2008). As reported by Levy, *et al*., (2007) who measured sensitivity of 11 marine microalgae to copper for 72hr, they found that *Minutocellus polymorphus* was the most sensitive species to copper and *Dunaliella tertiolecta* the least sensitive, with 72-h IC_{50} values (concentration to inhibit growth rate by 50%) of 0.6 and 530 µg Cu/L, respectively.

(ii) Toxicity of textile dyes used in the study

Textile dye usually containing highly coloured substance that can reduce light intensity absorbtion by the algae during photosynthesis process. The toxic effect of textile dye on algae are rarely reported by previous researchers. However, the treatment process to remove and reduce textile dye contamination in the aquatic system using algae or other organism such as bacteria and fungi are widely reported in literature (Lim, *et al*., 2010; Mostafa, *et al*., 2009; Aksu and Tezer, 2005; Acuner and Dilek, 2003).

In this study, our results show that for the algae cultures exposed to textile dye for short term duration (4 days), Metal complex dye was the most toxic textile dye to *Chlorella, Tetraselmis* and *Boergesenia* (with IC_{50} value of 0.0064 mg/L, 0.0079 mg/L and 6.7857 mg/L respectively) and Basic dye was the most toxic textile dye to *Ventricaria* (with IC_{50} value of 0.0059 mg/L).

However, Acidic dye was the least toxic textile dye to *Chlorella, Tetraselmis* and *Boergesenia* (with IC_{50} value of 0.0332 mg/L, 0.0094 mg/L and 20.4966 mg/L respectively) and Metal complex dye was the least toxic textile dye to *Ventricaria* (with IC_{50} value of 26.6757 mg/L).

For the algae cultures exposed to textile dye for long term duration (ten days), our results shows that, Metal complex dye was the most toxic textile dye to *Chlorella, Tetraselmis* and *Boergesenia* (with IC_{50} value of 0.0074 mg/L; 5.9064 mg/L and 36.3462mg/L respectively) and Basic dye was the most toxic textile dye to *Ventricaria* (with IC_{50} value of 20.6174 mg/L).

However, Acidic dye was the least toxic textile dye to *Chlorella*, *Tetraselmis*, *Boergesenia* and *Ventricaria* (with IC_{50} value of 0.0091 mg/L; 52.2552 mg/L; 126.5732mg/L and 46.1649 mg/L respectively).

(iii) Toxicity of organophosphate pesticides used in the study

Organophosphate pesticides is a compound containing phosphate used to kill pests like insect, plant patogent, weeds, mollusks, birds, mammals, fish, nematodes and microbes. It is widely used in the agriculture farm and it can enter the marine system via runoff and discharge of effluent into the river system and directly to the sea. The toxic effect of organophosphate pesticide on aquatic organisms which showed reduction in growth have been demostrated in several studies carried out by Coutris, *et al*., (2011); Kock, *et al.,* (2010); Abdel-Halim, *et al*., (2006) and etc.

In this study, based on the IC_{50} value obtained in all the algae cultures grown for four days in the Prov50 Medium containing selected organophosphate pesticides used in this study, Dichlovos was the most toxic organophosphate pesticides to *Chlorella*, *Tetraselmis* and *Ventricaria* (with IC_{50} value of 0.0088 mg/L, 0.0054 mg/L and 16.4286 mg/L respectively) and Malathion was the most toxic organophosphate pesticides to *Boergesenia* (with IC_{50} value of 0.2773 mg/L).

In contrast, Malathion was the least toxic organophosphate pesticides to *Chlorella, Tetraselmis* and *Ventricaria* (with IC_{50} value of 0.0918 mg/L, 0.0057 mg/L and 100.00 mg/L respectively) and Dichlovos was the least toxic organophosphate pesticides to *Boergesenia* (with IC_{50} value of 30.6250 mg/L).

For the algae cultures exposed to organophosphate pesticides for ten days, Dichlovos was the most toxic organophosphate pesticides to *Chlorella* and *Ventricaria* (with IC_{50} value of 0.0065 mg/L and 178.1703mg/L respectively) and Malathion was the most toxic organophosphate pesticides to *Tetraselmis* and *Boergesenia* (with IC_{50} value of 0.0057 mg/L and 0.0050mg/L respectively).

However, Malathion was the least toxic organophosphate pesticides to *Chlorella* and *Ventricaria* (with IC_{50} value of 0.0072 mg/L and 213.2829 mg/L respectively) and Dichlovos was the least toxic organophosphate pesticides to *Tetraselmis* and *Boergesenia* (with IC_{50} value of 0.0059 mg/L and 0.0052 mg/L respectively).

Our results which shows lowest IC_{50} value obtained in the algae cultures exposed to the organophosphate pesticides are similar as reported by Geoffroy, *et al*., (2002), who have exposed two types of herbicides, Oxyfluorfen (10 to 40µ/L) and diuron (5 to 30µ/L) to *Scenedesmus obliquus* for 24 hrs. Their results, showed a very low IC_{50} value obtained in the cultures exposed to both herbicides (Oxyfluorfen:15µ/L; and diuron: 10µ/L) and this indicated that the two compounds were very toxic to *Scenedesmus obliquus*.

5.3.2.2 Comparison of IC_{50} value of algae between short term (4 days) and long term (10 days) exposure

In comparison of IC_{50} value of algae between short term (4 days) and long term (10 days) exposure, in this study we observed that, the IC_{50} value of *Chlorella* [exposed to Acidic dye (72.59%), Dichlovos (26.14%) and Malathion (92.16%)]; *Tetraselmis* [exposed to Cr (62.55%)]; *Boergesenia* [exposed to Zn (72.65%), Dichlovos (99.98%)

and Malathion (98.02%)] and *Ventricaria* [exposed to Fe (70.65%)] in four days cultures was higher than in the ten days cultures. This condition indicate that the tolerance of *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* exposed to Cr, Fe, Zn, Acidic dye, Dichlovos and Malathion was decreased with increasing duration of exposure.

However, the IC_{50} value of *Chlorella* cultures [exposed to Cd (14.13%), Co (11.84 %), Cr (11.69%), Cu (92.19%), Fe (19.51%), Mn (6.90%), Zn (99.32%), Basic dye (13.51%) and Metal complex dye (13.51%); *Tetraselmis* [exposed to Cd (9.21%), Co (50.35%), Cu (89.75%), Fe (97.05%), Mn (42.14%), Zn (97.76%), Acidic dye (99.98%), Basic dye (99.98%), Metal complex dye (99.87%), Dichlovos (8.47%) and Malathion (0%)]; *Boergesenia* [exposed to Cd (3.57%); Co (98.13%), Cr (83.38%), Cu (99.87%), Fe (5.68%), Mn (99.78%), Acidic dye (83.81%), Basic dye (89.70%) and Metal complex dye (81.33%)] and *Ventricaria* [exposed to Cd (90.48%), Co (74.67%), Cr (71.15%), Cu (89.11%), Mn (77.09%), Acidic dye (80.68%), Basic dye (99.97%), Metal complex dye (1.59%), Dichlovos (90.78%) and Malathion (53.11%)] in the ten days cultures were higher than in four day cultures.

The higher value of IC_{50} value in ten days cultures than in the four days cultures shows that, the tolerance of *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* to these toxicants was increased with increasing of duration of exposure. This indicate that these four marine algae can tolerate and adapted to these toxicants after long term exposure.

5.3.2.3 Comparison of IC_{50} value of microalgae between *Chlorella vulgaris* UMACC 245 and *Tetraselmis tetrahele* UMACC 144

(i) Short term exposure (4 days).

Based on the IC_{50} value obtained in the each types of treatment tested, the IC_{50} value of *Tetraselmis* cultures exposed for four days to Cd (99.88%), Co (99.86%), Cr (99.92%), Cu (98.64%), Fe (99.77%), Mn (99.84%), Zn (99.58%), Basic dye (24.71%) and Metal complex dye (18.99%), was higher than the *Chlorella* cultures showing that *Tetraselmis* was more tolerant to Cd, Co, Cr, Cu, Fe, Mn, Zn, Basic dye and Metal complex dye in comparison to *Chlorella* after being exposed for short term duration.

However, for the microalgae cultures exposed to Acidic dye (71.69%), Dichlovos (38.64%) and Malathion (93.79%), the IC_{50} value in *Chlorella* cultures were higher than in the *Tetraselmis* cultures, indicating that *Chlorella* was more tolerant to Acidic dye, Dichlovos and Malathion than to Tetraselmis after being exposed for short term duration.

(ii) Long term exposure (10 days).

In the long term exposure algae culture to toxicants, the IC_{50} value of *Tetraselmis* cultures exposed for ten days to Cd (99.87%), Co (99.92%), Cr (99.75%), Cu (98.21%), Fe (99.99%), Mn (99.90%), Zn (98.61%), Acidic dye (99.98%), Basic dye (99.98%) and Metal complex dye (99.87%), was higher than in the *Chlorella* cultures showing that *Tetraselmis* was more tolerant to Cd, Co, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye and Metal complex dye in comparison to Chlorella after being exposed for long term duration.

Despite that, for the cultures exposed to Dichlovos (9.23%) and Malathion (20.83%) the IC_{50} value in *Chlorella* cultures were higher than in the *Tetraselmis* cultures shows that *Chlorella* was more tolerant to Dichlovos and Malathion in comparison to *Tetraselmis* after being exposed for long term duration.

5.3.2.4 Comparison of IC_{50} value of macroalgae between *Boergesenia forbesii* and *Ventricaria ventricosa*

(i) Short term exposure (4 days).

The IC_{50} value in the *Boergesenia* cultures exposed for four days to [Co (99.39%), Cr (44.44%), Cu (99.60%), Fe (55.07%), Mn (99.31%), Metal complex dye (74.56%), and Malathion (99.72%)] were higher than in the *Ventricaria* cultures indicate that *Boergesenia* was more tolerant to Co, Cr, Cu, Fe, Mn, Metal complex dye and Malathion in comparison to *Ventricaria* after being exposed for short tem duration.

However for the cultures exposed to Cd (88.70%), Zn (16.02%), Acidic dye (56.48%), Basic dye (99.91%), Dichlovos (46.36%) the IC50 value was higher in *Ventricaria* cultures than in *Boergesenia* cultures showing that *Ventricaria* was more tolerant to Cd, Zn, Acidic dye, Basic dye and Dichlovos than Boergesenia after exposure for short term duration.

(ii) Long term exposure (10 days).

The *Boergesenia* cultures exposed for ten days to Cd (12.59%), Co (91.75%), Cr (3.55%), Cu (65.11%), Mn (28.98%), Dichlovos (100%) and Malathion (100%), contained higher IC_{50} value compared to the *Ventricaria* cultures showing that

Boergesenia was more tolerant to Cd, Co, Cr, Cu, Mn, Dichlovos and Malathion in comparison to *Ventricaria* after being exposed for long term duration.

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Even so, for the cultures exposed to Fe (38.39%), Zn (52.89%), Acidic dye (63.53%), Basic dye (69.38%), Metal complex dye (25.42%) the IC_{50} value in the *Ventricaria* cultures were higher than in the *Boergesenia* cultures indicating that *Ventricaria* was more tolerant to Fe, Zn, Acidic dye, Basic dye and Metal complex dye in comparison to *Boergesenia* after being exposed for long term duration.

In this study, we observed that, cell size is one of the important characteristics to be evaluated to determine the sensitivity of algae to the toxicants. As shown in our results, the microalgae *Chlorella* (8.5±0.5µm) have smaller cell size compared to *Tetraselmis* (14±0.5µm) and macroalgae *Ventricaria* (0.3±0.1mm) is smaller than *Boergesenia* (0.8±0.1mm). Both small sized algae are more sensitive to toxicants compared to bigger sized species. As reported by Quigg, *et al*., (2006) the smaller cells are more sensitive to copper exposure due to their greater surface/volume ratio. The smaller size of the algae will have higher potential for the toxicant to attach to its cell surface and increased the absorbtion capacity of toxicant into the cell, which will increase production of ROS in the cell during photosynthesis process and directly increase the oxidative-stress level in the algae. Therefore lower value of IC_{50} obtained in the the *Chlorella* indicate that *Chlorella* is more sensitive to toxicants compared to *Tetraselmis*.

5.3.4 Carotenoid Content

Carotenoids are tetraterpenes, that is they are made up of eight isoprenoid (ip) (branched 5-carbon) units. They can be considered to be formed by the tail to tail condensation of two 20 carbon units, themselves formed by head to tail condensation of four isoprenoid (ip) residues. The first C40 polyene formed biosynthetically is phytoene which is stepwise desaturated to form lycopene which is probably the precursor of all carotenoids found in algae (Goodwin, 1974). According to Takaichi, (2011), in the Chlorophyceae, lycopene is synthesis using 1-deoxy-D-xylulose-5-phosphate (DOXP) pathway in the plastids. In this pathway, pyruvate and glycelaldehyde are converted to Isopentenyl pyrophosphate (IPP). IPP is a C_5-compound, is the source of isoprenoids, terpenes, quinones, sterols, phytol of chlorophylls, and carotenoids (Takaichi, 2011).

Carotenes relevant to chlorophyll-dependent photosynthesis are based on a C-40 structure. They are usually either hydrocarbon (carotenes, eg: α-carotene, ß-carotene, ε-carotene) or oxygenated hydrocarbons (carotenols or xanthophylls, eg: zeaxanthin, violaxanthin, neoxanthin) (Gregory, 1989; Hall and Rao, 1994; Takaichi, 2011). The main carotenoids pigments in algae chlorophyta are ß-carotene, α-carotene, lutein, violaxanthin, neoxanthin and zeaxanthin (Takaichi, 2011, Strain 1958; 1966; Goodwin, 1971; Hager and Stransky, 1970). ß-carotene, existing in fairly high concentration, is the only universal carotenoid of the algae.

Like chlorophyll, the condition such as nutrition, light intensity, UV radiation, metal toxicity and other environmental stress will affects the carotenoid content in the algae cell (Yoshii, *et al*., 2012; Steiger, *et al*., 1999; Grung, *et al*., 1993; Grung and Liaoen-Jensen, 1993; Gala and Giesy, 1993; Millie, *et al*., 1990). For the present study,

the effects of chemical contaminants such as metals, textile dyes and organophosphate pesticides on the carotenoid content of the exposed algae is evaluated.

5.3.3.1 Carotenoid content trend in macroalgae in the study

The carotenoid content of the *Boergesenia* grown for four days (0.0386ug/g) and ten days (0.0417ug/g) in the Prov50 Medium without toxicant (control cultures) were higher than in the cultures that exposed to all toxicants used in this study (Cd, Co, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion).

The higher value of carotenoid content obtained in the control cultures in comparison to the algae cultures that exposed to these selected toxicants indicate that these toxicants give adversed effect on carotenoid content of the algae after being exposed for short and long term duration.

However, for the *Ventricaria* cultures exposed to all toxicants used in this study except Basic dye, for four days (short term exposure), and also for ten days (long term exposure), the carotenoid content were higher than in the control cultures (four days: 0.0154ug/g; ten days: 0.0167 ug/g) between concentration 0.01 mg/L to 10 mg/L toxicant. This condition shows that additional input of metals like Cd, Co, Cr, Fe, Mn and Zn, which served as important nutrients for the Ventricaria at lower concentrations of toxicant (0.01 mg/L – 10 mg/L) and enhanced the carotenoid content in the algae.

In this study, different carotenoid content trends were observed in the macroalgae after being exposed to selected toxicants for four and ten days are summarised in Table 5.4. The first trend observed was, the carotenoid content of the algae cultures exposed to toxicants for four and ten days, decreased with increasing concentrations of toxicant. This condition indicate that, these toxicants could inhibit the carotenoid synthesis (carotenogenesis) in the algae following increasing concentrations of toxicant.

Table 5.4: Carotenoid content trend in the algae cultures exposed to selected toxicants

Carotenoid content trend	Four days cultures	Ten days cultures
The carotenoid content of the algae decreased with increasing concentrations of toxicant	● *Boergesenia* (exposed to Cd, Cu, Fe, Acidic dye, Basic dye and Malathion) ● *Ventricaria* (exposed to Basic dye)	● *Boergesenia* (exposed to Fe and Malathion).
The carotenoid content of the algae increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant.	● *Boergesenia* (exposed to Co, Cr, Mn, Zn, Metal complex dye and Dichlovos)	● *Boergesenia* (exposed to Cd, Co, Cr, Cu, Mn, Zn, Acidic dye, Basic dye, Metal complex dye and Dichlovos)

Our results which show the decrease of carotenoid content in the macroalgae cultures exposed to the different types of toxicants which potentially induced oxidative stress in the cell were similar to that reported by Piotrowska-Niczyporuk, *et al*., (2012) who demonstrated that the carotenoid content of the *Chlorella vulgaris* was reduced in response to metal (Cd, Cu, Pb) stress.

According to Cemeli, *et al*., (2009), carotenoids are the most potent biological quenchers of singlet O_2. Carotenoids interact with singlet O_2 either via a physical quenching mechanism, where the excited energy from singlet O_2 is transferred to the carotenoid and then dispersed to the surroundings as heat, or via chemical quenching, in which the carotenoid is destroyed in the process by addition of O_2 to its double bond system (Liebler,1993; Di Mascio, *et al*., 1989). Therefore, in this study it is suggested that one of the reason, the carotenoid content reduced in the cultures exposed to toxicant following increasing concentration of toxicants may be because the molecular structure of the carotenoid is destroyed due to attack by reactive singlet oxygen ($^1O_2$) or other free radicals that abundantly occurred in the chloroplast, after the algae are exposed to different concentrations of the toxicant-induced oxidative stress for short and long term duration.

The second pattern of the carotenoid content observed in the algae cultures exposed to the toxicants for short and long term duration was, the carotenoid content of the algae increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. The increase of carotenoid content at the four lower concentrations of toxicants used in this study shows that the metals like Cd, Co, Cr, Cu, Fe, Mn and Zn, which are the important nutrients for the algae can induce the carotenoid synthesis in the algae at lower concentrations of toxicant (0.01 mg/L to 10 mg/L). Higher concentrations of toxicant (100 mg/L to 500 mg/L) can inhibit the carotenoid synthesis in the algae. For the Acidic dye, Basic dye, Metal complex dye and Dichlovos, the molecular structure of these toxicants maybe degraded after long term exposure to the algae. The degraded by-products may be less toxic to the algae and may then induce carotenoid synthesis in the algae at lower concentration of

toxicant. However, at higher concentration of toxicant, the tolerance threshold of the algae is exceeded, leading to death of the algae.

The same results were also reported by Ip and Chen (2005), who exposed *Chlorella zofingiensis* in heterotrophic culture to the Fe^{2+}, various concentration of ROS generator (H_2O_2 and NaClO) for five days. They found that, the amount of carotenoids produced under heterotrophic condition at low concentrations of toxicant, were induced in some ROS-treated cultures. For instance, in the culture containing 0.001 mM NaClO, the contents of primary carotenoids were elevated. However at higher concentration of ROS-treated culture (0.01 to 1 mM), the carotenoid content decreased with increasing concentration of toxicants. They suggested that the amount of carotenoids produced under such conditions might be far outbalanced by the toxicity imposed by reactive singlet oxygen ($^1O_2$) in the cultures; thus, resulting in increased of cell mortality and decreased carotenogenesis. It seems that the biosynthesis of carotenoid also depends on the nature of ROS applied in the culture medium (Ip and Chen, 2005).

For the present study, different trends were observed in the *Boergesenia* (exposed to Co) and *Ventricaria* (exposed to Zn) for four days where the carotenoid content should be decreased with increasing concentrations of toxicant or following the same trend as the growth of the algae. However in these cultures, the carotenoid content was increased with increasing concentrations of toxicant up to 10 mg/L toxicant and then decreased at higher concentrations of toxicant. The same trend were also observed in the Boergesenia (exposed to Fe and Basic dye) and Ventricaria (exposed to Malathion), after being exposed for ten days (long term exposure).

The increase in carotenoid content at low concentrations of toxicant (0.01mg/L to 10 mg/L toxicant) which showed the opposite trend to the growth (Chl *a* content) of algae may be because, the carotenoid pigment was synthesised abundantly and accumulated in the cell, to compensate for reduction in photosynthesis caused by these toxicants which induced oxidative stress in the cell. The algae increased carotenoid content to capture more radiant energy to assist in photosynthesis, especially if Chl *a* pigment had also been reduced or destroyed (Takaichi, 2011). This is may be one of the adaptive and protective mechanismes carried out by algae to protect the cell from the oxidative damages caused by these toxicants.

As reported by Murthy *et al*., (2005), carotenoids fight against free radicals in more than one mechanism, such as by supplying missing electron to the free radicals from other molecules or by forming adduct with such radicles. In both the cases electron rich nature of carotenoids make them attractive to radicles, by which they protect the cell from radical damage (Di Masico, *et al*., 1989).

According to Gregory, (1989), the carotenes can be oxygenated in various ways to give a great diversity of xanthophylls. To some extent this is reversible and maybe part of the operation of their protective role against oxygen. The excited state of chlorophyll has a normally short lifetime and is relatively immune from attack by agents such as oxygen; however, the normal 'singlet' excitation state can change to a 'triplet' state with a longer lifetime. Carotenoids appear to deactivate the triplet state of chlorophyll and either annihilate the excitation (as heat) or combined with oxygen sacrificially, as xanthophylls cycle. Oxygenated carotenes (xanthophylls) are virtually universal. In higher plants and green algae, the principal form is lutein, with some

violaxanthin and neoxanthin. These carotenes have epoxy groups and take part in the xanthophylls cycle. This is associated with PSII in green plants and maybe a protective system against reactive oxygen (Gregory, 1989).

Despite that, at higher concentrations of toxicants (100mg/L to 500 mg/L toxicant), the carotenoid content was decreased. This is because of the production of the carotenoid pigment may be reduced in the cell or that carotenoid molecules structure maybe totally degraded due to inhibition by this toxicant on the algae that have been exposed to short or long durations.

5.3.3.2 Carotenoid content sensitivity and tolerance of macroalgae in the study

For present study, we also evaluated the sensitivity and tolerance of the algae to the toxicants by ranking the carotenoid content (based on the highest value obtained in each group of treatment between concentration 0.01 mg/L to 500 mg/L toxicant), to assess the effects of these toxicants on carotenoid content of the algae. Table 5.5 summarizes the carotenoid content sensitivity and tolerance in the algae cultures exposed to selected toxicants.

In this study, in the macroalgae cultures exposed to Cu (most toxic metal to *Boergesenia*), Basic dye (most toxic textile dye to *Ventricaria*) and Malathion (most toxic organophosphate pesticides to *Boergesenia*) for four days, the cultures had the lowest carotenoid content in comparison to other toxicants tested in this study.

Table 5.5: Carotenoid content sensitivity and tolerance in the macroalgae cultures exposed to selected toxicants

Algae cultures	Exposure duration	Carotenoid content (highest value obtained in the cultures)
Boergesenia forbesii	Four days	control (0.0386ug/g carotenoid), Mn (0.0173ug/g carotenoid)< Cu (0.0178ug/g carotenoid) < Basic dye (0.0211ug/g carotenoid) < Metal complex dye (0.0213ug/g carotenoid), Co (0.0225ug/g carotenoid) < Acidic dye (0.0229ug/g carotenoid) < Cd (0.0239ug/g carotenoid) < Fe (0.0273ug/g carotenoid) < Cr (0.0273ug/g carotenoid) < Malathion (0.0274ug/g carotenoid) < Dichlovos (0.0349ug/g carotenoid) < Zn (0.0380ug/g carotenoid).
	Ten days	control (0.0417ug/g carotenoid), Malathion (0.0008ug/mL carotenoid)< Dichlovos (0.0349ug/mL carotenoid) < Fe (0.026ug/g carotenoid) < Zn (0.027ug/g carotenoid) < Cr (0.027ug/g carotenoid) < Mn (0.032ug/g carotenoid) < Cu (0.033ug/g carotenoid) < Acidic dye (0.0325ug/g carotenoid) < Metal complex dye (0.0328ug/g carotenoid) < Basic dye (0.0337ug/g carotenoid) < Cd (0.0343ug/g carotenoid) < Co (0.035ug/g carotenoid).
Ventricaria ventricosa	Four days	control (0.0154ug/g carotenoid) Basic dye (0.0104ug/g carotenoid)< Acidic dye (0.0240ug/g carotenoid) < Cu (0.02385ug/g carotenoid) < Mn (0.0246ug/g carotenoid) < Metal complex dye (0.0284ug/g carotenoid) < Zn (0.0298ug/g carotenoid) < Cd (0.0350ug/g carotenoid) < Co (0.0290ug/g carotenoid) < Malathion (0.0294ug/g carotenoid) < Dichlovos (0.0388ug/g carotenoid) < Fe (0.0423ug/g carotenoid) < Cr (0.0435ug/g carotenoid).
	Ten days	control (0.0167ug/g carotenoid) Zn (0.0283ug/g carotenoid)< Cd (0.0350ug/g carotenoid) < Cr (0.0360ug/g carotenoid) < Acidic dye (0.0360ug/g carotenoid) < Cu (0.0370ug/g carotenoid) < Metal complex dye (0.0370ug/g carotenoid) < Fe (0.0390ug/g carotenoid) < Dichlovos (0.0400ug/g carotenoid) < Malathion (0.0420ug/g carotenoid) < Mn (0.0425ug/g carotenoid) < Co (0.0429ug/g carotenoid) < Basic dye (0.0460ug/g carotenoid).

Similar results were also observed in the cultures of the algae exposed to Malathion (most toxic organophosphate pesticides to Boergesenia) for ten days (long term exposure).

Low concentration of carotenoid content obtained in these cultures shows that, Cu, Basic dye and Malathion significantly give adverse effects on the carotenoid synthesis in the algae, after being exposed for short and long term duration. This suggested that, this condition occur maybe due to the activity of several functionally enzymes such as phytoene synthase, phytoene desaturase, lycopene ß-cyclase, zeaxanthin epoxidase, ß-carotene ketolase (Takaichi, 2011) and other enzymes which are involved in the carotenogenesis pathways of Chlorophyceae such as *Chlorella*, *Chlamydomonas*, *Dunaliella* and *Haematococcus* (Takaichi, 2011; Gregory, 1989) being inhibited by these toxicants and therefore interfered with the carotenoid synthesis process in the cell.

5.4 EFFECTS OF SELECTED TOXICANTS ON BIOCHEMICAL COMPOSITION IN THE ALGAE

Chemical contaminants (toxicants) such as metals, hydrocarbon, wastewater effluent, pesticides and other pollutants have long been known as a potent metabolic inhibitors to many organisms. Thus, toxicants can interfere with the metabolism of proteins, lipids, and carbohydrates in the cell (Yu, *et al*., 1987; Reddy, *et al*., 1989; Reddy and Venugopal,1990; Mathews, *et al*., 1990). Although the mechanism involved in the inhibition is not completely understood, toxicants-induced oxidative stress often inhibits macromolecules such as protein, lipid, carbohydrate, enzymes and other biochemical composition in the cell. Changes in macromolecules structure and function,

especially enzyme activity and intermediary metabolism caused by chronic chemicals contaminants exposure may lead to altered growth, development, and reproduction of the organism.

As reported in literature by many researchers, biochemical composition of the algae are usually related to the nutritional condition of mariculture or aquaculture organisms (Carboni, *et al*., 2011; Ismail, *et al*., 2011; Pettersen, *et al*., 2010). Algae play an important role in mariculture as food for many mollusks, crustaceans and some fish. Growth and development of these organism reared on microalgae are dependent on the proportion and availability of the biochemical constituents and digestibility of the cell (Chu and Dupuy, 1981; Wikfors, *et al*., 1984). The biochemical composition of algae are varied based on the algae species, light, temperature, environmental stress and growth stage. Variation in biochemical composition due to growth stage is frequently related to culture age and nutrient depletion, particularly if an organism is grown in batch culture (Harrison, *et al.,* 1990; Morris, *et al.,* 1983; Morris and Hopkins, 1983). Because aquatic consumers such as mussels, fish and others mariculture animals have a defined suite of nutritional requirements, the relative balance of all these biochemical components is important in identifying quality algal diets for the culture and propagation of mariculture organisms.

Therefore in this study, biochemical composition; carbohydrate, protein and lipid content obtained in the algae exposed to different concentrations (0, 0.01, 0.1,1,10,100 and 500 mg/L) of toxicants (metals, textile dye and organophosphate pesticides) for four days (short term exposure) and ten days (long term exposure) was measured to assess the effects of these toxicants on the biochemical composition of the algae.

5.4.1 Carbohydrate Content

A carbohydrate is an organic compound that consist of carbon, hydrogen and oxygen, with the empirical formula: C_n $H_{2n}O_n$. The carbohydrates (saccharides) are divided into four chemical groupings: monosaccharides, disaccharides, oligosaccharides and polysaccharides.

Carbohydrate is synthesised by photosynthetic organisms like plants and algae. Algae use photosynthesis and solar energy to produce glucose from carbon dioxide (CO_2). In the carbon fixation or photosynthesis process, CO_2 is reduced to carbohydrate (glucose) via carbon reduction cycle or Calvin cycle (Raven, 1974; Lobhan and Harrison, 1994). The glucose produced is stored mainly in the form of starch granules, in plastids such as chloroplasts and amyloplasts. Overall, algae carbohydrates were include: (i) cell wall polysaccharides (eg: cellulose, a polymer of 1,4 linked ß-D-glucose), (ii) reserve carbohydrate (eg: treated starches, ß-(1,3)-glucans, fructans, low-molecular weight carbohydrates) and (iii) extracellular carbohydrates (eg: glucose, galactose, mannose, xylose) (Stewart, 1974; Barsanti and Gualtieri, 2006).

Carbohydrate metabolism includes the various biochemical processess responsible for the formation, breakdown and interconversion of carbohydrate in living organims. The energy obtained from metabolism (e.g. oxidation of glucose) is usually stored temporarily within cells in the form of ATP (Stewart, 1974; Barsanti and Gualtieri, 2006).

As reported by previous researchers, the condition such as nutrition (Cheng, *et al.*, 2011), temperature (Martin, *et al*., 2012), metal toxicity (Nagajyoti, *et al*., 2009) and

other environmental stress will affects the carbohydrate content in the algae and plants. For the present study, the effects of chemical contaminants such as metals, textile dyes and organophosphate pesticides on the carbohydrate content in the exposed algae is evaluated.

5.4.1.1 Carbohydrate content trends in the study

For the present study, the carbohydrate content of the *Chlorella* (7.05% carbohydrate) and *Boergesenia* (11.948% carbohydrate) grown for four days in the Prov50 Medium without toxicant (control cultures) were higher than in the cultures that exposed to all toxicants used in this study (Cd, Co, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion). Similar pattern were also observed in the *Boergesenia* (15.93% carbohydrate), grown for ten days in the Prov50 Medium without toxicant (control cultures). The higher value of carbohydrate content obtain in the control culture in comparison to the algae cultures that exposed to these toxicants indicate that these toxicants give adversed effect on carbohydrate content of the algae after being exposed for short and long term duration.

However, for the *Tetraselmis* (exposed to Cu and Mn) and *Ventricaria* (exposed to Cd, Co, Cr, Cu, Fe, Mn, Basic dye, Metal complex dye, Dichlovos and Malathion), the carbohydrate content obtained in these cultures were higher than in the control cultures (*Tetraselmis*:9.79%carbohydrate;*Ventricaria*:2.52%carbohydrate) between concentration 0.01 mg/L to 1mg/L toxicant. The same results were also obtained in the ten days (long term exposure) cultures of *Chlorella* (exposed to Cd, Co, Cr, Cu, Mn, Zn, Acidic dye, Basic dye and Metal complex dye), *Tetraselmis* (exposed to Cd, Cr, Cu, Mn, Zn, Acidic

dye, Basic dye and Metal complex dye) and *Ventricaria* (exposed to Co, Cr, Fe, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion) where the carbohydrate content obtained in these cultures were higher than in the control cultures (*Chlorella*:22.41% carbohydrate; *Tetraselmis*:26.39% carbohydrate; *Ventricaria*:3.36% carbohydrate) between concentration 0.01 mg/L to 10 mg/L toxicant. This condition shows that additional input of metals, textile dye and organophosphate pesticides, at lower concentrations of toxicants (0.01 mg/L to 10 mg/L) can increase the carbohydrate content and stimulate the carbohydrate accumulation in the algae cell.

In this study, different carbohydrate content trends were observed in the algae after being exposed to selected toxicant for four days (short term exposure) and ten days (long term exposure) as summarizes in Table 5.6.

The first pattern observed was, the carbohydrate content in the algae cultures exposed to the toxicants for four and ten days, decreased with increasing concentrations of toxicants. Decrease of carbohydrate content with increasing concentration of toxicants shows that, these toxicants could reduce the carbohydrate synthesis (carbon fixation) in the algae following increasing concentrations of toxicant. Perhaps stress also increased respiration which then used up the carbohydrates that was synthesized previously.

The exposure of toxicants for short term and long term duration to the algae can give adverse effect on the carbohydrate metabolism which include various biochemical processess such as carbon fixation and glycolysis process (Harris, 1997; Stewart, 1974; Barsanti and Gualtieri, 2006). For example, in the glycolysis process, the toxicants can inhibit the oxidation metabolism of glucose molecules to obtained ATP and pyruvate

Table 5.6: Carbohydrate content trend in the algae cultures exposed to selected toxicants

Carbohydrate content trend	Four days cultures	Ten days cultures
The carbohydrate content of the algae decreased with increasing concentrations of toxicant	● *Chlorella* (exposed to Cr, Mn, Acidic dye, Basic dye and Malathion), ● *Tetraselmis* (exposed to Co, Fe, Acidic dye, Basic dye and Dichlovos) ● *Boergesenia* (exposed to Cd, Cu, Fe and Metal complex dye) for four days	● *Chlorella* (exposed to Fe), ● *Tetraselmis* (exposed to Co and Dichlovos) ● *Boergesenia* (exposed to Co, Basic dye and Metal complex dye).
The carbohydrate content of the algae increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant.	● *Chlorella* (exposed to Cd, Co, Cu, Fe, Zn, Metal complex dye and Dichlovos) ● *Tetraselmis* (exposed to Cd, Cr, Zn, Metal complex dye and Malathion) ● *Boergesenia* (exposed to Co, Cr, Mn, Zn, Acidic dye, Basic dye, Dichlovos and Malathion) ● *Ventricaria* (exposed to Zn and Acidic dye)	● *Chlorella* (exposed to Dichlovos and Malathion) ● *Tetraselmis* (exposed to Fe and Malathion) ● *Boergesenia* (exposed to Cd, Cr, Cu, Fe, Mn, Zn, Acidic dye, Dichlovos and Malathion) ● *Ventricaria* (exposed to Cd, Cu, Mn and Zn)

(Harris, 1997). Therefore the reduction of carbohydrate content in the algae will also reduced the metabolism process in the cell, which required ATP as energy source.

According to Nagajyoti, *et al*.,(2009) and Abdul Razak (1985), the decrease in the total carbohydrates may be due to inhibition of RUBP carboxylase activity, which resulting in reduction levels of carbohydrates. RUBP carboxylase is a most abundant key enzyme in photosynthesis, for carbohydrates assimilation in plants.

Carbon is a major structural element of carbohydrates. The decrease in carbohydrates contents in the algae that exposed to toxicants are suggested may be due to reduction of growth rates, photosynthesis capacity and the pigment composition (Chl *a* and carotenoid) in the algae, and all this factors can influence the CO_2 assimilation and carbohydrate formation in the algae cell .

The second pattern observed was, the carbohydrate content in the algae cultures exposed to the toxicants for four and ten days, increased with increasing concentrations of toxicant up to 1 mg/L of toxicant and then decreased at higher concentrations of toxicant. This condition shows that these metals like Cd, Cr, Cu, Fe, Mn and Zn, can enhanced the carbohydrate synthesis in the algae at lower concentrations of toxicant (0.01 mg/L to 10 mg/L). For the textile dyes and organophosphate pesticides, the molecular structure of these toxicants maybe degraded after long term exposure to the algae. The degraded by-product may be less toxic to the algae and may then induced the carbohydrate synthesis in the algae at lower concentration of toxicants. However, at higher concentration of toxicants (100 mg/L to 500 mg/L), the algae may not be able to

tolerate these toxicants anymore and finally died after long term exposure, and because of that will reduced the carbohydrate content in the algae.

For the present study, different trend were observed in the *Chlorella* (exposed to Cd, Cu, Fe, Metal complex dye and Dichlovos), *Tetraselmis* (exposed to Metal complex dye and Malathion), *Boergesenia* (exposed to Co, Zn, Acidic dye, Basic dye and Malathion) and *Ventricaria* (exposed to Cd, Fe, Basic dye and Metal complex dye) for four days where the carbohydrate content should be decreased with increasing concentrations of toxicant or follow the same trend as the growth of the algae. However in these cultures, the carbohydrate content was higher than in the control cultures and was increased with increasing concentrations of toxicant up to 1mg/L toxicant and then decreased at higher concentrations of toxicant. Similar pattern were also observed in the *Chlorella* (exposed to Cd, Mn, Acidic dye, Metal complex dye and Malathion), *Tetraselmis* (exposed to Fe, Mn, Zn and Metal complex dye), *Boergesenia* (exposed to Fe, Mn, Basic dye, Dichlovos and Malathion) and *Ventricaria* (exposed to Cd, Mn and Zn), after being exposed for ten days (long term exposure) to toxicants.

The increase in carbohydrate content at low concentrations of toxicant (0.01mg/L to 10 mg/L toxicant) which showed the opposite trend to the growth of algae was maybe due to carbohydrate (glucose) being synthesised abundantly and accumulated in the cell. This condition also indicated that the carbon fixation capacity rate in the algae was increased. Therefore, it is suggested that, the increase in carbohydrate content at low concentrations of toxicants (0.01mg/L to 10 mg/L) may be because of the carbohydrate molecules (glucose) was synthesised abundantly in the cells to be used in metabolic process (like glycolysis) in order to produce additional energy source to increase the

level of anti-oxidative stress enzymes activity to overcome the stress cause by these toxicants. This may be one of the adaptive and protective mechanisms carried out by algae to protect the cell from damage.

However at higher concentrations of toxicants (100 mg/L to 500 mg/L), the carbohydrate content decreased maybe because of the carbohydrate molecular structure is degraded and destroyed due to inhibition in growth and also reduction in metabolic process after the algae have been exposed to these toxicants for short term and long term duration.

5.4.1.2 Carbohydrate content sensitivity and tolerance in the study

For present study, we also evaluated the sensitivity and tolerance of the algae to the toxicants by ranking the carbohydrate content (based on the highest value obtained in each group of treatment between concentration 0.01 mg/L to 500 mg/L toxicant), to assess the effects of these toxicants on carbohydrate content of the algae. Table 5.7 summarizes the carbohydrate content sensitivity and tolerance in the algae cultures exposed to selected toxicants.

In this study, we observed that, in the algae cultures exposed to Cu (most toxic metal to *Boergesenia*), Metal complex dye (most toxic textile dye to *Tetraselmis*) and Dichlovos (most toxic organophosphate pesticides to *Chlorella* and *Tetraselmis*) for four days, the carbohydrate content obtained in the cultures have the lowest carbohydrate content in comparison to other toxicant tested in this study. The same results were also observed in the ten days (long term exposure) cultures of the algae exposed to Co (most

Table 5.7: Carbohydrate content sensitivity and tolerance in the algae cultures exposed to selected toxicants

Algae cultures	Exposure duration	Carbohydrate content (highest value obtained in the cultures)
Chlorella vulgaris UMACC245	Four days	control (7.05% carbohydrate) Acidic dye (0.33% carbohydrate) < Basic dye (0.89% carbohydrate)< Zn (1.21% carbohydrate) < Dichlovos (1.56% carbohydrate) < Cr (1.73% carbohydrate) < Cu (1.80% carbohydrate) < Mn (2.14% carbohydrate) < Cd (2.36% carbohydrate) < Malathion (3.29% carbohydrate) < Co (3.39% carbohydrate) < Fe (3.51% carbohydrate) < Metal complex dye (4.98% carbohydrate).
	Ten days	control (22.41% carbohydrate) Dichlovos (8.13% carbohydrate) < Malathion (10.02% carbohydrate) < Fe (22.19% carbohydrate) < Zn (24.40% carbohydrate) < Cd (25.86% carbohydrate) < Cu (26.01% carbohydrate) < Cr (26.50% carbohydrate) < Basic dye (26.69% carbohydrate) < Mn (28.29% carbohydrate) < Co (30.87% carbohydrate) < Acidic dye (35.83% carbohydrate) < Metal complex dye (42.72%carbohydrate).
Tetraselmis tetrahele UMACC 144	Four days	control (9.79% carbohydrate) Dichlovos (1.75% carbohydrate) < Malathion (2.94% carbohydrate) < Metal complex dye (3.65% carbohydrate) < Zn (3.75% carbohydrate) < Cd (4.19% carbohydrate) < Basic dye (4.392% carbohydrate) < Co (4.55% carbohydrate) < Acidic dye (4.68% carbohydrate) < Cr (6.57% carbohydrate) < Fe (8.58% carbohydrate) < Mn (10.50% carbohydrate) < Cu (13.32% carbohydrate).
	Ten days	control (26.39% carbohydrate) Malathion (4.22% carbohydrate) < Dichlovos (4.29% carbohydrate) < Fe (20.65% carbohydrate) < Co (24.54% carbohydrate) < Metal complex dye (29.78% carbohydrate) < Cu (30.33% carbohydrate) < Zn (31.07% carbohydrate) < Acidic dye (32.69% carbohydrate) < Cd (35.58% carbohydrate) < Mn (38.61% carbohydrate) < Basic dye (38.72% carbohydrate) < Cr (41.16% carbohydrate).
Boergesenia forbesii	Four days	control (11.948% carbohydrate) Zn (7.66% carbohydrate) < Mn (9.18% carbohydrate) < Cd (10.59% carbohydrate) < Cu (11.18% carbohydrate) < Co (12.10% carbohydrate) < Fe (12.16% carbohydrate) < Malathion (12.86% carbohydrate) < Acidic dye (13.17% carbohydrate) < Basic dye (14.58% carbohydrate) < Cr (14.80% carbohydrate) < Dichlovos (14.82% carbohydrate) < Metal complex dye (15.17% carbohydrate).
	Ten days	control (15.93% carbohydrate) Co (7.60% carbohydrate) < Cd (9.49mg/g) < Acidic dye (11.45% carbohydrate) < Mn (12.58mg/g) < Zn (13.04mg/g) < Fe (13.97mg/g) < Metal complex dye (14.51% carbohydrate) < Dichlovos (14.83% carbohydrate) < Malathion (14.93% carbohydrate) <Cr (15.07mg/g) < Cu (15.13mg/g) < Basic dye (15.165% carbohydrate).
Ventricaria ventricosa	Four days	control (2.52% carbohydrate) Zn (2.22% carbohydrate) < Acidic dye (2.45% carbohydrate) < Basic dye (2.55% carbohydrate) < Cu (3.13% carbohydrate) < Cr (3.26% carbohydrate) < Fe (3.26% carbohydrate) < Cd (3.27% carbohydrate) < Dichlovos (3.39% carbohydrate) < Malathion (3.40% carbohydrate) < Mn (3.46% carbohydrate) < Metal complex dye (3.59% carbohydrate) < Co (4.86% carbohydrate).
	Ten days	control (3.36% carbohydrate) Zn (2.51% carbohydrate) < Mn (2.97% carbohydrate) < Cd (3.34% carbohydrate) < Cu (3.36% carbohydrate) < Basic dye (3.37% carbohydrate) < Metal complex dye (3.38% carbohydrate) < Dichlovos (3.40% carbohydrate) < Malathion (3.40% carbohydrate) < Cr (3.49% carbohydrate) < Acidic dye (3.59% carbohydrate) < Co (5.00% carbohydrate) < Fe (6.49% carbohydrate).

toxic metal to *Boergesenia*), Zn (most toxic metal to *Ventricaria*), Dichlovos (most toxic organophosphate pesticides to *Chlorella*) and Malathion (most toxic organophosphate pesticides to *Tetraselmis*).

The lowest carbohydrate content obtained in the cultures exposed to the most toxic toxicants for each type of algae indicate that, these toxicants significantly inhibited and give adverse effect on the carbohydrate synthesis in the algae, after being exposed for short term and long term duration.

However, for the algae cultures exposed to Cu (most toxic metal to *Tetraselmis*) and Metal complex dye (most toxic textile dye to *Chlorella* and *Boergesenia*) for four days, the carbohydrate content obtained in the cultures have the highest carbohydrate content in comparison to other toxicant tested in this study. The similar results were also observed in the algae cultures exposed to Cr (most toxic metal to *Tetraselmis*), Metal complex dye (most toxic textile dye to *Chlorella*) and Malathion (most toxic organophosphate pesticides to *Boergesenia*), for ten days (long term exposure).

The highest carbohydrate content obtained in the cultures exposed to the most toxic toxicants for each type of algae shows that carbohydrate was synthesised abundantly in the algae exposed to these toxicants at low concentrations of toxicants (0.01mg/L to 10 mg/L) to be used in metabolic process (like glycolysis) in order to produce additional energy source to increase the level of anti-oxidative stress enzymes activity to reduced the stress level, caused by these toxicants. However, at higher concentration of toxicants (100 mg/L to 500 mg/L), the carbohydrate synthesis in the algae is inhibited due to reduction of algae growth.

Increase of carbohydrate content in the cultures exposed to these toxicants for short and long term duration may be also due to the different characteristics of cell wall composition and cell wall synthesis rate in the algae, where these factor also influence the sensitivity and tolerance of algae species to toxicants. As reported by Cheng, *et al*., (2011), some *Chlorella* strains have a cell wall with a trilaminar outer layer (TLS) that contains algaenan which is a highly resistant and non-hydrolyzable aliphatic biopolymer, while other strains have a cell wall with no TLS outer layer but a high percentage of polysaccharides (Yamada and Sakaguchi, 1982). Therefore it is suggested that, *Chlorella* may have this cell wall characteristic, the algae are more resistant to the metals, textile dyes and organophosphate pesticides toxicity.

As reported by Baskin, (2001); La Claire II, (1989) and Itoh, *et al*., (1984), coenocytic green algae such as *Boergesenia* have the ability to produce highly active cell wall synthesis especially in the wound-healing response, where the aplanospore formation process occurred in the cell. In this process, after the cell is wounded (due to physical distruction or chemical stress), the cytoplasm will be round up into protoplast, called aplanospore. The regeneration of aplanospore cell wall, which synthesize large cellulose microfibrils occurred within 2-3 hours after wounding (Itoh, *et al.*, 1984). The fast formation of the cell wall and the cell wall thickens, indicating active synthesis of cell wall, was very important in adaptation mechanism response carried out by algae in oder to reduce stress level in the cell. Therefore it is suggested that because *Boergesenia* have this cell wall characteristic, the algae are more tolerant to the toxicity of toxicants used in this study and as a result contained higher carbohydrate content.

In comparison of carbohydrate content in algae between short term (4 days) and long term (10 days) exposure, we observed that the carbohydrate content of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* grown for ten days in the Prov50 medium without toxicant (control cultures) was 68.55%, 62.89%, 25.00% and 25.00% respectively higher than in the four days cultures. This condition indicate that the increased of carbohydrate content following increasing of time was due to the increased in cell number and cell size of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* cultures.

In addition, in the non-renewal culture system, the nutrient level in the culture medium will be decreased with increase of time (Stewart, 1974; Barsanti and Gualtieri, 2006). According to Cheng, *et al*., (2011), nitrogen limitation is known to decrease photosynthetic efficiency and alter the metabolism of carbon in algae. In general, lower nitrogen levels favor higher carbohydrate accumulation (Stewart, 1974). Therefore in this study, it is suggested that maybe the increase of carbohydrate content of the algae following increase of time and growth was also due to the limiting nitrogen level in the culture medium, which in this study, we use non-renewal culture system during the experiment is conducted.

The carbohydrate content of *Tetraselmis* cultures grown for four days and ten days in Prov50 medium without toxicant (control cultures) was 28.06% and 15.09% respectively higher than in the *Chlorella* cultures. The higher value of carbohydrate content obtained in *Tetraselmis* grown for short term duration (four day), make it suitable to be used as fish feed in mariculture industry due to their fast growth ability and containing high content of carbohydrate nutrient.

5.4.2 Protein Content

Protein are linear polymers of amino acids. Each of the 20 different amino acids found in proteins has a different R group, which can be either hydrophobic, hydrophilic uncharged or hydrophilic charged. These amino acids can be linked together in any sequence via peptide bonds to form the wide variety of polypeptides that make up monomeric and multimeric proteins (Hardin, *et al*., 2012; Schultz and Liebman, 1997).

Most proteins are enzymes that catalyze biochemical reactions and plant metabolism. Other proteins maintain cell shape and provide signaling functions within the plant (Edwards, 2008).

Upon oxidative stress, an increased production of reactive oxygen species (superoxide, hydrogen peroxide, and hydroxyl radical) and reactive nitrogen species (nitric oxide and peroxynitrite) will disrupt the cellular redox homeostasis and provoke damage to cellular components like lipids, nucleic acids and proteins. Oxidative damage to proteins can affect all amino acids, sulfur-containing amino acids and aromatic amino acids, as they are the most susceptible to oxidation (Klaassen, 2008).

As demostrated by previous researchers, conditions such as nutrition (Piorreck, *et al*., 1984; D'Souza and Kelly, 2000), light (Krupinska and Humbeck, 1994; Korbee, *et al*., 2005) metal toxicity (Bajguz and Godlewska-Zylkiewicz, 2004), UV-radiation (Agrawal, 1992) and other environmental stress will affect the protein content in the algae and plants. For the present study, the effects of chemical contaminants induced oxidative stress, such as metals, textile dyes and organophosphate pesticides on the protein content in the exposed algae is evaluated.

5.4.2.1 Protein content trends in the study

For the present study, the protein content of *Chlorella* (31.01% protein) and *Tetraselmis* (44.89% protein), grown for four days in the Prov50 Medium without toxicant (control cultures) were higher than in *Chlorella* (exposed to Zn, Metal complex dye, Dichlovos and Malathion) and *Tetraselmis* (exposed to Cd, Fe, Zn, Acidic dye, Basic dye, Dichlovos and Malathion). Similar pattern were also observed in, *Chlorella* (63.068% protein) and *Tetraselmis* (60.45% protein) grown for ten days in the Prov50 Medium without toxicant (control cultures) which were higher than in *Chlorella* (exposed to Cd, Co, Cr, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion), *Tetraselmis* (exposed to Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion).The higher value of protein content obtain in the control cultures in comparison to the algae cultures exposed to these toxicants indicate that these toxicants give adverse effect on protein content of the algae after exposure for short and long term duration.

However, for the *Chlorella* (exposed to Cd, Co, Cr, Cu, Fe, Mn, Acidic dye and Basic dye), *Tetraselmis* (exposed to Cr, Cu, Mn and Metal complex dye), *Boergesenia* and *Ventricaria* (exposed to all toxicant used in the study: Cd, Co, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion), the protein content obtained in these cultures were higher than in the control cultures between concentration 0.01 mg/L-10 mg/L toxicant. The same trends were also observed in the ten days (long term exposure) cultures of *Chlorella* (exposed to Cu), *Tetraselmis* (exposed to Cd and Co), *Boergesenia* and *Ventricaria* (exposed to all toxicant used in the study), where the protein content obtained in these cultures were higher than in the control cultures between concentration 0.01 mg/L to 10 mg/L toxicant. This condition

shows that at low concentrations (0.01 mg/L to 10 mg/L) of metals, textile dyes and organophosphate pesticides, may be supplying useful nutrients and therefore can increase the protein content and stimulate the protein accumulation in the algae cell.

In this study, different protein content trends were observed in the algae after being exposed to selected toxicant for four and ten days as summarized in Table 5.8.

The first trend observed was, the protein content in the algae cultures exposed to the toxicants for four and ten days, decreased with increasing concentrations of toxicant. Reduction of protein content with increasing concentrations of toxicant shows that, these toxicants could inhibit the protein synthesis in the algae following increasing concentrations of toxicant. In addition, reduction of protein is also due to a failure of protein maintenance (Klaassen, (2008).

The exposure of toxicants for short term and long term duration to the algae can give adversed effect on the protein metabolism. According to Klaassen, (2008), the reaction of reactive oxygen species and reactive nitrogen species with both amino acid side chains and peptidic backbonecould cause protein oxidation (Schultz and Liebman, 1997 ; Liu, *et al.*, 2008).

In the hydrogen abstraction reaction, neutral free radical ($HO^{\bullet}$, $NO_2^{\bullet}$,$CO_3^{-\bullet}$) can readily abstract H atoms from endogenous compounds, converting those compounds into radicals. Abstraction of hydrogen from thiols (R-SH) creates thiyl radicals (R-S.), which upon radical recombination with HO. Form sulfenic acids (R-S-OH) that are precursor of disulfides (R-S-S-R). Radicals can remove hydrogen from CH_2 groups of free amino acidfs of from amino acids residues in proteins and convert them to carbonyls.

Table 5.8: Protein content trend in the algae cultures exposed to selected toxicants

Protein content trend	Four days cultures	Ten days cultures
The protein content of the algae decreased with increasing concentrations of toxicant	● *Chlorella* (exposed to Dichlovos and Malathion) ● *Tetraselmis* (exposed to Acidic dye, Basic dye and Dichlovos) ● *Ventricaria* exposed to the toxicant such as Mn, Basic dye and Metal complex dye	● *Chlorella* (exposed to Cd, Cr, Fe, Mn, Zn, Acidic dye and Basic dye) ● *Tetraselmis* (exposed to Cr, Cu, Fe, Basic dye, Metal complex dye and Dichlovos) ● *Boergesenia* exposed to the toxicant such as Co, Cr, Cu and Malathion ● *Ventricaria* exposed to the toxicant such as Co, Cr, Fe and Metal complex dye
The protein content of the algae increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant.	● *Chlorella* (exposed to Zn and Metal complex dye) ● *Tetraselmis* (exposed to Cd, Co, Fe, Zn and Malathion) ● *Boergesenia* cultures exposed to Cd, Co, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye and Dichlovos, ● *Ventricaria* cultures exposed to Cd, Co, Cr, Cu, Fe, Zn, Acidic dye, Dichlovos and Malathion	● *Chlorella* (exposed to Co, Metal complex dye, Dichlovos and Malathion) ● *Tetraselmis* (exposed to Mn, Zn, Acidic dye and Malathion) ● *Boergesenia* cultures exposed to Cd, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye and Dichlovos ● *Ventricaria* cultures exposed to Cd, Cu, Mn, Zn, Acidic dye, Basic dye, Dichlovos and Malathion

These carbonyls react covalently with amines, forming cross-links with DNA or other proteins (Schultz and Liebman, 1997; Liu, *et al.*, 2009).

Our results show that decrease protein content with increasing concentration of toxicants agree well with the observations by previous studies carried out by Bajguz and Godlewska-Zylkiewicz (2004), who exposed *Chlorella vulgaris* to 10^{-6} to10^{-4} M lead for 48h. Lead displayed the greatest inhibitory activity at a concentration of 10^{-4} M between the 12^{th} and 48^{th} hour of cultivation. The lowest activity in decreasing protein content was demonstrated at a concentration of 10^{-6} M lead. Similar results was reported by Piotrowska-Niczyporuk, *et al*., (2012) who showed, the decrease in proteins was obtained in *Chlorella vulgaris,* in response to heavy metals (Cd, Cu, Pb) stress.

The second trend observed was, the protein content in the algae cultures exposed to the toxicants for four and ten days, increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. This condition shows that these metals like Co, Fe, Mn and Zn, can induce the protein synthesis in the algae at lower concentrations of toxicant (0.01 mg/L to 10 mg/L). For the Acidic dye, Metal complex dye and Dichlovos, the degraded by-products may be less toxic to the algae and may then induce protein synthesis in the algae at lower concentration of toxicant. However, at higher concentration of toxicant (10 mg/L –500 mg/L), the tolerance threshold of the algae is exceeded, leading to death of the algae.

Our results show that increase protein content with increasing concentration of toxicants at lower concentration of toxicants are similar with the observations shown by Sabatini, *et al.,* (2009) who exposed *Scenedesmus vacuolatus* and *Chlorella kessleri* to

Cu (6.2 to 414µM) for 7 days. In *Scenedesmus vacuolatus* the increase of Cu concentration induced an augmentation of protein content per 10^6 cells. They suggested, *Scenedesmus vacuolatus* increased its intra cellular protein content, could represent a mechanism to reduce metal effects through the induction of protective protein synthesis. Besides, the increase of these proteins may be reflecting an increase in phytochelatins (Ahner and Morel, 1995; Cobbett, 2000) involved in mechanisms of cellular detoxification via the formation of metal complexes.

For the present study, different trends were observed in *Chlorella* (exposed to Cd, Cu, Fe, Zn, Metal complex dye and Dichlovos), *Tetraselmis* (exposed to Mn, Zn and Metal complex dye), *Boergesenia* (exposed to Cd, Co, Cr, Cu, Fe, Acidic dye, Basic dye and Dichlovos) and *Ventricaria* (exposed to Cd, Co and Fe) for four days where the protein content should be decreased with increasing concentrations of toxicant or followed as the same trend as the growth of the algae. However in these cultures, the protein content was higher than in control cultures and was increased with increasing concentrations of toxicant up to 10 mg/L toxicant and then decreased at higher concentrations of toxicant. Similar trends were also observed in *Chlorella* (exposed to Metal complex dye and Malathion), *Tetraselmis* (exposed to Co, Mn and Zn), *Boergesenia* (exposed to Fe, Acidic dye, Basic dye, Metal complex dye and Dichlovos) and *Ventricaria* (exposed to Cd, Cu, Mn, Zn, Basic dye and Metal complex dye), after exposure for ten days (long term duration).

The increase of protein content at low concentrations of toxicant (0.01mg/L to 10 mg/L toxicant) which shows the opposite trend with the growth of algae may be because the additional input of metals, textile dye and organophosphate pesticides at the lower

concentrations of toxicants (0.01 mg/L to 10 mg/L) enhanced the protein synthesis in the algae. In addition, the increase of protein content in the algae was higher than in control cultures maybe because of protein was synthesised abbundantly in the cell to produce more anti-oxidative stress enzyme molecules (which made from the protein molecule) to reduced the stress level cause by these toxicants. This is may be one of the adaptation and protective mechanisme carried out by algae to protect the cell from the oxidative damages induced by these toxicants.

However at higher concentration of toxicant, the protein content is decreased may be because of the protein are denaturated and the protein synthesis is inhibited by these toxicant, after exposure for short term and long term duration.

5.4.2.2 Protein content sensitivity and tolerance in the study

For the present study, we also evaluated the sensitivity and tolerance of the algae to the toxicants by ranking the protein content (based on the highest value obtained in each group of treatment between concentration 0.01 mg/L to 500 mg/L toxicant), to assess the effects of these toxicants on protein content of the algae. Table 5.9 summarizes the protein content sensitivity and tolerance in the algae cultures exposed to selected toxicants.

In this study, we observed that, in the algae cultures exposed to Dichlovos (most toxic organophosphate pesticides to *Chlorella* for four days and also in the algae cultures exposed to Metal complex dye (most toxic textile dye to *Tetraselmis*) for ten days, the protein content obtained in the cultures, have the lowest protein content in comparison to other toxicants tested in this study. The lowest protein content obtained in the cultures

Table 5.9: Protein content sensitivity and tolerance in the algae cultures exposed to selected toxicants

Algae cultures	Exposure duration	Protein content (highest value obtained in the cultures)
Chlorella vulgaris UMACC245	Four days	control (31.01% protein) Dichlovos (15.38 % protein)< Malathion (15.88 % protein) < Zn (25.45% protein) < Metal complex dye (27.72% protein) < Cr (31.17% protein) < Cd (33.54% protein) < Basic dye (33.70% protein) < Fe (34.32% protein) < Co (36.30% protein) < Mn (37.09% protein) < Cu (40.58% protein) < Acidic dye (42.59% protein).
	Ten days	control (63.068% protein) Malathion (21.01% protein) < Dichlovos (21.35% protein) < Acidic dye (51.74% protein) < Metal complex dye (52.20% protein) < Zn (53.05% protein) < Fe (56.01% protein) < Basic dye (57.92% protein) < Cr (58.21% protein) < Cd (59.01% protein) < Co (62.11% protein) < Mn (62.32% protein) < Cu (67.54% protein).
Tetraselmis tetrahele UMACC 144	Four days	control (44.89% protein) Dichlovos (16.29% protein) < Malathion (17.71% protein) < Co (32.32% protein) < Cd (35.99% protein) < Basic dye (36.89% protein) < Acidic dye (42.01% protein) < Zn (42.93% protein) < Fe (44.51% protein) < Metal complex dye (46.42% protein) < Cu (49.25% protein) < Cr (50.29% protein) < Mn (64.42% protein).
	Ten days	control (60.45% protein) Dichlovos (19.34% protein) < Malathion (22.34% protein) < Metal complex dye (25.07% protein) < Zn (40.41% protein) < Basic dye (42.06% protein) < Cr (42.43% protein) < Acidic dye (49.12% protein) < Fe (49.19% protein) < Mn (51.25% protein) < Cu (59.32% protein) < Cd (62.35% protein) < Co (65.71% protein).
Boergesenia forbesii	Four days	control (1.82% protein) Cr (5.35% protein) < Zn (5.45% protein) < Cu (5.82% protein) < Basic dye (6.08% protein) < Fe (6.09% protein) < Metal complex dye (6.26% protein) < Mn (6.38% protein) < Acidic dye (7.16% protein) < Cd (7.50% protein) < Malathion (8.00% protein) < Dichlovos (8.04% protein) < Co (9.11% protein).
	Ten days	control (2.43% protein) Zn (5.84% protein) < Fe (6.00% protein) < Mn (6.60% protein) < Acidic dye (6.88% protein) < Cr (6.95% protein) < Cu (7.36% protein) < Basic dye (7.53% protein) < Cd (8.04% protein) < Dichlovos (8.36% protein) < Metal complex dye (8.48% protein) < Malathion (8.55% protein) < Co (8.67% protein).
Ventricaria ventricosa	Four days	control (2.52% protein) Basic dye (3.87% protein) < Metal complex dye (4.51% protein) < Zn (5.34% protein) < Cu (5.49% protein) < Acidic dye (5.50% protein) < Mn (5.54% protein) < Fe (5.65% protein) < Cr (5.68% protein) < Co (5.82% protein) < Cd (8.00% protein) < Malathion (8.26% protein) < Dichlovos (8.30% protein).
	Ten days	control (3.78% protein) Zn (3.95% protein) < Acidic dye (4.00% protein) < Fe (4.11% protein) < Cr (4.16% protein) < Mn (4.30% protein) < Co (4.40% protein) < Metal complex dye (8.36% protein) < Basic dye (8.39% protein) < Cd (8.45% protein) < Dichlovos (8.54% protein) < Cu (8.58% protein) < Malathion (8.59% protein).

exposed to the most toxic toxicants for each types of algae indicate that, these toxicants significantly inhibited and give adversed effect on the protein synthesis in the algae, after being exposed for short term and long term duration.

There are two main adverse effects of toxicants on macromolecules (eg: protein, carbohydrate, lipid) in the cell: (i) dysfunction of target molecules and (ii) destruction of target molecules (Liu, *et al.*, 2008).

For example, protein function is weaken when confirmation or structure is altered by interaction with the toxicant. Many proteins have critical moieties, especially thiol groups, which are essential for catalytic activity or assembly (polymerization) to macromolecular complexes (Schultz and Liebman, 1997; Liu, *et al.*, 2009). Many other proteins are inactivated by thiol-reactive chemicals, causing impaired maintainance ofthe cell's energy and metabolic homeostasis and/or altered signal transduction. In addition, formation of protein sufenic acids by the oxidative stress product H_2O_2 is thus considered as an initiating redox signaling (Forman, *et al*., 2004).

Toxicants can alter the primary structure of molecules by means of cross-linking and fragmentation. H_2O_2 and hydroxyl radicals that produced from the toxicants can induce cross-linking by converting proteins into either reactive electrophiles (e.g. protein sulfenic acids and protein carbionyls), which react with a nucleophilic group (e.g. thiol, amine) in another macromolecules. Radicals may induce cross-linking of macromolecules by converting them into radicals, which react with each other by radical recombination. Cross-linking imposes both structural and functional constraints on the linked molecules (Schultz and Liebman, 1997; Liu, *et al.*, 2008).

In contrast, in the algae cultures exposed to Metal complex dye (most toxic textile dye to *Tetraselmis*), Dichlovos (most toxic organophosphate pesticides to *Ventricaria*) and Malathion (most toxic organophosphate pesticides to *Boergesenia*) for four days, the protein content obtained in the cultures have the highest protein content in comparison to other toxicants tested in this study. The same results were also observed in the algae cultures exposed to Co (most toxic textile dye to *Boergesenia*), Basic dye (most toxic textile dye to *Ventricaria*), Metal complex dye (most toxic textile dye to *Tetraselmis* and *Boergesenia*), Dichlovos (most toxic organophosphate pesticides to *Chlorella*) and Malathion (most toxic organophosphate pesticides to *Tetraselmis* and *Boergesenia*), after exposure for ten days (long term exposure).

The highest protein content obtained in the cultures exposed to the most toxic toxicants for each types of algae indicate that, protein was synthesis abbundantly in the algae cell and accumulated in the cell. This also indicate the molecular repair mechanism is occurred in the cell. Damaged molecules may be repaired in different ways. For example, chemical alteration (such as oxidation of protein thiols and methylation of DNA), are simply reversed (Schultz and Liebman, 1997 ; Liu, *et al.*, 2008)

For the protein repair mechanism, thiol groups are essential for the function of numerous proteins such as receptors, enzymes, cytoskeletal proteins and transcription factors (TFs). Oxidation of protein thiols (Prot-SHs) to protein disulfites (Prot-SS, Prot1-SS-Prot2), protein-glutahione mixed disulfides (Prot-SSG) and protein sulfenic acids (Prot-SOH) as well as oxidation of methaionine in proteins to metathionine sulfoxide can be reversed by enzymatic reduction (Watson, *et al*., 2004); Gravina and Mieyal, 1993; Moskovitz, 2005). Protein disulfites (Prot-SS, Prot1-SS-Prot2), protein sulfenic acids

(Prot-SOH) and protein-metathionine sulfoxides (Prot-Met=O) are reduced by thioredoxin with metathionine sulfoxide reductases catalizing the latter process. Protein-glutahione mixed disulfides (Prot-SSG) are reduced by glutaredoxin, which is also called thioltransferase. Because the catalytic thiol groups in these proteins become oxidized, they are reduced by NADPH, which is generated by $NADP^+$-dependent isocitrate dehydrogenase localized in various cell compartment (cytosol, mitochondria, peroxysome) (Klaassen, 2008).

At higher concentration of toxicants (100 mg/L – 500 mg/L), the protein synthesis in the algae is inhibited due to reduction of algae growth and also degradation of protein structure.

In comparison of protein content in algae between short term (4 days) and long term (10 days) exposure, our results shows that in the control cultures, the protein content of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* grown for ten days was 50.84%, 25.75%, 25.01% and 33.33% respectively higher than in the four days cultures.

5.4.3 Lipid Content

Lipids are not true polymers but are often considered as macromolecules due to their high molecular weight and their frequent association with maccromeleccules, particularly proteins. Fatty acids are lipids consisting of a long hydrocarbon chain of 12-20 carbon atoms with a carboxylic acid group at one end. They are energy-rich

molecules found in the triacylglycerols that make up phospholipids founds in all cellular membranes (Hardin, *et al*., 2012; McGarry, 1997)

Fatty acids are synthesis by plants and algae. In the plants, when there is excess carbohydrate, it will be converted to triglycerides. This involves the synthesis of fatty acids from acetyl-CoA and the esterification of fatty acids in the production of triglycerides, a process called lipogenesis. Fatty acids are synthezied by fatty acids synthase that polymerize and then reduce acetyl-CoA units. The acyl chains in the fatty acids are extended by a cycle of reactions that add the acetyl group, reduce it to an alcohol, dehydrate it to an alkene group and then reduce it again to an alkane group (Hardin, *et al*., 2012; McGarry, 1997).

As reported by previous researchers, condition such as nutrition (Liu, *et al*., 2008; Piorreck, *et al*., 1984), temperature (Sato and Murata,1980), light (Khotimchenko and Yakovleva, 2005) metal toxicity (Gennity, *et al*., 1985) and other environmental stress will affects the lipid synthesis and lipid content in the algae and plants. For the present study, the effects of chemical contaminants such as metals, textile dyes and organophosphate pesticides on the lipid content in the exposed algae is evaluated.

5.4.3.1 Lipid content trends in the study

In general, the lipid content of the *Tetraselmis* (5.67% lipid), grown for four days and the lipid content of the *Chlorella* (12.58% lipid), *Tetraselmis* (9.84% lipid), *Boergesenia* (6.33% lipid) and *Ventricaria* (9.55% lipid) grown for ten days in the Prov50 Medium without toxicant (control cultures), were higher than in the cultures that

were exposed to all toxicants used in this study (Cd, Co, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion). The higher value of lipid content obtained in the control culture in comparison to the algae cultures that were exposed to these toxicants indicate that these toxicants give adverse effect on lipid content of the algae after being exposed for short and long term duration.

However, for the *Chlorella* (exposed to Cd, Cr, Acidic dye, Basic dye and Metal complex dye), *Boergesenia* (exposed to Co, Fe, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion) and *Ventricaria* cultures (exposed to Cd, Co, Cr, Cu, Fe, Mn, Zn, Acidic dye, Metal complex dye, Dichlovos and Malathion) for four days, the lipid content obtained in these cultures were higher than in the control cultures (*Chlorella*:4.24% lipid; *Boergesenia*:3.63% lipid; *Ventricaria*: 3.10% lipid) between concentration 0.01 mg/L to 10 mg/L toxicant. This condition shows that additional input of metals, textile dye and organophosphate pesticides, at low concentrations (0.01 mg/L to 10 mg/L) can increased the lipid content and stimulate the lipid accumulation in the algae cell.

In this study, different lipid content trends were observed in the algae after being exposed to selected toxicant for four and ten days as summarizes in Table 5.10. The first trens observed was, the lipid content in the algae cultures exposed to the toxicants for four and ten days, decreased with increasing concentrations of toxicant. This condition shows that, these toxicants could inhibit the lipid synthesis in the algae following increasing concentrations of toxicant.

Table 5.10: Lipid content trend in the algae cultures exposed to selected toxicants

Lipid content trend	Four days cultures	Ten days cultures
The lipid content of the algae decreased with increasing concentrations of toxicant	● *Chlorella* (exposed to Zn and Dichlovos) ● *Tetraselmis* (exposed to Cr, Cu, Fe, Basic dye, Metal complex dye, Dichlovos and Malathion) ● *Boergesenia* (exposed to Cd, Cu and Mn) ● *Ventricaria* (exposed to Basic dye)	● *Chlorella* (exposed to Cr, Acidic dye, Basic dye and Malathion) ● *Tetraselmis* (exposed to Cd, Cr, Zn and Basic dye) ● *Boergesenia* (exposed to Cd, Co, Cr, Zn, Acidic dye, Basic dye, Dichlovos and Malathion) ● *Ventricaria* (exposed to Co, Fe, Mn, Zn, Metal complex dye and Dichlovos)
The lipid content of the algae increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant.	● *Chlorella* (exposed to Co, Cu, Fe, Mn and Malathion) ● *Tetraselmis* (exposed to Cd, Co, Mn, Zn and Acidic dye) ● *Boergesenia* (exposed to Cr)	● *Chlorella* (exposed to Cd, Co, Cu, Fe, Mn, Zn, Metal complex dye and Dichlovos) ● *Tetraselmis* (exposed to Co, Cu, Fe, Mn, Acidic dye, Metal complex dye, Dichlovos and Malathion) ● *Boergesenia* (exposed to Cu, Fe, Mn and Metal complex dye) ● *Ventricaria* (exposed to Cd, Cr, Cu, Acidic dye and Basic dye)

The reaction of toxicants with fatty acids via hydrogen abstraction reaction produces lipid radicals and initiates lipid peroxidation. Lipid peroxidation results from the reaction of reactive oxygen species (superoxide, hydrogen peroxide, and hydroxyl radical) and reactive nitrogen species (nitric oxide and peroxynitrite) with fatty acids (Liu , *et al*., 2008a).

According to Hardin, *et al*., (2012) and McGarry, (1997), fatty acids can also be degraded by beta oxidation. Beta oxidation is the metabolic process by which fatty acids are broken down in the mitochondria and/or in peroxisome to generate acetyl- CoA. For the most part, fatty acids are oxidized by a mechanism that is similar to, but not identical with, a reversal of the process of fatty acid synthesis. That is, two-carbon fragments are removed sequentially from the carboxyl end of the acid after steps of dehydrogenation, hydration, and oxidation to form a beta-keto acids, which is split by thiolysis. The acetyl-CoA is then ultimately converted into ATP, CO_2, and H_2O using the citric acid cycle and the electron transport chain. Hence the Krebs Cycle can start at acetyl-CoA when lipid is being broken down for energy if there is little or no glucose available (Hardin, *et al.*, 2012; McGarry, 1997). Therefore, the exposure of toxicants for short term and long term duration to the algae can give adverse effects on the lipid metabolism.

The second trend observed was, the lipid content in the algae cultures exposed to the toxicants for four and ten days, increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. This condition shows that these metals like Cd, Co, Cr, Cu, Fe, Mn and Zn, and also the degraded by-products of textile dye and organophosphate pesticides may be less toxic to the algae and may then enhance the lipid synthesis in the algae at lower concentrations of

toxicant (0.01 mg/L to 10 mg/L). However, at higher concentration of toxicants (100 mg/L to 500 mg/L), the tolerance threshold of the algae is exceeded, leading to death of the algae. Therefore the lipid content decreased.

For the present study, different trend were observed in the *Chlorella* (exposed to Co, Cu, Metal complex dye and Malathion), *Tetraselmis* (exposed to Mn) and *Ventricaria* (exposed to Cd), for four days where the lipid content should be decreased with increasing concentrations of toxicant or followed as the same trend as the growth of the algae. However in these cultures, the lipid content was higher than in control cultures and increased with increasing concentrations of toxicant up to 1 mg/L toxicant and then reduced at higher concentrations of toxicant. The same results were also observed in the ten days cultures of *Chlorella* (exposed to Cd, Cu, Mn, Metal complex dye and Malathion), *Tetraselmis* (exposed to Co), *Boergesenia* (exposed to Fe) and *Ventricaria* (exposed to Cd).

The increased in lipid content at low concentrations of toxicant (0.01mg/L to 10 mg/L) which shows the opposite pattern with the growth of algae may be because lipid was synthesised abundantly and accumulated in the cell. This condition also indicate that the lipid accumulation rate in the algae was increased after being induced by these toxicants. Therefore, it is suggested that, the increase of lipid molecules (such as triacylglycerols) accumulated in the cell, can be used as alternative energy source (if the glucose content in the cell reduced) to increase the metabolic process in the cell in order to increase the anti-oxidative stress enzymes activity to overcome the stress cause by these toxicants. This is may be one of the adaptive and protective mechanisme carried out by algae to protect the cell from damages.

According to Klaassen (2008), there are several adaptation mechanism carried out by the organisms to reduce the oxidative stress that occur in the cell. One of the adaptation mechanism is repairing the oxidized lipid. Peroxidized lipids are repaired by a complex process that operates with a series of reductants, glutathione peroxidase and reductase. Phospholipids containing fatty acids hydroperoxides are preferentially hydrolyzed by phospholipase A2, with the peroxidized fatty acids replaced by normal fatty acids. In the process, NADPH is needed to repair the reductants that are oxidized (Klassen, 2008; Hardin, *et al*., 2012; McGarry, 1997).

However at higher concentration of toxicants (100 mg/L to 500 mg/L), the lipid content is decreased may be because of the lipid molecular structure are degraded and destroyed due to inhibition in growth and also reduction in metabolic process after the algae have been exposed to these toxicants for short term and long term duration.

5.4.3.2 Lipid content sensitivity and tolerance in the study

For the present study, we also evaluated the sensitivity and tolerance of the algae to the toxicants by ranking the lipid content (based on the highest value obtained in each group of treatment between concentration 0.01 mg/L-500 mg/L toxicant), to assess the effects of these toxicants on lipid content of the algae. Table 5.11 summarizes the lipid content sensitivity and tolerance in the algae cultures exposed to selected toxicants.

In this study, we observed that, in the algae cultures exposed to Cu (most toxic metal to *Boergesenia*), Basic dye (most toxic textile dye to *Ventricaria*) and Dichlovos (most toxic organophosphate pesticides to *Tetraselmis*) for four days, the lipid content

Table 5.11: Lipid content sensitivity and tolerance in the algae cultures exposed to selected toxicants

Algae cultures	Exposure duration	Lipid content (% Lipi/DW) (highest value obtained in the cultures)
Chlorella vulgaris UMACC245	Four days	control (4.24% lipid) Malathion (0.60% lipid)< Fe (1.34% lipid) < Basic dye (2.12% lipid) < Cu (2.27% lipid) < Dichlovos (2.94% lipid) < Co (3.45% lipid) < Zn (3.67% lipid) < Acidic dye (4.13% lipid) < Mn (4.21% lipid) < Metal complex dye (4.37% lipid) < Cr (4.73% lipid) < Cd (5.51% lipid).
	Ten days	control (12.58% lipid) Cr (1.61% lipid) < Malathion (1.71% lipid) < Dichlovos (2.46% lipid) < Co (2.82% lipid) < Metal complex dye (3.48% lipid) < Cu (3.73% lipid) < Basic dye (5.51% lipid) < Acidic dye (5.56% lipid) < Fe (6.68% lipid) < Zn (6.99% lipid) < Cd (7.83% lipid) < Mn (8.43% lipid).
Tetraselmis tetrahele UMACC 144	Four days	control (5.67% lipid) Zn (1.77% lipid) < Acidic dye (1.89% lipid) < Metal complex dye (1.97% lipid) < Mn (2.27% lipid) < Fe (2.44% lipid) < Dichlovos (2.97% lipid) < Malathion (3.01% lipid) < Co (4.48% lipid) < Cd (4.49% lipid) < Cu (4.74% lipid) < Basic dye (4.78% lipid) < Cr (5.04% lipid).
	Ten days	control (9.84% lipid) Dichlovos (1.72% lipid) < Malathion (2.09% lipid) < Metal complex dye (2.29% lipid) < Cu (3.55% lipid) < Fe (3.71% lipid) < Acidic dye (3.72% lipid) < Cd (3.97% lipid) < Basic dye (4.38% lipid) < Zn (5.19% lipid) < Co (5.23% lipid) < Mn (5.39% lipid) <Cr (7.17% lipid).
Boergesenia forbesii	Four days	control (3.63% lipid) Cu (1.95% lipid) < Mn (2.55% lipid) < Cr (2.96% lipid) < Cd (3.11% lipid) < Fe (3.86% lipid) < Co (3.94% lipid) < Zn (5.29% lipid) < Metal complex dye (6.21% lipid) < Acidic dye (6.53% lipid) < Basic dye (6.77% lipid) < Dichlovos (9.67% lipid) < Malathion (11.80% lipid).
	Ten days	control (6.33% lipid) Malathion (1.67% lipid) < Co (2.04% lipid) < Cr (2.11% lipid) < Fe (2.26% lipid) < Dichlovos (2.55% lipid) < Zn (2.78% lipid) < Mn (3.25% lipid) < Basic dye (3.56% lipid) < Acidic dye (4.07% lipid) < Metal complex dye (4.27% lipid) < Cd (5.10% lipid) < Cu (5.97% lipid).
Ventricaria ventricosa	Four days	control (3.10% lipid) Basic dye (1.33% lipid) < Mn (3.47% lipid) < Dichlovos (3.54% lipid) < Cu (3.67% lipid) < Acidic dye (3.72% lipid) < Co (3.98% lipid) < Cr (4.67% lipid) < Malathion (4.69% lipid) < Metal complex dye (4.78% lipid) < Cd (6.43% lipid) < Zn (6.66% lipid) < Fe (9.06% lipid).
	Ten days	control (9.55% lipid) Co (2.33% lipid) < Acidic dye (2.76% lipid) < Malathion (3.37% lipid) < Cu (3.60% lipid) < Cd (3.63% lipid) < Metal complex dye (4.28% lipid) < Fe (4.86% lipid) < Mn (5.56% lipid) < Dichlovos (6.28% lipid) < Basic dye (6.33% lipid) < Cr (8.12% lipid) < Zn (8.84% lipid).

obtained in the cultures have the lowest lipid content in comparison to other toxicants tested in this study. Similar results were also obtained in the ten days (long term exposure) cultures of the algae exposed to Co (most toxic metal to *Boergesenia*), Dichlovos (most toxic organophosphate pesticides to *Ventricaria*) and Malathion (most toxic organophosphate pesticides to *Boergesenia*).

The lowest lipid content obtained in the cultures exposed to the most toxic toxicants for each types of algae indicate that, these toxicants significantly inhibited and give adverse effects on the lipid synthesis in the algae, after being exposed for short term and long term duration. Some target molecules like lipids are susceptible to spontaneous degradation after chemical attack. Free radical such as $ClCOO^{\bullet}$ and $HO^{\bullet}$ can initiated peroxidative degradation of lipids by hydrogen abstraction from fatty acids (Recknagel, *et al*., 1989).

However, the algae cultures exposed to Dichlovos (most toxic organophosphate pesticides to *Chlorella*) and Malathion (most toxic organophosphate pesticides to *Boergesenia*) for four days, the lipid content obtained in the cultures have the highest lipid content in comparison to other toxicants tested in this study. The same trends were also observed in the, algae exposed to Cr (most toxic metal to *Tetraselmis*), Zn (most toxic metal to *Ventricaria*), Basic dye (most toxic textile dye to *Ventricaria*), Metal complex dye (most toxic textile dye to *Boergesenia*), Dichlovos (most toxic organophosphate pesticides to *Chlorella*) and Malathion (most toxic organophosphate pesticides to *Tetraselmis*), for ten days (long term exposure).

The highest lipid content obtained in the cultures exposed to the most toxic toxicants for each types of algae indicate that, additional input of toxicants which containing metal or by-products of degraded textile dye and organophosphate pesticides stimulated the lipid accumulation in the cell. Therefore, the lipid content increased in the algae. As reported by Liu, *et al*., (2008b) who exposed *Chlorella vulgaris* to different concentrations of Fe^{3+} ($1.2x10^{-8}$ mol/L to $1.2x10^{-5}$ mol/L), they found that the increase in total lipid of *C. vulgaris* stimulated by high iron concentration was mainly attributed to neutral lipids accumulation. They suggested that some metabolic pathways related to the lipid accumulation in *C. vulgaris* were probably modified by high level of iron concentration in the initial medium.

In addition, the high content of lipid obtained at low concentration of toxicants (0.01 mg/L – 10 mg/L), may be due to degradation of the membrane structure of the algae. Phospholipids and fatty acids are the fundamental structural components of membrane cell (Chia, *et al.*, 2013). The lipid compounds are easily oxidize by free radicals and also other reactive metal that produces by these toxicants. During oxidation of lipid, the large molecule structure of lipids are breakdown to free fatty acids and accumulated in the cell. As a results the lipid content increased in the cell (Hardin, *et al*., 2012; McGarry, 1997).

In comparison of lipid content in algae between short term (4 days) and long term (10 days) exposure, we observed that the lipid content of the *Chlorella*, *Tetraselmis*, *Boergesenia* and *Ventricaria* grown for ten days in the Prov50 medium without toxicant (control cultures) was 66.29%, 42.41%, 42.59% and 67.57% respectively higher than in

the four days cultures. This condition shows that the increase of lipid content following increasing duration of time was due to the increase in growth of the algae.

According to Gatenby, *et al.*, (2003), typically algae cultures become depleted in nutrients, as they enter stationary stages of growth, and total lipid and carbohydrate will increased while the protein will be decline (Liu, *et al.,* 2008a; Ogbonna and Tanaka 1996; Lourenco, *et al*., 1997). As demonstrated by Liu, *et al*., (2008b) and Illman, *et al*., (2000), reduction of nitrogen in the medium increased the lipid content in algae strains investigated (*Chlorella emersonii*: 63%; *Chlorella minutissima*: 56% and *Chlorella vulgaris*: 40% lipid/DW).

In addition to this factor, in the non-renewal culture system, the nutrient level in the culture medium will be decreased with increase of time (Stewart, 1974; Barsanti and Gualtieri, 2006). Therefore in this study, it is suggested that may be the increased of lipid content in the algae after being grown for ten days (where the algae already reach stationary stage), was also due to the limiting nutrient level in the culture medium, which in this study, we use non-renewal culture system.

For the present study, the lipid content of *Tetraselmis* grown for four day, was 25.16% higher than in the Chlorella. The higher value of lipid content in *Tetraselmis*, indicate *Tetraselmis* is suitable to be use in mariculture industry as the fish feed due to their fast growth ability and containing high content of lipid.

5.5 EFFECTS OF SELECTED TOXICANTS ON DNA DAMAGE :RAPD ASSAY IN ALGAE

The increasing presence of genotoxic chemicals in the marine environment has led to the development of rapid and easy monitoring methods. In this study, we have applied the random amplified polymorphism DNA (RAPD) and AP-site counting method to evaluate the DNA damage effects induced by genotoxic chemicals such as metals, textiles dye and organophosphate pesticides in four types of marine algae *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa*.

The random amplified polymorphic DNA (RAPD) is a useful assay for the detection of genotoxin-induced DNA damage and mutations. The advantages of RAPD assay is that, the assay which is suitable for any extracted DNA (with good sufficient quality) requires very little source of materials. As random primers (10-mer oligonucleotide primers) are used, specific details of the DNA damage or the sequence of the genome under investigation are not needed. In addition, no radioactivity or chemicals or enzymatic degradation of the DNA is required before analysis. The analysis also can be performed non-destructively, is non-radioactive, which can be useful for the screening of large number of valuable samples. The RAPD assay is also applicable to any organism with a potential to detect a wide range of DNA damages and mutations including point mutations and large rearrangements (Unyayar, *et al*., 2006; Hagger, *et al*., 2005). These technologies are relatively cheap and do not require the use of special and expensive equipment (Atienzar, *et al*., 2000; Zhou, *et al*., 2011).

Although the generation of RAPD profiles has often been criticised as unreliable (Ellsworth, *et al.*, 1993), subsequent reports have demostrated that after rigorous optimization of PCR parameters, the assay performs well in terms of number of amplified bands, product yield and clarity of profile with a wide range of organisms. Previous studies reported by several researchers show that this assay has been successfully used to detect genotoxic induced DNA damage in marine organism including bacteria (Atienzar, *et al.,* 2002b), zooplankton (Atienzar and Jha 2004; Atienzar *et al*., 2002), larval barnacles (Atienzar, *et al*., 2002a), mussels (Hagger, *et al.*, 2005), fish (Rong and Yin 2004; Ferrero, *et al.*, 1998), seaweed (Atienzar, *et al*., 1997, Atienzar, *et al.*, 1998, Atienzar, *et al.*, 2000a, Atienzar, *et al*., 2002b), and can be adapted to different types of chemical contaminants (Zhou, *et al*., 2011; Atienzar and Jha, 2004; Rong and Yin, 2004; Atienzar, 2002a; Atienzar, 2002a; Atienzar, *et al*., 2001), UV radiations (Atienzar, *et al.*, 2000a; Atienzar, *et al.*, 1998; Atienzar, *et al*.,1997) and stresses (Liu, *et al*., 2005; Atienzar and Jha, 2006).

In the RAPD assay, the process of DNA extraction is a very important step to determine the success of this assay. In this study, the suitability of the DNA extraction methods was evaluated based on the quality of the extract purity and its concentrations. Concentration and purity of the DNA extracted are usually measured at OD_{260nm} and evaluated by the 260 nm/280 nm absorbance ratio. The purity grade obtained from the microalgae and macroalgae extraction is in the range of 1.75–1.95 and the yield obtained is about 1 mg DNA/g algae sample. This indicates the high DNA purity grade of the extraction. The purity and integrity of the template DNA are crucial for good RAPD analysis (Zhou, *et al*., 1997).

In total, eight selected random primers were used in the analysis of the results for each algae used in this study. After optimisation of the PCR conditions and the use of the suitable oligonucleotide primers for each species-specific DNA template, the RAPD assay was shown to perform well as indicated by the number of bands and variations in the band intensity in the RAPD profiles.

In addition, a single base change in the sequence of the 10-mer primers in most cases caused significant changes in the RAPD profiles. Caetano-Anolles *et al*. (1992) and Atienzar, *et al*., (2002a) reported that, at an annealing temperature of 55 °C, only the perfectly matched RAPD primer was successful in amplifying a synthetic template. As in the present study an annealing temperature of 42 to 44 °C for 10-mer primers was used, it is likely that most of the amplified PCR products (and particularly the brighter bands) derived from perfect, or almost perfect, pairing between the DNA and primer sequence. After DNA extraction, PCR reaction were performed; the Taq DNA polymerase amplified the DNA regions from 150 up to 500 basepairs with low efficiency and at 500 up to 3000 basepair, the amplified bands were at high efficiency.

5.5.1 RAPD Profile Pattern in the Study

In all cases, RAPD profiles generated by toxicant-exposed algae were different from those obtained using control DNA. DNA pattern generated by each treatment group were reproducible, although each RAPD profile was obtained from an individual algal sample. In this study, the reproducibility of the RAPD profiles was generally optimal among the different treatments. In addition, the use of two DNA concentrations that differ by a factor of four, as recommended by Welsh, *et al*., (1995), led to identical

RAPD profiles due to the robustness of the assay. Since the RAPD assay is considered to be a semi-quantitative assay (Atienzar and Jha, 2004), our approach in this study was to use it as a qualitative rather than a quantitative assay.

Previous studies have shown that changes in band patterns observed in RAPD profile analyses reflect DNA alterations from single base changes (point mutations) to complex chromosomal rearrangements (White, *et al*., 1990; Welsh, *et al*., 1990; Atienzar, *et al*., 1999). For example, Atienzar, *et al*., (2000) reported that a set of experiments performed in vitro revealed that DNA alterations such as DNA breakage, benzo (a)pyrene adducts and thymine dimers as well as mutation in the sequence of the primer induced significant changes in RAPD profiles. Similarly, in the present study, DNA damage induced by metals, textiles dye and organophosphate pesticides were shown by changes in RAPD profiles like the appearance of new band, disappearance of bands, similiarity of band with the control and variation in band intensity which occurred in the profiles generated by exposed algae in comparison with control cultures.

The result of the RAPD profiles may differ among individuals, depending on the (i) presence/absence of priming sites, (ii) priming complementary completeness/incompleteness or (iii) the distance between priming sites (Wolf, *et al*., 2004). Therefore, RAPD bands were lost (disappeared) or gained when point mutations, inversions, deletions, additions or gross chromosomal rearrangements affect the presence/absence of primer sites, their complementarity to primers and/or the distance between priming sites. RAPD bands of different molecular weights are interpreted as separate loci which are scored on a present (amplification)—absent (non-amplification) basis (Fritsch, L.H. Rieseberg 1996).

5.5.2 RAPD: Appearance of New Band

New PCR products are amplified maybe because some oligonucleotide priming sites became accessible to oligonucleotide primers after structural change induced by DNA adducts and:or by non genotoxic events such as transposition and DNA amplification. In addition, some changes in DNA sequence may occurr due to mutations, large deletions (bringing two pre-existing annealing sites closer), and/or homologous recombination (Atienzar, *et al*., 1999) in the genome. According to Bowditch, *et al.*,(1993), the RAPD assay has the potential to detect mutations outside the priming site. Following exposure to mutagens, DNA replication and error-prone DNA repair are generally involved in generating mutations (Livneh, *et al*., 1993). Mutations can only be responsible for the appearance of new bands if they occur at the same locus in a sufficient number of cells (a minimum of 10% of mutations may be required to get a new PCR product visible in agarose gel) to be amplified by PCR (Bowditch, *et a*l., 1993).

In this study, it is suggested that the appearance of new bands occur in the RAPD profiles because of DNA fragmentation which are caused by toxicants that induce structural changes in the DNA template. A toxicant may interfere with the template function of DNA by covalent binding of chemicals to DNA, which will cause nucleotide mispairing during replication. For example, attack of DNA bases by free radicals can result in the formation of imidazole ring-opened purines or ring-contracted pyrimidines, which will block DNA replication (Klaassen, 2008). Therefore after being amplified by PCR, small DNA fragments will be observed in the RAPD profiles.

In the present study, in comparison to the control cultures, appearance of new bands were detected in the RAPD profiles of the algae exposed to the toxicant for short

(four days) and long (ten days) term exposure. There are two different trends of appearance of new bands were observed in the RAPD profiles of the algae exposed to the toxicant for four days and long ten days as summarizes in Table 5.12.

The first trend observed was, the appearance of new bands in algae cultures exposed to the toxicant for four and ten days, decreased with increasing concentrations of toxicant. Reduction of the new bands amplified indicate that, these toxicants could inhibit the replication process of DNA strand following increasing concentrations of toxicant.

The second trend observed was, the appearance of new bands in algae cultures exposed to the toxicant for four and ten days, increased with increasing concentrations of toxicant up to 1 mg/L of toxicant and then decreased at higher concentrations of toxicant. Increase in the appearance of new bands occurred for the three lowest concentrations of toxicant (at concentrations of 0.01, 0.1, 1 mg/L toxicant) showing that, the number of the small DNA fragments was increased following increasing concentration of toxicant up to 1 mg/L toxicant. Therefore the % appearance of new bands will be increased following increasing concentration of toxicant due to increasing damage of the DNA strand.

However, at the high concentrations of toxicant (10 to 500 mg/L toxicant), the appearance of new bands decrease may be because these toxicants inhibit DNA repair and replication process in the algae cell. In addition, the growth of the algae is inhibited at high concentration of toxicants (10 to 500 mg/L), which will reduce the synthesis and replication process of the DNA strand in the cell. Beside that, the DNA strand structure may be totally damaged or lost after being exposed to higher concentrations of toxicant.

Table 5.12: Appearance of new band trend of the RAPD profiles in the algae cultures exposed to selected toxicants

Appearance of new band trend	Four days cultures	Ten days cultures
Decreased with increasing concentrations of toxicant	● *Chlorella* exposed to Mn, Acidic dye and Dichlovos ● *Tetraselmis* exposed to Cd, Cr, Acidic dye and Dichlovos ● *Boergesenia* exposed to Cd, Zn, Acidic dye, Basic dye and Malathion ● *Ventricaria* exposed to Zn, Basic dye, Metal complex dye and Malathion	● *Chlorella* exposed to Cd, Mn and Metal complex dye ● *Tetraselmis* exposed to Cd, Cr, Cu and Metal complex dye ● *Boergesenia* exposed to Basic dye, Metal complex dye and Dichlovos ● *Ventricaria* exposed to Cd, Cu, Acidic dye, Basic dye and Dichlovos
Increased with increasing concentrations of toxicant up to 1 mg/L of toxicant and then decreased at higher concentrations of toxicant.	● *Chlorella* exposed to Cd, Fe, Basic dye, Metal complex dye and Malathion, ● *Tetraselmis* exposed to Cu, Basic dye, Metal complex dye and Malathion, ● *Boergesenia* exposed to Cu, Metal complex dye and Dichlovos ● *Ventricaria* exposed to Cd, Cu, Acidic dye and Dichlovos	● *Chlorella* exposed to Fe, Acidic dye, Basic dye, Dichlovos and Malathion ● *Tetraselmis* exposed to Acidic dye, Basic dye, Dichlovos and Malathion ● *Boergesenia* exposed to Cd, Cu, Zn, Acidic dye and Malathion ● *Ventricaria* exposed to Zn, Metal complex dye and Malathion

Therefore less DNA strands will be obtained in the cell and will directly reduce the % appearance of new band in the RAPD profiles of the algae.

5.5.3 RAPD: Disappearance of band

For the present study, in comparison with the control cultures in the RAPD profiles, different trends of disappearance of PCR amplified products in the algae cultures exposed to selected toxicants for four and ten days, were observed. Table 5.13. summarizes the different trends of disappearance of bands in the RAPD profiles of the algae exposed to the toxicant for four days and long ten days.

The first trend observed was, the disappearance of bands in algae cultures exposed to the toxicant for four and ten days, was increased with increasing concentrations of toxicant. Increase in % disappearance of bands in the RAPD profiles shows that, less DNA strands were amplified, which indicates that these toxicants could inhibit the replication process in the cell following increasing concentration of toxicant due to the structural changes in the DNA template. Therefore, the damage level of the DNA strand in the cell will increase with increasing concentrations of toxicant, resulting in increased in the % disappearance of bands in the RAPD profiles of the algae.

The second trend observed was, the disappearance of bands in algae cultures exposed to the toxicant for four and ten days, increased with increasing concentration of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. The increase of % disappearance of band at the lower concentrations of

Table 5.13: Disappearance of band trend of the RAPD profiles in the algae cultures exposed to selected toxicants

Disappearance of band trend	Four days cultures	Ten days cultures
Increased with increasing concentrations of toxicant	● *Chlorella* exposed to Cd, Fe, Mn,Acidic dye, Basic dye, Dichlovos and Malathion ● *Tetraselmis* exposed to Cd,Cr, Cu, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion ● *Boergesenia* exposed to Cu, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion ● *Ventricaria* exposed to Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion	● *Chlorella* exposed to Cd, Fe, Mn, Acidic dye, Basic dye, Metal complex dye and Dichlovos ● *Tetraselmis* exposed to Cd, Cr, Cu, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion ● *Boergesenia* exposed to Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion ● *Ventricaria* exposed to Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion
Increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant.	● *Chlorella* exposed to Metal complex dye ● *Boergesenia* (exposed to Cd)	● *Chlorella* cultures exposed to Malathion

toxicant (0.01 mg/L to 10 mg/L), shows that, the damage level of the DNA strand in the cell was increased following increasing concentration of toxicant up to 10 mg/L toxicant.

However at higher concentrations of toxicant (100 mg/L to 500 mg/L), the DNA strand template in the cell maybe totally destroyed or lost caused by these toxicants, and therefore the % disappearance of bands in the RAPD profiles decreased. In addition, inhibition of growth at higher concentration of toxicant will also contribute to the loss of DNA strands in the cell.

Our results show that increase in disappearance of bands with increasing concentration and time of exposure of toxicants agree well with the observations by previous studies carried out by researchers who demonstrated a dose-dependent relationship between the disappearance of RAPD band with increasing concentration and time of exposure of nitrofurazone (Zhou, *et al*.,2011), Cadmium (Liu, *et al*.,2005), Cuprum (Atienzar *et al.*, 2001) and UV radiation (Atienzar, *et al.*, ,2000a) using RAPD analysis. The disappearance of band mainly affected the high molecular weight bands. Disappearance of the largest amplicons were probably due to the presence of bulky adducts which can block or reduce the process of the *Taq* DNA polymerase (Chary, *et al*., 1995). However, some smaller amplicons were even much more affected, thus suggesting non random interactions between the chemical contaminants intermediate and DNA (Smith, *et al*., 2000; Atienzar, *et al.*, 2002a; Atienzar, *et al.*, 2002b; Atienzar, *et al.*, 2002c).

In addition, according to Atienzar, *et al*., (2000a) the disappearance of band (i) can only be detected or observed if the same structural changes occur in 75-95% of the

cells or (ii) if same mutation arise in the same percentage of cells. As suggested by Liu, *et al*,. (2005), disappearance of bands are likely to be due to one or a combination of the following events: (i) changes in oligonucleotide priming sites due mainly to genomic rearrangements and less likely to point mutations; (ii) DNA damage in the primer binding sites; (iii) interactions of DNA polymerase in test organism with damaged DNA. For example, the relatively high frequency of disappearance of bands may reveal that the survival of the individuals was greatly affected. The growth experiments support this observation.

5.5.4 RAPD: Similarity of band

In the present study, different trend of % similarity of band in RAPD profiles in comparison to the control were observed in the algae cultures exposed to the toxicant for four and ten days. Table 5.14 summarizes the different trend similarity of bands in the RAPD profiles of the algae exposed to the toxicants for short term (four days) and long term (ten days) exposure.

The first trend observed was, the similarity of bands in algae cultures exposed to the toxicant for four and ten days, was decreased with increasing concentrations of toxicant. Decrease in the % similiarity of the bands in comparison to control indicated that, these toxicants may have induced structural changes in the DNA template and this condition will interrupt the DNA strand synthesis and replication process in the cell, which will then directly increase the damage level of the DNA strands in the cell. For that reason, less DNA strands can be amplified in the cell following increasing

Table 5.14: Similarity of band trend in comparison to control of the RAPD profiles in the algae cultures exposed to selected toxicants

Similarity of band trend	Four days cultures	Ten days cultures
Decreased with increasing concentrations of toxicant	● *Chlorella* exposed to Cd, Fe, Mn, Acidic dye, Metal complex dye and Dichlovos ● *Tetraselmis* exposed to Cd, Cu, Acidic dye, Basic dye and Dichlovos ● *Boergesenia* exposed to Cu, Zn, Metal complex dye and Malathion ● *Ventricaria* exposed to Cd, Cu, Zn, Basic dye, Metal complex dye, Dichlovos and Malathion	● *Chlorella* exposed to Cd, Fe, Mn, Acidic dye, Basic dye, Metal complex dye and Dichlovos ● *Tetraselmis* exposed to Cd, Basic dye and Metal complex dye ● *Boergesenia* exposed to Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye and Malathion ● *Ventricaria* exposed to Cu, Zn, Acidic dye, Basic dye and Malathion
Increased with increasing concentrations of toxicant up to 1 mg/L of toxicant and then decreased at higher concentrations of toxicant.	● *Chlorella* exposed to Basic dye and Malathion ● *Tetraselmis* exposed to Cr, Metal complex dye and Malathion ● *Boergesenia* exposed to Cd, Acidic dye, Basic dye and Dichlovos ● *Ventricaria* exposed to Acidic dye	● *Chlorella* exposed to Mn and Malathion ● *Tetraselmis* exposed to Cr, Cu, Acidic dye, Dichlovos and Malathion ● *Boergesenia* exposed to Dichlovos ● *Ventricaria* exposed to Cd, Metal complex dye and Dichlovos

concentration of toxicant, resulting in reduction in the % similarity of bands in the RAPD profiles of the algae.

The second trend observed was, the similarity of bands in algae cultures exposed to the toxicant for four and ten days, was increased with increasing concentrations of toxicant up to 1 mg/L of toxicant and then decreased at higher concentrations of toxicant. The increase of % similarity of the bands, following increasing concentration of toxicant up to 1 mg/L toxicant shows that, the synthesis and replication process of the algae may not have been inhibited by these toxicants or may be at this lower concentration of toxicant, the algae adapted and tolerated these toxicants (for long term exposure algae).

At low concentrations of toxicant (0.01 mg/L to 1 mg/L), the metals like Cd, Cu, Fe and Zn may be used as additional trace mineral supplement to induce the replication of DNA strand in the cell. For instance, Zn and Cu are important cofactors for many enzymes (Chaney, 1997) including DNA polymerases which are involved in the DNA replication mechanism in the cell. It is suggested that the DNA repair mechanism was also involved in the process of adaptation in the algae cell to these toxicants until a certain threshold, beyond which the cell lost their resistance and died. Therefore the % similiarity of the bands in comparison to the control, in the RAPD profiles of the algae exposed to toxicant for four days and ten days, increased with increasing concentration of toxicant up to 1 mg/L toxicant.

However, at the higher concentrations of toxicants (10 mg/L to 500 mg/L), the algae could not tolerate to the high concentrations of toxicant and growth is inhibited. Inhibition of algae growth will directly reduce the DNA strand synthesis in the cell and

finally may destroy the DNA strand structure in the cell following increasing concentration of toxicant. Therefore, the % similiarity of the bands in comparison to the control, in the RAPD profiles of the algae, will be decreased due to the increased damage level of the DNA strands in the algae.

Our results are similar to that reported by Rong and Yin (2004) who showed that the RAPD pattern from zebrafish exposed to genotoxic chemicals such as cyclophosphamide (ranged from 0.1 to 10 mg/L) and dimethoate (ranged from 1.0 to 100 mg/L) displayed some changes in band patterns in comparison to control, including the loss of stable bands. As mentioned in previous reports by other researchers, damage to the genomic DNA will then result in changes of the binding site and PCR product, and furthermore alter the electrophoresis pattern (Bacerril, *et al*., 1999; Savva, 1998).

5.5.5 RAPD: Intensity of band

In this study different trends of variation of band intensity were observed in the RAPD profiles of algae exposed to selected chemical contaminants for four and ten days. The band intensity differences between treatment and control groups were analyzed using image analysis software and only well resolved intense RAPD bands were used when analyzing RAPD profiles as suggested by Ge, *et al*., (2003) and Aagaard, *et al*., (1998).

According to Donahue, *et al*., (1994) and Nelson, *et al*., (1996), the variation in band intensity may be influenced by the presence of DNA adducts which can act to block or reduce (bypass event) the polymerization of DNA in the PCR reaction. The bypass

event is a complicated process which depends on the enzymatic properties of DNA polymerase, the structure of the lesion and the sequence context of its location (Ide, *et al.*, 1991). Atienzar, *et al*., (2002a,b,c) reported that the DNA damage may interfere with the DNA polymerase activity, thus altering the number of newly synthesized amplicons. For example, a single DNA photoproduct (Govan, *et al*., 1990) or cisplatin adduct (Jennerwein and Eastman, 1991) can block the PCR amplification. The intensity differences in the RAPD profiles are suggested as the results from product copy number differences, competition between PCR product, heterozygosity, co-migration or partial mismatching of primer sites (Harris, 1999; Wolf, *et al*., 2004).

For the present study, the % intensity of band in the RAPD profiles of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* cultures grown for four days and ten days in the Prov50 Medium without toxicant (control cultures) have been set as 100%. The % intensity of band in the RAPD profiles of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* cultures grown for four days in the Prov50 Medium containing all toxicants used in this study (Cd, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion) were higher than in the algae cultures grown in the Prov50 Medium without toxicant (control cultures). Similar results was obtained in the *Chlorella* (exposed to Mn, Basic dye, Metal complex dye and Malathion), *Tetraselmis* (exposed to Cd, Cr, Cu, Acidic dye, Basic dye, Metal complex dye and Dichlovos), *Boergesenia* and *Ventricaria* exposed to all toxicants used in this study (Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion) after being exposed to the toxicants for ten days (long term exposure). This condition showed that the algae exposed to these toxicants had increased % intensity of band in the RAPD profiles. This condition occurred because after being exposed to these toxicants, a

long DNA strand will be lysed into small DNA fragments and these small DNA fragments were amplified abundantly in the cell. Therefore the % intensity of band in the RAPD profiles of the exposed algae was higher than in the control cultures.

However, for the *Chlorella* cultures exposed to Cd for four days, and also in the *Chlorella* cultures (exposed to Cd, Fe, Acidic dye and Dichlovos) and *Tetraselmis* (exposed to Malathion) for ten days, the % intensity of band in the RAPD profiles were lower than in the control cultures.This condition showed that these toxicants, inhibited the replication process in the cell, which then reduced the intensity of band in the RAPD profiles of the exposed algae. This may be because after being exposed to Cd (one of the most toxic metal to *Chlorella*) and other toxicants, long DNA strands may be totally destroyed and directly reduce the number of small DNA fragments in the cell. For that reason, less DNA strands were amplified in the cell. As a result, the % intensity of band in the RAPD profiles of the exposed algae was lower than in the control cultures due to the loss of the DNA strands in the cell.

In the present study, different variation of intensity of band were observed in the RAPD profiles. Table 5.15 summarizes the different trend intensity of bands in the RAPD profiles of the algae exposed to the toxicants for short term (four days) and long term (ten days) exposure.

The first trend observed was, the intensity of bands in algae cultures exposed to the toxicant for four and ten days, was decreased with increasing concentrations of toxicant. The results show that, these toxicants could inhibit the replication process in the

Table 5.15: Intensity of band trend of the RAPD profiles in the algae cultures exposed to selected toxicants

Intensity of band trend	Four days cultures	Ten days cultures
Decreased with increasing concentrations of toxicant	● *Chlorella* exposed to Mn and Acidic dye ● *Boergesenia* exposed to Cd	● *Chlorella* exposed to Acidic dye ● *Tetraselmis* exposed to Malathion ● *Ventricaria* exposed to Dichlovos
Increased with increasing concentrations of toxicant	● *Chlorella* exposed to Metal complex dye ● *Tetraselmis* exposed to Malathion) ● *Boergesenia* exposed to Cu, Zn, Acidic dye, Metal complex dye and Dichlovos	● *Ventricaria* exposed to Malathion
Increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant.	● *Chlorella* exposed to Cd, Fe, Basic dye, Dichlovos and Malathion ● *Tetraselmis* exposed to Cd, Cr, Cu, Acidic dye, Basic dye, Metal complex dye and Dichlovos ● *Boergesenia* exposed to Basic dye and Malathion ● *Ventricaria* exposed to Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion	● *Chlorella* exposed to Cd, Fe, Mn, Basic dye, Metal complex dye, Dichlovos and Malathion ● *Tetraselmis* exposed to Cd, Cr, Cu, Acidic dye, Basic dye, Metal complex dye and Dichlovos ● *Boergesenia* exposed to Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion ● *Ventricaria* exposed Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye and Dichlovos

cell which reduced the intensity of band in the RAPD profiles of the algae following increasing concentrations of toxicant.

The second trend observed was, the intensity of bands in algae cultures exposed to the toxicant for four and ten days, was increased with increasing concentrations of toxicant. This indicated that, after being exposed to these toxicants, a long DNA strand was lysed into small DNA fragments and the small DNA fragments were be amplified abundantly in the cell. Therefore, the % intensity of band in the RAPD profiles of the algae was increased due to the increased DNA damage level in the cell following increasing concentration of toxicant.

The third trend observed was, the intensity of bands in algae cultures exposed to the toxicant for four and ten days, was increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. Increased in the % intensity of band in the RAPD profiles, in the alga exposed to four lowest concentration of toxicants (0.01 mg/L to10 mg/L toxicant) showing that, there are high number of the small DNA fragments are amplified following increasing concentration of toxicants up to 10 mg/L toxicants, which indicate the elevated of DNA damage level in the cell. Therefore the % intensity of bands increased up to 10 mg/L toxicants. However, at higher concentration of toxicants (100 mg/L- 500 mg/L), the % intensity decreased due the lost of DNA fragment in the cell .

According to Atienzar *et al.,* (2002a) and Atienzar *et al.,* (2002b), increased in band intensity observed in the RAPD profiles, shows that the level of DNA adduct induced by toxicants may block the PCR enzyme at certain sites and allow a more

efficient amplification of non-damaged genomic DNA. Alternatively, the increase in band intensity could be also due to a better availability of 10-mer primers (Wolf, *et al*., 2004). The increase in band intensity could be attributed to conformational DNA changes possibly improving the access of the primer(s) to the binding sites near the adducts (Pietrasanta, 2000).

5.5.6 RAPD: Genomic template stability

Genomic Template Stability is related to the level of DNA damage, the efficiency of DNA repair and replication. Although the RAPD assay does not provide information on the nature and extent of these genotoxic induced DNA alterations, it can sometimes be used in a quantitative way (Atienzar, *et al*., 2002a,b,c). As suggested by Atienzar, *et al.,* (1999) , in the present study, the differences to estimate the percentage of genomic template stability, calculated as 100− (100*a*/*n*), where *n* is the number of bands in the control DNA (i.e. non-exposed individuals) and *a* is the average number of DNA changes in the exposed individuals.

5.5.6.1 Genomic template stability trends in the study

For the present study, the % genomic template stability in the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* cultures grown for four days in the, Prov50 Medium without toxicant (control cultures) have been set as 100%.

The % genomic template stability of the algae cultures grown in the Prov50 Medium without toxicant (control cultures) for four and ten days were higher than in the

Chlorella, Tetraselmis, Boergesenia and *Ventricaria* cultures exposed to all toxicants used in this study (Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion) showing that, these toxicants give adverse effects on genomic template stability and increased the damage level of the DNA strand in the cell.

There were different trends in the genomic template stability observed in the present study as summarizes in Table 5.16. The first trend observed was, the % Genomic template stability of the algae cultures exposed to the toxicants for four days (short term exposure) and ten days (long term exposure), decreased with increasing concentration of toxicants.

Decrease in % genomic template stability of the exposed algae shows that, these toxicants could affected the stability of the DNA structure in the cell, following increasing concentrations of toxicant. This condition may have occurred because of the high frequency of change in RAPD profiles due to high level of DNA damage in the cell (as shown by high % disappearance of band or low % appearance of new band in the RAPD profiles) which will contribute to the reduction of the % genomic template stability following increasing concentrations of toxicant. This indicated that genomic template stability is significantly affected by chemical contaminants which agrees well with the results reported by Zhou, *et al.*, (2011) who demonstrated the reduction in genomic template stability in the marine ciliate, *Euplotes vannus* with increasing nitrofurazone concentration in the range of 0 to 24mg/L and time of exposure from 24 to 96 h.

Table 5.16: Genomic template stability trend in the algae cultures exposed to selected toxicants

Intensity of band trend	Four days cultures	Ten days cultures
Decreased with increasing concentrations of toxicant	● *Chlorella* exposed to Cd, Mn, Acidic dye, Basic dye and Dichlovos ● *Tetraselmis* exposed to Cd, Cr, Cu, Basic dye, Dichlovos and Malathion ● *Boergesenia* exposed to Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye and Malathion ● *Ventricaria* exposed to Cd, Cu, Zn, Basic dye, Metal complex dye, Dichlovos and Malathion	● *Chlorella* exposed to Cd, Fe, Mn, Acidic dye, Basic dye, Metal complex dye and Dichlovos ● *Tetraselmis* exposed to Cd, Cr, Basic dye and Metal complex dye ● *Boergesenia* exposed to Cd, Cu, Zn, Acidic dye and Malathion ● *Ventricaria* exposed to Cu, Basic dye, Metal complex dye and Dichlovos
Increased with increasing concentrations of toxicant up to 1 mg/L of toxicant and then decreased at higher concentrations of toxicant.	● *Chlorella* exposed to Fe, Metal complex dye and Malathion ● *Tetraselmis* exposed to Acidic dye and Metal complex dye ● *Boergesenia* exposed to Dichlovos ● *Ventricaria* exposed to Acidic dye	● *Chlorella* exposed to Malathion ● *Tetraselmis* exposed to Cu, Acidic dye, Dichlovos and Malathion ● *Boergesenia* exposed to Basic dye, Metal complex dye and Dichlovos ● *Ventricaria* exposed to Cd, Zn, Acidic dye and Malathion

The second trend observed was, the % genomic template stability of the algae cultures exposed to the toxicants for four days (short term exposure) and ten days (long term exposure), increased with increasing concentrations of toxicant up to 1 mg/L of toxicants and then decreased at higher concentrations of toxicants. The results indicate that, at low concentrations of toxicant (0.01 mg/L to 1 mg/L toxicant), the algae still can grow and tolerate these low levels of toxicants in their cell. Therefore the DNA strand structures in the cell can still replicate and repair in order to adapt to these increasing concentrations of toxicants. As shown in RAPD profiles results, at concentrations of toxicant between 0.01 mg/L to 1 mg/L, the % appearance of new band and % disappearance of band trends were variable, which will influence the value of average number of RAPD changes used to calculate the % genomic template stability. As a result, the % genomic template stability will be increased or fluctuated between that range of concentrations until a certain level (1 mg/L toxicants).

However at the higher concentrations of toxicants (10 to 500 mg/L toxicants), the% genomic template stability decrease may be because the algae were not able tolerate to these high concentration of toxicants and the replication and repair process on the DNA strand was inhibited, which directly will destroy the DNA strand structure as shown by high % of disappearance of band and low % of similarity of the band.

In the present study, different trends were observed in the *Chlorella* [exposed to Fe (for 4 days), Metal complex dye (for 4 days) and Malathion (for 4 days and 10 days)]; *Tetraselmis* [exposed to Metal complex dye (for 4 days)]; *Boergesenia* [exposed to Basic dye (for 10 days) and Dichlovos (for 10 days)] and *Ventricaria* [exposed to Cd (for 10 days) and Zn (for 10 days)] where the genomic template stability should be decreased

with increasing concentrations of toxicant or followed the same trend as the growth of algae. However in these cultures, the genomic template stability increased with increasing concentrations of toxicant up to 1mg/L and then decreased at higher concentrations of toxicant.

Genomic template stability is one of the good parameters to measure the DNA damage levels in the exposed cells (Atienzar, *et al*, 1999). Therefore the trend that shows increasing % genomic template stability at low concentrations of toxicant (0.01mg/L to 1 mg/L toxicant), is an opposite pattern to the growth of the algae, may be because of the DNA repair process occurring in the less damage DNA strand structure. DNA repair is the most important mechanism for the cell to solve the problem of how to cope with the extensive DNA damage that it sustains (Preston and Hoffmann, 2008). If the damage is less severe, it can be repaired by a range of processes that are part of generalized cellular DNA damage response network that returns the DNA to its undamaged state (error-free repair) or to an improved but still altered state (error-prone repair). However, if the DNA damage is extensive, the cell can undergo apoptosis (programmed cell death) and effectively releasing it from becoming a mutant cell (Evan and Littlewoods, 1998).

In addition, the low concentration of metals like Cu, Fe and Zn (which was important trace mineral required for the cell) can be used to induce the synthesis of new DNA strand and replication process in the DNA. These low concentrations of trace minerals may also be involved in the repair mechanism in the cell (Chaney, 1997). Therefore the DNA strand structure changes and stability in the cell (which shown by % genomic template stability value) will be counter-balanced between DNA damage (shows by disappearance of band) and DNA repair mechanism following increasing

concentrations of toxicants until a threshold level (1 mg/L toxicant). The DNA repair and replication mechanism may help the algae to tolerate and reduce the DNA damage induced by these toxicants up to concentrations of 1 mg/L. This may be one of the adaptive or protective mechanisms carried out by the algae to protect the cell from DNA damages induced by toxicants. However at higher concentrations of toxicants (10 mg/L - 500 mg/L toxicant), the DNA strand structure in the algae were totally damaged or disappeared which will contribute to the reduction in the % genomic template stability measured.

Previous studies have also shown that changes in RAPD profiles induced by toxicants can be regarded as changes in genomic DNA template stability. For instance, Atienzar *et al*., (1999) demonstrated that the DNA template stability parameter shows significant effects at benzo{*a*}pyrene exposure levels to clonal *Daphnia magna*., *Daphnia magna* (Atienzar, 2002c); *Mytilus galloprovincialis* (Atienzar, *et al*., 2002b). Atienzar *et al*., (2000a) also reported that the genomic template stability in the a marine alga *Palmaria palmata* decreased with increasing UV radiation and exposure time, which indicated that genomic template stability was significantly affected by UV radiation . Similar effect on DNA damage measured using genomic template stability as a biomarker was reported for the marine mollusc embryos *Mytilus edulis* exposed to tritiated water (0.37 to 370 kBq/mL of HTO) for one hour (Hagger, *et al*., 2005); cyclophosphamide (0.1 to 10 mg/L) and dimethoate (1.0 to 100 mg/L) exposed to zebrafish *Danio rerio* for 96h (Rong and Yin , 2004) and man-made estrogens, exposed to larval barnacles *Elminius modestus* for 8 days (Atienzar, *et al*, 2002a) indicated that genomic template stability was significantly affected by the toxicants and may potentially be a useful biomarker to detect DNA damage in this study.

In comparison of the genomic template stability of the algae between short term (4 days) and long term (10 days) exposure to the toxicant, the results shows that, the % genomic template stability of the *Chlorella* (exposed to Cd, Mn, Acidic dye, Basic dye, Dichlovos and Malathion), *Tetraselmis* (exposed to Cr and Dichlovos), *Boergesenia* (exposed to Cu, Zn, Acidic dye, Metal complex dye and Dichlovos) and *Ventricaria* (exposed to Cd, Zn, Acidic dye, Basic dye, Dichlovos and Malathion), for ten days was higher than in the four days cultures, showing that % genomic template stability, increased following increasing duration of exposure to these toxicant. This condition occurred due probably to adaptation and tolerance to the toxicant and therefore the DNA damage level in the cell was reduced. As a result, the % genomic template stability of the algae exposed to toxicants for ten days was higher than in the four days cultures.

The % genomic template stability of the *Chlorella* (exposed to Fe and Metal complex dye), *Tetraselmis* (exposed to Cd, Cu, Acidic dye, Basic dye, Metal complex dye and Malathion), *Boergesenia* (exposed to Cd, Basic dye and Malathion) and *Ventricaria* (exposed to Cu and Metal complex dye), for four days was higher than in ten days cultures, indicating that the % genomic template stability, was decreased following increasing duration of exposure to these toxicants. This may be because, after being exposed for short term duration, the growth of the algae was not yet inhibited by these toxicants and therefore the DNA damage level in the cell could still be controlled via synthesis of new DNA strands. In addition, maybe repair mechanism occurred in the algae cell exposed to these toxicants, to reduce the DNA damage level in cell. Therefore the % genomic template stability of the algae exposed to toxicant for four days was higher than in the ten days cultures.

However after being exposed for long term duration (10 days), the algae were not able tolerate to these toxicant and growth was inhibited. Inhibition of the growth of the algae will directly destroy the DNA strand structure in the cell following increasing concentration of toxicant. Therefore, the % genomic template stability of the algae will be decreased due to the increased damage level of the DNA strand in the algae and also due to the inhibiton of growth in algae.

5.5.7 Limitation of RAPD Assay

Although RAPD assay has many advantages over allozyme electrophoresis, it also has some important limitations. Reproducibility is one of its major problems and it is influenced by PCR conditions (Harris, 1999). However, as reported by Benter, *et al*., (1995); Gravesen, *et al*., (2000); Fraga, *et al*., (2002) and Blixt, *et al*., (2003), even if RAPD reproducibility can be achieved, replicates should always be included and bands that fail to be reproduced consistently, should not be considered (Aagaard, *et al*., 1998; Bouzat, 2001; Ge, *et al*., 2003; Maltagliati, *et al.*, 2003; Wolf, *et al*., 2004). Most of the studies which experienced some problems of reproducibility suggested that the results were due to artefacts, contamination or mutation in genomic DNA (Scott, *et al*.,,1992, Riedy, *et al*.,1992). Welsh, *et al*., (1995) suggested that , if profiles from the same genomic template, or from different individuals of the same species are different, the reproducibility of the assay should be confirmed by repeating the PCR reaction by using two different template concentrations, differing by at least two-fold. The use of two DNA concentrations that differ by at least a factor of two can be used to identify spurious or non-reproducible bands (Atienzar and Jha, 2006). Therefore, in order to obtain reliable results, DNA isolation and PCR conditions must be strictly standardized,

use of blanks in each PCR run, improving electrophoretic separation and band staining procedures (Backeljau, *et al*., 1995) and replicates should be carefully analyzed with respect to presence/absence and intensity differences of RAPD bands (Atienzar and Jha, 2006).

As reported by Fritsch and Rieseberg, (1996), a second limitation of RAPD assay arises from the dominant/recessive character of RAPD bands. As a result, heterozygotes cannot be distinguished from homozygotes of the dominant allele (Fritsch and Rieseberg 1996; Lynch, *et al*., 1994). Thirdly, the nature of the genomic change that is scored is not known. Fourthly, RAPD may not screen the genome as randomly as expected. Most RAPD primers have a high GC content, required for the successful annealing at low temperatures, they may tend to screen GC-rich regions which are not evenly distributed across the genome (Harris 1999) and often contain fast mutating sites (Backeljau *et al.*, 1995), therefore genotoxicity effects observed may be upwardly biased.

According to Wolf *et al*., (2004), due to these limitations RAPD assay can be considered as a relatively cheap but "quick and dirty" method to preliminary screen populations for genotoxic effects, if replicates and non-exposed individuals are carefully monitored and analyzed as well. As reported by Atienzar and Jha (2006) the major challenge of identifying the precise reasons for the changes which may be induced in the RAPD profiles may remain unresolved. Indeed, different types of DNA lesions and mutations can induce the same type of alterations in RAPD profiles (e.g. variation in band intensity, band appearance and disappearance) (Atienzar and Jha, 2006). For example, in the present study, although it seems obvious that the DNA damage level increased with increasing concentrations of toxicant, it is not known if mutations

occurred. Jones and Kortenkamp (2000) reported that the RAPD assay can detect mutations only if they occur in at least 2% of the DNA which makes this assay less sensitive than the Ames or hprt test.

Previous studies reported by several researchers suggested that genotoxicity related RAPD alterations require further investigation using cloning, sequencing and/or probing techniques, in order to verify and determine the extent and nature of the DNA alterations (Liu *et al*.,2005; Atienzar and Jha, 2006; Zhou, *et al.*,2011). Therefore, other methods measuring genotoxic end-points, such as detection of DNA adducts, gene mutations or cytogenetic effects, are required for the identification of changes at DNA level (Atienzar and Jha, 2006).

In conclusion, the RAPD method has been successfully used as a sensitive assay of detecting toxicants induced DNA damage in the algae and shows potential as a reliable and reproducible assay for genotoxicity. In addition, similiarly as suggested by Liu *et al*., (2005), Atienzar and Jha, (2006) and Zhou *et al*., (2011), we also strongly encourage the use of the RAPD assay in conjunction with other biomarkers at higher level of biological organization such as growth and biochemical composition, as our studies have demonstrated a link between growth parameters and changes in RAPD profiles in the algae exposed to different types of toxicants. Finally, as reported by Atienzar and Jha (2006), it is important for all who use this technique to appreciate that the RAPD method generates qualitative or semi-quantitative data rather than quantitative data.

5.6 EFFECTS OF SELECTED TOXICANTS ON DNA DAMAGE : AP-SITE CONTENT IN ALGAE

In the present study, AP-site counting assay using DNA Damage Quantification Kit- AP-Site counting (Dojindo Molecular Technologies Inc., Japan) was used to detect the DNA damage in *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa*, induced by genotoxic chemicals such as metals, textiles dye and organophosphate pesticides.

In general, free radicals from both endogenous metabolism and environmental agents like genotoxic contaminants can cause damage to DNA. As reported by Heflich (1991), chemicals can produced DNA alterations either directly (DNA-reactive) as adducts or indirectly by intercalation of a chemical between the base pairs. Many electrophilic chemicals react with DNA, forming covalent addition products (adducts).

The DNA base involved and the positions on DNA bases can be specific for a given chemicals. Such specificity of DNA damage can results in a spectrum of mutations that is chemical specific which is like a variety of fingerprint (Dogliotti, *et al*., 1998). Some alkylated bases can mispair, causing mutations when DNA is replicated. Alkylated bases can also lead to secondary alteration in DNA. For example, the alkyl group of an N7-alkylguanine adduct, which ia a major adduct formed by many alkylating agents, labilizes the bond that connects the bases to deoxyribose, and therefore stimulating base loss. Base loss from DNA leaves an apurinic or apyrimidinic site, commonly called as an AP-site. The insertion of incorrect bases into AP site can causes mutations (Laval, *et al*.,

1990) and it is suggested to be an important intermediate in mutagenesis and carcinogenesis (Loeb and Preston, 1986; Kamiya, *et al*., 1992).

The abasic (AP) site in DNA is the most common lesion and can be generated spontaneously under physiological conditions (Asaeda, *et al*., 1998) or through the action of endogenous or exogenous factors (Melo, *et al* ,2007). It has been estimated that up to 200,000 abasic sites are created in a cell each day (Friedberg, *et al*., 2006; Lindahl, 1993, Nakamura and Swenberg, 1999).

Previous studies reported by several researchers show that AP-Site counting assay has successfully been used to detect genotoxic induced DNA damage in many organism including bacteria (Saito, *et al*.,1997; Weinfeld, *et al*., 1997; Parsian, *et al*., 2002; Gralnick and Downs, 2003; Pfeifer and Greiner, 2005; Cunniffe, *et al*., 2007); HeLa cell (Asaeda, *et al*., 1998); mammalian T4 cell (Hatahet, *et al*., 1999); calf thymus DNA (Kow and Dare, 2000; Kanazawa, *et al*., 2000; Chakravarti, *et al*., 2005); cancer cell (Fortini, *et al*., 2003; Zhao, *et al*., 2007; Wang, *et al*., 2009); yeast (Boiteux and Guillet, 2004; Sugimoto, *et al*., 2005) and have been tested to different types of chemical contaminants such as radioactive from hotspring (Saito, *et al*.,1997); ionizing radiation (Weinfeld, *et al*., 1997; Zhou, *et al*., 2005); Methylmethane sulfonate (MMS) (Asaeda, *et al*., 1998); UV light (Lloyd, 1998); Cr (Casaderall, *et al*., 1999); HNO_2 and NO (Suzuki, *et al*., 2000), drugs (Martelli, *et al*., 2000), styrene (Vodicka, *et al*., 2001); phenanthroline (Martelli, *et al*., 2002), para-nitrobenzamide (Martelli, *et al*., 2002); Fe (Gralnick and Downs, 2003) and dibenzo (a,1) pyrene (Chakravarti, *et al*., 2005).

In this study, for the AP-sites counting assay, the concentrations and quality of genomic DNA obtained is very important to determine the success of this assay. The results showed that the purity and concentration of the DNA extracted from the microalgae and macroalgae (seaweed) is in the range of 1.85–1.95 and the concentration obtained was about 1 mg DNA/g algae sample. This indicates the high DNA purity grade of the extraction (Zhou, *et al*., 1997). The purity and integrity of the template DNA are important for good AP-site counting assay.

5.6.1 AP-Site Content Trends in the Study

There were different trends of the AP-site content observed in this study after the algae were exposed to selected toxicants for four and ten days.

In general, the AP-site content of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* cultures grown for four days (sort term exposure) and ten days (long term exposure) in the Prov50 Medium containing all toxicants used in this study (Cr, Cu, Fe, Mn and Zn) were higher than in the algae cultures grown in the Prov50 Medium without toxicant (control cultures). This indicated that the algae exposed to these selected toxicants had increased DNA damage (AP-site content) level in the cell after short term and long term exposure.

After being exposed for four days, the AP-site content of the *Chlorella* (exposed to Mn), *Tetraselmis* (exposed to Cu), *Boergesenia* (exposed to Zn) and *Ventricaria* (exposed to Cu) increased with increasing concentrations of toxicant. Similar trends were observed for the *Ventricaria* cultures exposed to Cu for ten days. This indicated that Mn,

Cu and Zn could induce and increase the DNA damage (AP-site content) level in the algae following increasing concentrations of toxicant. This condition may be because after being exposed to toxicant for short and long term duration, these toxicants caused loss of bases in the DNA strand structure, which finally would destroy the DNA strand structure in the cell and therefore increased the DNA damage (AP-site content) level in the cell following increasing concentration of toxicants. Aktipis (1997) reported that the alkylating agents like chemical contaminants may affect the structure of the bases as well as disrupt phosphodiester bonds, leading to the fragmentation of the strands. In addition, certain alkilating agents can interact covalently with both strands, creating interstrand bridges (Aktipis 1997). Similar results was reported by Fundador and Rusling (2007) who showed that removal of nucleobases from the DNA backbone leads to the formation of abasic sites. The rate of abasic site formation is significantly increased with increasing chemically damaged nucleobases (Fundador and Rusling, 2007).

For the *Chlorella* (exposed to Fe), *Boergesenia* (exposed to Cu) and *Ventricaria* (exposed to Zn) for four days, the AP-site content of the algae increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. The same trends were observed for the *Chlorella* (exposed to Fe and Mn), *Tetraselmis* (exposed to Cr and Cu), *Boergesenia* (exposed to Cu and Zn) and *Ventricaria* (exposed to Zn), after being exposed for ten days to the toxicants.

Increased AP site content at the four lowest concentrations of toxicant (0.01 mg/L to 10 mg/L toxicant) indicated that the DNA strand structure in the cell may be increasingly damaged following increasing concentrations of toxicant until at threshold concentration (10 mg/L). However at higher concentration of toxicant (100 mg/L to 500

mg/L toxicants), the DNA damage (AP-site content) level in the cell may be reduced because of the total damage or disappearance of the DNA template. The damage or disappearance of the DNA template will prevent the Aldehyde Reactive Probe (ARP), which reacts specifically with an aldehyde group (the open ring form) of the AP sites, from attachement, so AP-site content in the exposed algae cell cannot be measured. The low content of AP-site also correlated with the growth of the algae where at concentrations of 100 mg/L and 500 mg/L toxicant, the growth of algae were very low or the algae died. Therefore the low quality of the template DNA obtained in the algae at the concentrations of 100 mg/L and 500 mg/L toxicant will influence the measurement and sensitivity of this assay.

Despite of that, different AP-site content trends were observed in the *Tetraselmis* cultures exposed to Cr for four days where the AP-site content decreased with increasing concentrations of toxicant. This indicated that, Cr could reduce the DNA damage (AP-site content) level in the algae following increasing concentrations of toxicant. In this study, Cr was the least toxic metal to *Tetraselmis tetrahele* UMACC 144. As reported by Casadevall, *et al*., (1999), the chromate anion itself is unreactive towards DNA where their results shows that the calf thymus DNA exposed to Cr-Hydroxyl radicals did not seem to be important for the generation of DNA damage as the characteristic modified DNA bases could not be detected by using gas chromatography–mass spectrometry (Casadevall, *et al,*1999). However, in earlier *in vitro* work with isolated DNA, Casadevall *et al*., (1999) showed that chromium (VI) in combination with glutathione (GSH) or ascorbate (AsA) was able to induce similar numbers of single strand breaks and apurinic: apyrimidinic sites (AP-sites). The precise mechanism by which DNA damaging species are formed is unclear (Casadevall, *et al*.,1999).

Therefore, for the present study, the hydrolysis of the N-glycosidic bond (Lindahl and Nyberg 1972) as well as chemical modification of bases by Cr that destabilized the N- glycosidic bond (Tarpley *et al*., 1982; Foster, *et al*., 1983; Osborne and Merrifield, 1985; Lawley and Brookes, 1963; Singer, 1976) in the DNA template of this algae maybe less affected. Asaeda, *et al*., (1998) reported that, the intracellular level of AP sites would be elevated as a result of base modifications and their subsequent repair. This repair mechanism may help the algae to tolerate Cr for short time duration following increasing concentration of toxicant.

In the base excision repair pathway, AP sites are formed as intermediates by the action of DNA N-glycosylases (Sancar and Sancar, 1988; Friedberg *et al*., 1995). Repair of DNA base damage occurs primarily by a multistep pathway where in the sequential action of a number of enzymes ensures that a damaged base is removed, the resulting abasic site is cleaved, DNA ends are trimmed, correct nucleotides are inserted, and the remaining nicked DNA is ligated to restore the DNA to its native state (Wyatt and Pittman, 2006).

AP-site are generally restored in the repair pathway initiated by AP endonuclease in cells (Friedberg, 1985). If unrepaired, abasic sites are strong block to DNA synthesis and constitute lethal lesions (Schaaper and Loeb, 1981; Sagher and Strauss 1983; Moran and Wallace, 1985, Hevroni and Livneh, 1988; Laspia and Wallace 1989). Unrepaired abasic sites also can lead to further DNA damage in the form of double strands breaks when there are two or more sites within close proximity to each other on opposite strands (Chaudhry and Weinfeld, 1997, McKenzie and Strauss, 2001), as well as inhibition of topoisomerases (Wilstermann and Osheroff, 2003), replication and transcription (Yu, *et*

al., 2003). It has also been shown that translesion DNA synthesis occurs at an abasic site with a low frequency, resulting in mutation. In this case, purine nucleotides particularly dAMP, are preferentially incorporated opposite the lesion (Boiteux and Laval, 1982; Schaaper, *et al.*, 1983; Kunkel, 1983; Randall, *et al*., 1987; Takeshita, *et al*., 1987; Lawrence, *et al.*, 1990).

For the present study, different patterns were observed in the *Chlorella* (exposed to Fe and Mn), *Tetraselmis* (exposed to Cu), *Boergesenia* (exposed to Cu and Zn) and *Ventricaria* (exposed to Cu) for four days where the AP-site content should be decreased with increasing concentrations of toxicant or follow the same trend as the growth of the algae. However in these cultures, the AP-site content was increased with increasing concentrations of toxicant or the AP-site content was increased up to 10 mg/L toxicant and then decreased at higher concentrations of toxicant. Similar patterns were also observed in the *Chlorella* (exposed to Mn), *Tetraselmis* (exposed to Cr and Cu), *Boergesenia* (exposed to Cu) and *Ventricaria* (exposed to Cu and Zn) for ten days where the AP-site content shows the opposite pattern with the growth of algae.

The increase in AP-site content at low concentrations of toxicant (0.01mg/L to 10 mg/L toxicant) which shows the opposite pattern to the growth of algae, indicated that AP-site assay is a more sensitive assay in comparison to the growth parameter measured, for the purpose of detecting and assessing the effects of toxicants on the algae. This results also shows that, eventhough the toxicants may interfere with the template function of DNA, which can damage DNA through several mechanism, including the formation of DNA-xenobiotic adducts, by causing strand breaks and by oxidation of DNA bases; the cells are still able to grow and tolerate these conditions until its

threshold level (for this study, up to 10 mg/L toxicant). This adaptation process in the algae may be correlate with the repair mechanism occurring in the cell where once DNA damage has occurred, wheather from chemical exposure or other causes (respiration, UV radiation, viral interaction,etc), several subsequent outcomes can occur including: (i) the damage can be properly repaired, (ii) the damage can lead to cell death, (iii) resulting change in DNA structure (base sequence) can become fixed and passes on to daughter cell, i.e mutation occur (Klaassen, 2008). Therefore, from the results of this study, we are suggesting that AP-site assay is a useful and sensitive assay for detecting toxicant induced DNA damage in the algae.

In this study, we observed that the algae cultures exposed to Fe (most toxic metals to *Chlorella* and Cu (most toxic metal to *Tetraselmis, Boergesenia* and *Ventricaria,* for four days contained the highest AP-site content in comparison to other toxicants tested in this study. The same results were shown in the algae cultures exposed for ten days to Cr (most toxic metals to *Tetraselmis*) and Cu (most toxic metal to *Ventricaria*). This condition indicated that Cu, Cr and Fe can induce damage in the DNA structure of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* used in this study following increasing concentrations of toxicants and following duration of exposure.

In contrast, the algae cultures exposed to Cu (most toxic metal to *Boergesenia*), Fe (most toxic metal to *Chlorella*) and Zn (most toxic metal to *Venntricaria*) for ten days contained the lowest AP-site content in comparison to other toxicant tested in this study shows that, Cu, Fe and Zn significantly inhibited and destroyed the DNA strand structure in the cells after being exposed for long term duration.

5.6.2 Comparison of AP-site Content Between Short Term and Long Term Exposure to Toxicants

In comparison of AP-site content in algae between short term (4 days) and long term (10 days) exposure, we observed that the AP-site content of the *Chlorella*, *Boergesenia* and *Ventricaria* cultures grown for ten days in Prov50 Medium without toxicant (control cultures), was 44.32%, 16.63% and 25.00% respectively, and higher than in the four days cultures showing that the DNA damage (AP-site content) level in the *Chlorella*, *Boergesenia* and *Ventricaria* increased with increasing growth of algae. This condition may be because after being grown for long term duration, the oxidative stress level in the cell increased due to increase capacity of photosynthesis and respiration rate of the algae. Therefore the DNA damage (AP-site content) level in the algae cell also will be increased.

In contrast, the AP-site content of the *Tetraselmis* cultures grown for four days in Prov50 Medium without toxicant (control cultures), was 20.61% higher than in the ten days cultures, showing that the DNA damage (AP-site content) level in the *Tetraselmis* decreased with increasing growth of algae. This condition may be because during exponential phase (4 days), the growth rate of the *Tetraselmis* cultures was increased and therefore the DNA damage (AP-site content) level in the algae cell was higher due to the active photosynthesis process of the algae which led to high production of reactive oxygen species (ROS) in the cells. The high content of ROS can induce oxidation of the DNA strand structure, which will directly increase the DNA damage level in the cell. However after being grown for ten days in the Prov50 Medium, the growth rate of the *Tetraselmis* cultures was reduced because of the algae cultures had reached stationary phase period and this condition allowed the *Tetraselmis* to repair the DNA damage that

occured in the cell. Therefore the DNA damage (AP-site content) level in the algae cell was be reduced.

In general, the AP-site content of *Chlorella* (exposed to Fe and Mn), *Tetraselmis* (exposed to Cr and Cu), and *Ventricaria* (exposed to Cu and Zn) for ten days was higher than in the four days cultures showing that, the DNA damage (AP-site content) level in the the cultures exposed to these toxicants, increased with increasing time of exposure. This condition shows that after being exposed for long term duration to these toxicants, the algae were adapting to tolerate these toxicants, therefore the DNA damage (AP-site content) level in the algae cell was continuously increased following increasing growth of the algae.

However, for the Boergesenia (exposed to Cu and Zn), the AP-site content in four days cultures was higher than in ten days cultures, showing that, DNA damage (AP-site content) level in the the cultures exposed to these toxicants, decreased with increasing time of exposure. This is because of after being exposed to Cu (most toxic metal to Boergesenia) for long term duration (ten days), the algae could not tolerate the conditions, which inhibited the growth of algae. The reduction in the DNA damage (AP-site content) level in the algae may be due to the abundant loss of DNA strand structure in the cell.

However, for the Boergesenia cultures exposed to Zn (least toxic metal to Boergesenia), after being exposed to toxicant for long term duration (ten days), the algae adapted to the conditions, allowing the Boergesenia to repair the DNA damage that

occured in the cell. Therefore the DNA damage (AP-site content) level in the algae cell was also reduced and was lower than in the four days cultures.

In conclusion, the AP-site assay used in this study has been successfully used as a sensitive assay for detecting chemical contaminant induced DNA damage in the algae cell showing potential as a reliable and reproducible assay for genotoxicity. Thus, AP-sites assay can serve as good biomarkers for the quantification of DNA damage. The present study suggests that the AP-site assay applied in conjunction with other genotoxic biomarkers using other molecular techniques would prove as a powerful genotoxicological tool.

5.7 EFFECTS OF SELECTED TOXICANTS ON SOD ACTIVITY IN ALGAE

The increasing exposure of toxicants to the marine algae can induce production of ROS in the algae cell. Although activated oxygen compounds such as superoxide, hydrogen peroxide, and hydroxyl radical are produced as a by-product of normal cell metabolism (from a number of physiological reactions such as the electron flow in the chloroplasts and mitochondria and from some redox reactions in cells. Their levels are enhanced by exposure to chemical and environmental stress. These oxidant radicals can oxidize membrane lipids and cause denaturing of nucleic acids and proteins (Fridovich, 1986).

In order to defend themselves from the damaging effects of ROS, all aerobic organisms have evolved complex antioxidant defense system that include enzymatic and nonenzymatic components (Halliwell and Gutteridge, 1999). Antioxidant enzymes

include superoxide dismutase that convert O_2^- to H_2O_2, catalases and peroxidases that detoxify peroxides including H_2O_2, and enzymes involved in the production and maintainance of reduced glutathione (GSH) such as glutamate-cysteine ligase (GCL) and glutathione reductase (GR). Low molecular weight, nonenzymatic antioxidants include vitamins A, C, and E, ascorbic acid (ascorbate), glutathione and phenolic compounds (Noctor and Foyer, 1998).

One of the most important enzyme, Superoxide dismutase (SOD) (EC 1.15.1.1), catalyzes the dismutation of superoxide (O_2^-) to hydrogen peroxide (H_2O_2) and molecular oxygen (O_2) (Bowler, *et al*., 1992) and consequently reduces the activated oxygen molecules in the cell (McKersie *et al*., 1993). SOD is not only the first line of defence but also the only enzyme capable of catalyzing this reaction. Therefore SOD holds a key position within the antioxidant network.

The SOD activity assay is a useful assay for the detection of adaptation responses to toxicant-induced oxidative stress at a subcellular level. The advantages of SOD assay is that, the assay which is suitable for any extracted algae enzyme (with good sufficient quality and quantity) requires very little source of materials. As the SOD Assay Kit-WST (Dojindo Molecular Technologies Inc., Japan) are used, specific details of the SOD enzyme of the algae under investigation are not needed. In addition, no radioactive materials are required before and during analysis. The analysis also can be performed non-destructively, which can be useful for the screening of large number of valuable samples. This technology is relatively cheap and does not require the use of special and expensive equipment.

However, the SOD assay has some important limitations. SOD in particular is difficult to analyze, maybe due to problems in obtaining sufficient quantities of good quality enzyme samples for reliable and reproducible enzymatic assays (Janknegt, *et al*., 2007). In order to reduce its limitation, optimization of the assay needs to be carried out especially in cell disruption (sonication) process, protein extraction procedures, extraction buffers preparation, SOD standard preparation, SOD assay procedures and the assay temperature needs to be controlled during the experiment.

Although SOD activity results have often been criticised as unreliable and unreproducible, subsequent reports have demostrated that after rigious optimization of the enzyme extraction method, the assay performs well in terms of consistent and reproducible results with wide range of organisms. Previous studies reported by several researchers show that SOD assays have successfully been used to detect toxicant induced oxidative damage in green microalgae; including *Chlorella vulgaris* (Qian, *et al*., 2008 ; Qian, *et al*., 2009 ; Piotrowska-Niczyporuk, *et al*., 2012), *Nannochloropsis* sp. (Perelman, *et al*., (2006), *Tetraselmis gracilis* (Sigaud-Kutner, *et al*., 2005), *Scenedesmus* sp (Li, *et al*., 2005; Tripathi, *et al*., 2006 ; Sabatini, *et al*. , 2009) and also green macroalgae such as *Cladophora glomerata* (Choo, *et al*., 2004); *Enteromorpha ahlneriana* (Choo, *et al*., 2004) ; *Caulerpa racemosa var. cylindracea* (Cavas and Yurdakoc (2005), Undaria *pinnatifida* (Je, *et al*., 2009), *Ulva compressa* (Mellado, *et al*., 2012), *Ulva pertusa* (Schweikert and Burritt, 2012) and etc.

In this study, we have applied the superoxide dismutase activity analysis to evaluate the adaptation and defense response of four marine algae *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria*

ventricosa to toxicants (metals, textiles dye and organophosphate pesticides) induced oxidative stress. The results of this study describe for the first time the presence of SOD activity in *Chlorella vulgaris* UMACC 245, *Tetraselmis tetrahele* UMACC 144, *Boergesenia forbesii* and *Ventricaria ventricosa*.

5.7.1 SOD activity trend in the study

In general, the SOD activity of the *Chlorella, Tetraselmis, Boergesenia* and *Ventricaria* cultures grown for four days and ten days in the Prov50 Medium containing all toxicants used in this study (Cd, Cr, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion) were higher than in the algae cultures that were grown in the Prov50 Medium without toxicant (control cultures); except for the *Chlorella* (exposed to Acidic dye, Basic dye, and Dichlovos for four days) and *Tetraselmis* (exposed to Malathion for ten days), which have the SOD activity value lower than in control culture between concentration 0.01 mg/L to 500mg/L toxicants.

The higher value of SOD activity obtained in the exposed algae in comparison to control cultures show that these toxicants could induce SOD enzyme synthesis and increased the SOD activity in the algae cell after being exposed for short term (4 days) and long term duration (ten days). In this study, exposure of toxicants (between 0.01 mg/L to 500mg/L toxicants) increased the SOD activity by up to 10 times compared with the control, suggesting that some oxidative damage occurred in the algae cell which was caused by increased production of superoxide anion (O_2^-).

Our results show, the increase in SOD activity higher in comparison to control agree well with the observations reported by Qian, *et al*., (2008) who have demostrated that maximum SOD activity obtained in the *Chlorella vulgaris* exposure to glufosinate (herbicides) at concentration of 10 to 20µg/mL for 12 to 96hrs was 2.90 times higher than in control culture. Qian, *et al.,* (2009) also showed that SOD activity of *Chlorella vulgaris* exposed to N-phenyl-2-naphthylamine (allelochemical) for seven days at concentration 2.5 mg/L N-phenyl-2-naphthylamine increased the activity of SOD 2.47 over that of the control. However, exposure to 4.0 mg/L N-phenyl-2-naphthylamine, decreased the activity of SOD.

For the algae cultures that contained lower SOD activity value than the control cultures, exposure to toxicants like Basic dye and Malathion, could inhibited the SOD enzyme synthesis in the *Chlorella* and *Tetraselmis*. The low value of SOD activity in these cultures also paralleled the low growth rate measured in these cultures, indicating that SOD enzymes in these algae were reduced due to inhibition of algae growth.

Similar to other environmental stress, toxicants used in this study caused oxidative damage in algae, either directly or indirectly, by triggering increased levels of ROS. This was also observed during metal toxicity reported by Mellado, *et al.* 2012; Piotrowska-Niczyporuk, *et al*., 2012; Sabatini, *et al*., 2009) and also organophosphate pesticide toxicity (Schweikert and Burritt, 2012; Qian, *et al*., 2008).

In the present study, different SOD activity trends were observed in the algae exposed to selected toxicants for four days (short term exposure) and ten days (long term exposure). Table 5.17 summarizes the SOD activity trends in the algae cultures exposed to selected toxicants.

The first trend observed was, the SOD activity of the algae cultures exposed to the toxicants for four days (short term exposure) and ten days (long term exposure), decreased with increasing concentration of toxicants. Reduction of SOD activity in the algae with increasing concentrations of toxicants indicate that, these toxicants could inhibit the SOD enzyme synthesis in the algae. This condition occurred maybe because, after being exposed for four days (short term exposure) to the toxicants, the algae were stressed and growth was inhibited resulting in adverse effects and reduction of the SOD enzyme synthesis in the cell. Therefore the SOD activity in the algae was decreased following increasing concentration of toxicants.

For the algae exposed for ten days (long term exposure) to toxicants, the reduction in the SOD activity with increasing concentration of toxicants occured because the algae could not tolerate to the toxicant after long term exposure, and growth of the algae was reduced or inhibited and as a results, the SOD enzyme synthesis in the cell was inhibited or SOD enzymes were degraded following increasing concentrations of toxicants. Decrease in SOD activity following increasing concentration of toxicants also indicate that the antioxidant scavenging capacity, in these algae is reduced.

Table 5.17: Superoxide dismutase (SOD) activity trends in the algae cultures exposed to selected toxicants

So activity trend	Four days cultures	Ten days cultures
Decreased with increasing concentrations of toxicant	● *Tetraselmis* (exposed to Metal complex dye), ● *Boergesenia* (exposed to Zn, Basic dye, Metal complex dye and Dichlovos) ●*Ventricaria* (exposed to Cd, Zn, Dichlovos and Malathion)	● *Chlorella* (exposed to Fe) ● *Tetraselmis* (exposed to Basic dye) ● *Boergesenia* (exposed to Acidic dye and Malathion) ● *Ventricaria* (exposed to Zn).
Increased with increasing concentrations of toxicant	●*Chlorella* exposed to Cd ● *Tetraselmis* exposed to Cd, Cu, Acidic dye and Basic dye ● *Boergesenia* exposed to Cd and Cu ● *Ventricaria* exposed to Cu	● *Chlorella* exposed to Cd ● *Tetraselmis* exposed to Cd ● *Boergesenia* exposed to Cu and Zn ●*Ventricaria* exposed to Cu and Zn
Increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant.	●*Chlorella* exposed to Fe, Mn, Metal complex dye and Malathion ● *Tetraselmis* exposed to Cr, Dichlovos and Malathion ● *Boergesenia* exposed to Acidic dye and Malathion ● *Ventricaria* exposed to Acidic dye, Basic dye and Metal complex dye	● *Chlorella* exposed to Mn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion ● *Tetraselmis* exposed to Cr, Cu, Acidic dye, Metal complex dye, Dichlovos and Malathion ● *Boergesenia* exposed to Cd, Basic dye, Metal complex dye and Dichlovos ● *Ventricaria* exposed to Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion

However, different opinion was given by Johnstone, *et al*., (2006), who suggested that the decrease in antioxidant activity of *Euglena gracilliss* exposed to 6% (w/v) $FeSO_4$, could be due to a change in the Fe sequestration of cells, possibly causing free iron to participate in Fenton chemistry. According to Johnstone, *et al.*, (2006) and Ishikawa, *et al*., (1993), certain green algae like *Euglena gracillis* are able to sequester Fe. Ishikawa, *et al.,* (1993) reported that *Euglena gracilis* regulates cellular iron levels by incorporating bound iron in Fe–proteins complexes, in doing so, oxidative stress is ameliorated by preventing iron from participating in Fenton chemistry. Therefore in the present study, the reduction of SOD activity with increasing concentration of toxicant during short term exposure, may also be due to the ability of algae like *Tetraselmis*, *Boergesenia* and *Ventricaria* being able to sequester toxicants in their cells.

The second trend observed was, the SOD activity of the algae cultures exposed to the toxicants for four days (short term exposure) and ten days (long term exposure), increased with increasing concentrations of toxicants. The increase of SOD activity in the algae with increasing concentrations of toxicants show that, these toxicants could induce or stimulate the SOD enzyme synthesis in the algae following increasing concentrations of toxicant, in order to protect the cells from the oxidative damage. This condition occurred maybe because, after being exposed to the toxicants for short term and long term duration, the production of ROS induced by these toxicants was increased with increasing concentration of toxicants, maybe via photosynthesis process that continuously occured during the growth phase of the algae. In order to cope with the increasing production of ROS during photosynthesis process, the algae increased the synthesis of SOD enzymes to reduce the oxidative damage created in the cell.

Our results show that increase in SOD activity with increasing concentration and time of exposure of toxicants agrees well with the observations by previous studies carried out by researchers who demonstrated a dose-dependent relationship between the SOD activity with increasing concentration and time. Sabatini, *et al.,* (2009) demonstrated that SOD activity in *Scenedesmus vacuolatus* and *Chlorella kessleri* increased with increasing concentration of Cu after being exposed to 6.2 to 414µM Cu for 7 days. Similar results reported by Bajguz (2011) who exposed *Chlorella vulgaris* to culture media containing 10^{-6} to 10^{-4} M Cu, Pb and Cd individually for 24 hrs, where he found the activities of SOD in algae increased with an increasing concentration of metals. Tripathi, *et al.,* (2006) also shows that after being exposed to Cu (2, 5, 10 µM) and Zn (5, 25 µM) for 6 hrs and 7 days, the SOD activity in *Scenedesmus* sp. increased with increasing concentration of Cu and Zn. Therefore in this study, increased activity of SOD can be expected to reduce oxidative stress to algal cell. Elevated levels of SOD have been correlated with increased levels of oxidative stress resistance in severa1 cases (Gupta, *et al,* 1999; Jahnke, *et al.,* 1991, Jansen, *et al.,* 1989) and the enhancement of SOD activity was particularly higher in long-term than in short term experiment. These results support the hypothesis that SOD play integral roles in the protection of algae from oxidative stress.

This results also indicate that, over expression of SOD in algae led to increased protection from oxidative stress (Van Breusegem, *et al*., 1999; Pastori, *et al.,* 2000) after long term exposure to toxicants. Indeed, maintenance of a high antioxidant capacity in cells has been related to increased tolerance against different kinds of environmental stress (Okamoto, *et al.,* 2001; Pedrajas, *et al*., 1993; Dat, *et al*., 1998; Thomas, *et al*.,

1998). Therefore it is an important adaptive response to withstand adverse conditions in the algae.

The third trend observed was, the SOD activity of the algae cultures exposed to the toxicants for four days (short term exposure) and ten days (long term exposure), increased with increasing concentrations of toxicant up to 10 mg/L of toxicant and then decreased at higher concentrations of toxicant. Similar trends were also observed in the after being exposed for ten days to toxicants.

The increase of SOD activity at the low concentrations (0.01 mg/L to 10 mg/L) of toxicants indicate that, these toxicants can induce the SOD enzymes synthesis in the algae which directly increased the SOD activity in the cell to reduce the oxidative damage to the cell until certain threshold (in this study are up to 10 mg/L toxicant). However at higher concentration of toxicants (100 mg/L to 500 mg/L), these toxicants inhibited the SOD enzyme synthesis in the algae and because of that SOD activity in the cell was reduced.

As demonstrated by Xu, *et al.,* (2013) who exposed Perfluorooctanoic acid (PFOA), a kind of persistent organic pollutants to green algae *Chlorella pyrenoidosa* and *Selenastrum capricornutum* at the logarithmic growth stage. After a 192-h exposure to a low concentration of PFOA, the activities of SOD and catalase were greater than those of the control. However, at higher concentrations of PFOA, activities of SOD and catalase were strongly inhibited. These results indicate that long-term exposure to low levels of PFOA may induce excessive generation of ROS in algal cells, causing oxidative damage to cells.

Similar results reported by Schweikert and Burritt (2012) who investigated the impact of Coumaphos, a commonly used organophosphate, on the macrophyte *Ulva pertusa*. In a seven day experiment, *U. pertusa* cultures were exposed to four environmentally relevant concentrations of Coumaphos (0.01 mg/L, 0.05 mg/L, 0.1 mg/L, 0.5 mg/L). SOD activity increased in response to the higher concentrations of Coumaphos tested and remained elevated for the duration of the experiment. These results demonstrate that low levels of the insecticide Coumaphos can cause oxidative damage and increase the antioxidant scavenging capacity, in *U. pertusa*.

Similar to the mechanisms observed in plants and other algae, increased toxicant in the environment evokes oxidative stress and an increase in the antioxidant defenses of algae. However, these responses are not enough to prevent oxidative damage, as evidenced by the decrease in the growth (chlorophyll a) and the biochemical composition (protein) decrease at higher concentrations of toxicant, after being exposed foor short term (four days) and long term (ten days) duration to toxicants.

For the present study, different patterns were observed in the *Chlorella* (exposed to Cd, Fe, Mn, Acidic dye, Basic dye, Metal complex dye, Dichlovos and Malathion), *Tetraselmis* (exposed to Cd, Cu, Acidic dye, Basic dye, Dichlovos and Malathion), *Boergesenia* (exposed to Cd, Cu and Malathion) and Ventricaria (exposed to Cu, Zn, Basic dye and Metal complex dye) for four days where the SOD activity should be decreased with increasing concentrations of toxicant or followed the same trend as the growth of the algae. However in these cultures, the SOD activity was increased with increasing concentrations of toxicant or the SOD activity was increased with increasing concentrations of toxicant up to 10 mg/L toxicant and then decreased at higher

concentrations of toxicant. The same pattern was also observed in the *Chlorella* (exposed to Cd, Acidic dye, Basic dye, Metal complex dye and Malathion), *Tetraselmis* (exposed to Cd, Cu and Metal complex dye), *Boergesenia* (exposed to Cu, Zn, Basic dye, Metal complex dye and Malathion) and *Ventricaria* (exposed to Cd, Cu, Zn, Dichlovos and Malathion) after being exposed for ten days to toxicants, which showed opposite trend to the growth of the algae.

The increase in SOD activity at lower concentrations of toxicant (0.01mg/L to 10 mg/L toxicant) which showed the opposite trend to the growth of algae, may be because the SOD enzyme was synthesised abbundantly in the cell. Due to increased ROS production in the cell that was induced by these toxicants during photosynthesis, the algae increased the synthesis of the SOD enzymes to reduce the oxidative damage in the cell. Even though the growth of the algae have been reduced, the algae are still continuously synthesising the SOD enzymes and hence will increase the SOD activity in the cell, in order to overcome the oxidative damage to the algae. This may be one of the important adaptive and protective mechanisms carried out by algae to protect the cell from the oxidative damages. Therefore the SOD activity in the algae will be increased following increasing concentration of toxicants up to the threshold concentration (i.e 10 mg/L toxicant) that the algae can tolerate. According to Weiss, *et al*., (2004); Jamieson (1998) and Davies (1999), the cellular response to low concentrations of oxidative stress results in cell proliferation and adaptation which provides increased resistance to oxidant exposure.

However, at higher concentrations of toxicant (100 mg/L to 500 mg/L toxicant), the SOD activity was decreased with increasing concentrations of toxicant because at these high levels the algae cannot tolerate the toxicity and the synthesis of SOD enzymes is inhibited and the molecule structure of this enzyme is degraded, resulting in direct reduction of the SOD activity in the cell. Therefore the SOD activity in the algae will be decreased following increasing concentration of toxicants.

5.7.2 SOD activity sensitivity and tolerance in the study

In this study, after being exposed to toxicants for four days, we observed that in the algae cultures exposed to Fe (most toxic metal to *Chlorella*), Dichlovos (most toxic organophosphate pesticides to *Tetraselmis* and *Ventricaria*) and Malathion (most toxic organophosphate pesticides to *Boergesenia*), the SOD activity obtained in the cultures were lowest in comparison to other toxicants tested.

Similar results were shown in the cultures exposed for ten days to toxicant where, in the algae cultures exposed to Cu (most toxic metal to Tetraselmis), Zn (most toxic metal to *Ventricaria*), Basic dye (most toxic textile dye to *Ventricaria*), Metal complex dye (most toxic textile dye to *Tetraselmis*), Dichlovos (most toxic organophosphate pesticides to *Chlorella* and *Ventricaria*) and Malathion (most toxic organophosphate pesticides to *Tetraselmis* and *Boergesenia*), had the lowest SOD activity in comparison to other toxicants tested in this study. This condition indicated that, these toxicants significantly inhibited and give adverse effect on the SOD enzyme synthesis and SOD activity in the algae, following increasing concentration of toxicant after being exposed for short term and long tem duration to toxicants.

In contrast, for the algae cultures exposed to Cu (most toxic metal to *Boergesenia*), Basic dye (most toxic textile dye to *Ventricaria*) and Metal complex dye (most toxic textile dye to *Chlorella*) for four days, the SOD activity obtained in the cultures had the highest SOD activity in comparison to other toxicants tested in this study. The same results were also observed in the algae cultures exposed to Cu (most toxic metal to *Ventricaria*), Fe (most toxic metal to *Chlorella*) and Metal complex dye (most toxic textile dye to *Chlorella*), for ten days where the SOD activity obtained in the cultures were highest in comparison to other toxicants tested. This condition indicated that SOD enzyme was synthesised abundantly in the algae cell after exposure to these toxicants for four days (short term exposure) and ten days (long term exposure) in order to reduce the oxidative damage to the cells.

In present study we found that Fe was the most toxic metal to *Chlorella*. Exposure of *Chlorella* for short term duration (four days), inhibited growth and synthesis of SOD molecule in the cells (as indicate by lowest SOD activity in the cell). However after being exposed for long term duration, the growth and SOD activity of the algae increased which indicated that *Chlorella* can tolerate Fe toxicity after long term exposure due to the increased level SOD activity in the cell. As reported by Estevez, *et al.*, (2001), Fe^{2+} as the active form of Fe are relatively small ions with a marked tendency to form six-coordinate complexes with ligands containing O, N, and S. According to Halliwell and Gutteridge (1986), the reaction of O_2^- with H_2O_2 that forms ·OH is often catalyzed by metals, especially Fe^{2+} . As a consequence, Fe- bearing biomolecules, such as metalloenzymes and electron-transport proteins, may be the first sites of O_2^- damage in cells (Halliwell and Gutteridge, 1986; Kuo, *et al*., 1987; Fridovich, 1989; Gutteridge and Halliwell, 1990; Gardner and Fridovich, 1991). Following oxidative damage to Fe-

bearing proteins, free Fe^{2+} can adversely react with other cellular components, causing additional damage. O_2^- may also disrupt Fe-S centers directly in proteins such as Fd, aconitase, succinate dehydrogenase, and PSI (Liochev, 1996). Therefore, our results showing lowest growth rate and SOD activity in *Chlorella* exposed for four days indicate that Fe significantly gave adverse effect on growth and detoxification capacity of *Chlorella*. However, the results showed an increased SOD activity in *Chlorella* after being exposed to Fe for ten days, indicating that oxidative damage was increased by Fe availability. Increase in the antioxidant scavenging capacity in *Chlorella* is suggested as one of the adaptive mechanisms carried out by *Chlorella* to detoxify the excess Fe available in the cell and reduce the oxidative damage to the cell.

In comparison of SOD activity in algae between short term (4 days) and long term (10 days) exposure, we observed that the SOD activity of the *Chlorella* and *Tetraselmis* cultures grown for four days in Prov50 Medium without toxicant (control cultures), was 6.83% and 20.91% respectively higher than in the ten days cultures. This indicated that the SOD activity in the *Chlorella* and *Tetraselmis* decreased with increasing growth of algae. This condition occurred because of during exponential phase (four days), the photosynthesis activity in the *Chlorella* and *Tetraselmis* cultures was very active and because of that the algae produced abundant ROS in the cells via the photosynthesis process. In order to reduce this oxidative stress occurring in the cell, the algae will increase the synthesis of the SOD enzymes in the cell. Therefore the SOD activity in the algae will be higher during exponential phase (four days). However after being grown for ten days in the Prov50 Medium, the photosynthesis activity in the *Chlorella* and *Tetraselmis* cultures will be reduced because the algae cultures already reached stationary phase during that period.

In contrast, the SOD activity of the *Boergesenia* and *Ventricaria* cultures grown for ten days in Prov50 Medium without toxicant (control cultures), was 12.82% and 45.04% respectively higher than in the four days cultures indicating that, the SOD activity in the *Boergesenia* and *Ventricaria* increased with increasing growth of algae. This condition occurred because of after being grown for long term duration, the cell number and cell size of the macroalgae culture will be increased and the photosynthesis activity in the *Boergesenia* and *Ventricaria* cultures also will be increased and therefore the SOD activity in the algae cell will be higher. In addition may be the nutrient content in the medium decreased with time. The limited nutrients especially essential nutrients like nitrogen and phosphorus, will reduce and inhibit the growth of the algae. In order to cope with that stress, algae will increase their antioxidant scavenging capacity, to reduce the oxidative damage to the cell, that is induced by limitation of nutrients in the growth medium.

The present results which shows the SOD activity in algae cultures exposed to toxicant for long term exposure was higher than in the short term exposure cultures are simililar to that demonstrated by Tripathi, *et al.,* (2006) who found that during short- (6 h) and long-term (7 d) exposure of *Scenedesmus* sp. to Cu^{2+} (2.5µM and 10 µM) and Zn^{2+} (5µM and 25µM), the SOD activity of *Scenedesmus* sp. was >30% higher during long-term than during short term exposure to Cu^{2+} and Zn^{2+}. *Scenedesmus* sp.seems to have acquired tolerance against metal-induced oxidative stress in the long-term experiment due to maybe enhanced scavenging of ROS or sequestration of metals in intracellular compartments (Tripathi, *et al*., 2006).

Similar results were reported by Okamoto, *et al*., (2001) who evaluated the enzymes SOD, ascorbate peroxidase, glutathione and peridinin levels in isolated chloroplasts of the unicellular alga *Gonyaulax polyedra* exposed to the toxic metals Hg^{2+}, Cd^{2+}, Pb^{2+}, and $Cu^{2+}$for 48hrs and 30 days. They found that, heavy metals are able to induce oxidative stress in chloroplasts of *G. polyedra*, particularly under acute conditions (48hrs) where the cells subjected to acute metal stress displayed a slight increase in SOD activity. However during chronic condition (30 days), the cell exposed to metals exhibited high activity of the SOD in the cell.

Therefore in this study, we suggest that the response of SOD, the first line of defense against ROS in microaalgae and macroalgae, to toxicant-induced oxidative stress depends on the algal species and the exposure time.

In summary, our results provide additional evidence for oxidative stress mediating toxicant (especially metal) toxicity in algae. The increased SOD activities suggest a correlation between dose-dependent relationship and oxidative stress in algae cells. High concentration of toxicants and long term exposure to toxicant is damaging and appears to exceed the antioxidant defense. However, elevated SOD activities seem to be important in the attenuation of oxidative damage to algae cell under short term and long term exposure conditions of toxicant-induced stress. Such antioxidant response at the subcellular site where oxidative stress is triggered could contribute to the overall tolerance of this alga during conditions of toxicants (especially in metal) stress.

In conclusion, the SOD assay has been successfully used as a sensitive assay for detecting toxicant-induced oxidative stress and shows potential as a reliable and

reproducible assay for the algae. Furthermore, the present study suggests that the SOD assay applied in conjunction with other biomarkers such as growth and biochemical composition from higher levels of biological organization would prove a powerful ecotoxicological tool.

5.8 COMPARISON BETWEEN GROWTH, BIOCHEMICAL COMPOSITION, DNA DAMAGE AND SOD ACTIVITY IN ALGAE

To detect the sensitivity of the ends-points (growth rate, biochemical compostion, DNA damage and SOD activity) used in this study to be applied as suitable biomarker, each parameter used in the present study, [growth rate (based on Chl a), carotenoid content, carbohydrate content, protein content, lipid content, DNA damage: genomic template stability (which reflect RAPD changes), DNA damage:AP-site content and SOD activity] were compared. Changes in these values were calculated as percentage of their control value (set as 100%).

In the present study, to be used as suitable biomarker, several important factors are taken into consideration such as: (i) the value of the end-points (eg: carbohydtare content) obtained in the culture exposed to toxicants for short term (four days) and long term term (ten days) duration must be lower than in the control culture (set as 100%). (ii) The end-points (eg: carbohydrate) trends, must be decreased with increasing concentration of toxicant. Table 5.18 summarized the list of end-points (bioassay) that are suitable to be used as biomarker to detect specific toxicants using specific test organism (algae). Table 5.19 summarizes the ranking of end-points (bioassay) for each algae species.

Table 5.18: Suitability of end points (bioassay) to be use as biomarker to detect toxic contaminants for each type of algae

End-points	Test organism (Algae)	Suitable toxicants to be detected after short term (4 Days) exposure	Suitable toxicants to be detected after long term (10 Days) exposure
Growth rate	*Chlorella*	Cd, Cu, Fe, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos, Malathion	Cd, Cu, Mn, Acidic dye, Metal complex dye, Malathion
	Tetraselmis	Mn, Metal complex dye, Dichlovos, Malathion	
	Boergesenia	Cd, Co, Cu, Fe, Basic dye, Malathion	Fe, Basic dye, Dichlovos, Malathion
	Ventricaria	Basic dye	
Carotenoid	*Chlorella*	Cd, Cu, Fe, Mn, Zn, Acidic dye, Basic dye, Metal complex dye, Dichlovos, Malathion	Mn, Acidic dye, Basic dye, Metal complex dye, Malathion
	Tetraselmis	Mn, Metal complex dye, Dichlovos, Malathion	Malathion
	Boergesenia	Cd, Cu, Fe, Acidic dye, Basic dye, Malathion	Fe, Malathion
	Ventricaria	Basic dye	
Carbohydrate	*Chlorella*	Cr, Mn, Acidic dye, Basic dye, Malathion	Fe,
	Tetraselmis	Co, Fe, Acidic dye, Basic dye, Dichlovos	Co, Dichlovos
	Boergesenia	Cd, Cu	Co, Basic dye, Metal complex dye
Protein	*Chlorella*	Dichlovos, Malathion	Cd, Cr, Fe, Mn, Zn, Acidic dye, Basic dye
	Tetraselmis	Acidic dye, Basic dye, Dichlovos	Cr, Cu, Fe, Basic dye, Metal complex dye, Dichlovos
Lipid	*Chlorella*	Zn, Dichlovos	Cr, Acidic dye, Basic dye, Malathion
	Tetraselmis	Cr, Cu, Fe, Basic dye, Metal complex dye, Dichlovos, Malathion	Cd, Cr, Zn, Basic dye
	Boergesenia	Cd, Cu, Mn	Cd, Co, Cr, Zn, Acidic dye, Basic dye, Dichlovos, Malathion
	Ventricaria	Basic dye	Co, Fe, Mn, Zn, Metal complex dye, Dichlovos
RAPD-GTS	*Chlorella*	Cd, Mn, Acidic dye, Basic dye, Dichlovos	Cd, Fe, Mn, Acidic dye, Basic dye, Metal complex dye, Dichlovos, Malathion
	Tetraselmis	Cd, Cr, Cu, Basic dye, Dichlovos, Malathion	Cd, Cr, Basic dye, Metal complex dye
	Boergesenia	Cd, Cu, Zn, Acidic dye, Basic dye, Metal complex dye, Malathion	Cd, Cu, Zn, Acidic dye, Malathion
	Ventricaria	Cd, Cu, Zn, Basic dye, Metal complex dye, Dichlovos, Malathion	Cu, Basic dye, Metal complex dye, Dichlovos
AP-Site	*Chlorella*	Mn	Mn
	Tetraselmis	Cu	
	Boergesenia	Zn	
	Ventricaria	Cu	Cu
SOD activity	*Chlorella*	Cd	Cd
	Tetraselmis	Cd, Cu, Acidic dye, Basic dye	Cd
	Boergesenia		Cu
	Ventricaria	Cu	Cd, Cu

Table 5.19: The ranking of end-points (bioassay) for each algae species.

Algae	Duration of toxicant exposure	Ranking of the end-points
Chlorella vulgaris	Short term exposure (4 days)	CAR > GR = GTS > AP > CHO > PRO = LIP > SOD
	Long term exposure (10 days)	GTS > PRO > GR > CAR > LIP = SOD > AP > CHO
Tetraselmis tetrahele	Short term exposure (4 days)	GTS> LIP> AP= SOD > CHO> GR = CAR> PRO
	Long term exposure (10 days)	PRO > LIP > CHO > CAR
Boergesenia forbesii	Short term exposure (4 days)	GR = CAR = AP = GTS > CHO = LIP
	Long term exposure (10 days)	GTS > LIP > GR > CHO > CAR > SOD
Ventricaria ventricosa	Short term exposure (4 days)	GTS > AP > SOD > GR = CAR = LIP
	Long term exposure (10 days)	GTS > AP = LIP > SOD

AP-Site (AP); Carbohydrate (CHO); Carotenoid (CAR); Genomic template stability (which reflect RAPD changes) (GTS); Growth Rate (GR); Protein (PRO); Lipid (LIP); Superoxide dismutase (SOD)

5.9 SUMMARY OF THE STUDIES

The toxicity mechanisms of chemical contaminants (metal, textile dye and organophosphate pesticides) involves the generation of reactive oxygen species (ROS) through the intervention of metal ions in Fenton's reaction

↓

The increased levels of ROS produces oxidative damage to macromolecules such as carbohydrate, protein, lipids and nucleic acids which finally leads to the damage of different cellular organelles.

↓

Inhibited photosynthesis mechanisms leads to mortality of the cell after long term exposure to these chemical contaminants

↓

Reduction in biochemical composition leads to interference of cell functions
Eg: Carbohydrate: Inhibition of glucose synthesis and decrease cell metabolisms
Eg: Protein : Oxidation of amino acids and proteins (the addition of carbonyl groups)
Eg: Lipid: oxidation of unsaturated lipid component of membrans (lipid peroxidation)

↓

DNA damage in cell : Changes in DNA structure [eg: single and double- strand breaks in DNA backbones, cross-link between DNA bases or between DNA bases & protein, chemical addition to DNA bases (adduct)]

↓

To defend themselves from damaging effect of ROS, all aerobic organisms have evolved complex antioxidant defense system that include enzymatic complex such as (SOD).

↓

End-points such as growth rate, carbohydrate content, protein content, lipid content, DNA damage [genomic template stability (which reflect RAPD changes) & AP-site content] and SOD activity assay may be used as biomarkers for detection of chemical contamination, based on taxonomic species and duration of exposure

5.10 APPRAISAL OF STUDY

This has been an extensive and laborious study which involved a large number of three groups of toxicants and four tropical marine algal species. This is the first extensive study on tropical marine algae.The results are very important contributions to the development of bioassays for a range of toxicants for implementation in the tropics.

Besides commonly used end-points (biomarker) like growth rate, IC50 values and biochemical composition, this study included the study of DNA damage [Genomic template stability (which reflect RAPD changes) and AP-site content] and SOD responses, making this the first through study for tropical marine algae.

This study generated abundant data, which led to difficulty in data analysis as well as reporting in the 'Results' Section and complicated and lengthy discussions. With more time, the discussions can be merged for clearer understanding by the reader.

5.12 AREAS FOR FUTURE RESEARCH

1. Future studies should be designed to understand the mechanism of response and adaptation of the four algae to the twelve toxicants.

2. Recovery experiments should be conducted to investigate existence of repair mechanism as well period of tolerance to each toxicant by each alga.

3. Understanding the molecular basis of response and adaptation to the twelve toxicants

4. Conduct field trials on selected species and selected end-points

6.0 CONCLUSIONS

In this study, the effect of the seven metals, three textile dyes and two organophosphate pesticides were investigated on the growth, biochemical composition, DNA damage and Superoxide dismutase (SOD) enzyme activity in four marine algae, namely *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa.*

Studies were conducted using short term (four days) and long term (ten days) exposure under laboratory condition. The results were used to assess the suitability of these bioassay end points (growth, biochemical composition, DNA damage and SOD enzyme activity) for detection in *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa.*

The following are highlight of this study:

(1) Suitability of End Points

(i) Different end-points have different suitability for the four species tested (Table 5.18 and Table 5.19).

(ii) Growth rate was shown to be a good end-points to use for *Chlorella vulgaris* and *Boergesenia forbesii.* It was better for short term exposure.

(iii) Carbohydrate content was suitable for *Chlorella vulgaris, Tetraselmis tetrahele* and *Boergesenia forbesii* at short term and long term exposure.

(iv) Protein was a useful for end-points for two microalgae namely, *Chlorella vulgaris* and *Tetraselmis tetrahele* especially for long term exposure.

(v) Lipid was useful for end-points for *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa* for short term and long term exposure.

(vi) Genomic template stability was shown to be useful bioassay end-points for *Chlorella vulgaris* and *Boergesenia forbesii* for long term exposure, *Tetraselmis tetrahele* (short term exposure) and *Ventricaria ventricosa* (short term exposure and long term exposure).

(vii) Superoxide dismutase (SOD) enzyme activity was useful for end-points for *Tetraselmis tetrahele* for short term exposure, *Boergesenia forbesii* (long term exposure), *Chlorella vulgaris* and *Ventricaria ventricosa* (short term exposure and long term exposure).

(2) Suitability of the Species (Table 6.1)

(i) Metal

(a) Cadmium (Cd)

Short term exposure: *Chlorella> Ventricaria> Tetraselmis> Boergesenia*

Long term exposure: *Chlorella> Tetraselmis> Boergesenia> Ventricaria*

(b) Cobalt (Co)

Short term exposure: *Chlorella> Boergesenia> Tetraselmis> Ventricaria*

Long term exposure: *Chlorella> Boergesenia> Tetraselmis> Ventricaria*

(c) Chromium (Cr)

Short term exposure: *Chlorella> Tetraselmis> Boergesenia> Ventricaria*

Long term exposure: *Chlorella> Tetraselmis> Boergesenia> Ventricaria*

(d) Copper (Cu)

Short term exposure: *Chlorella> Boergesenia> Tetraselmis> Ventricaria*

Long term exposure: *Chlorella> Tetraselmis> Boergesenia> Ventricaria*

(e) Iron (Fe)

Short term exposure: *Chlorella> Tetraselmis> Boergesenia> Ventricaria*

Long term exposure: *Chlorella> Ventricaria> Boergesenia> Tetraselmis*

Table 6.1: Algae sensitivity to toxicant based on IC_{50} value

Algae cultures	Exposure duration	IC50 value
Chlorella vulgaris UMACC245	Four days	Metals: Fe (0.0066mg/L) < Co (0.0067mg/L) < Cr (0.0068mg/L) < Zn (0.0070mg/L) < Cu (0.0076mg/L) < Cd (0.0079mg/L) < Mn (0.0081mg/L) Textile dye: Basic dye (0.0064mg/L)= Metal complex dye (0.0064mg/L) < Acidic dye (0.0332mg/L) Organophosphate pesticides: Dichlovos (0.0088mg/L) < Malathion (0.0918mg/L)
	Ten days	Metals: Co (0.0076mg/L) < Cr (0.0077mg/L) < Fe (0.0082mg/L) < Mn (0.0087mg/L) < Cd (0.0092mg/L) < Cu (0.0973mg/L) < Zn (1.0353mg/L). Textile dye: Basic dye (0.0074mg/L) < Metal complex dye (0.0074mg/L) < Acidic dye (0.0091mg/L) Organophosphate pesticides: Dichlovos (0.0065mg/L) < Malathion (0.0072mg/L)
Tetraselmis tetrahele UMACC 144	Four days	Metals: Cu (0.5574mg/L) < Zn (1.6632mg/L) < Fe (2.8923mg/L) < Co (4.8514mg/L) < Mn (5.1250mg/L) < Cd (6.5138mg/L) < Cr (8.288mg/L). Textile dye: Metal complex dye (0.0079mg/L) < Basic dye (0.0085mg/L) < Acidic dye (0.0094mg/L) Organophosphate pesticides: Dichlovos (0.0054mg/L) < Malathion (0.0057mg/L)
	Ten days	Metals: Cr (3.1040mg/L) < Cu (5.4390mg/L) < Cd (7.1740mg/L) < Mn (8.8580mg/L) <Co (9.7710mg/L) <Zn (74.3920mg/L) < Fe (97.9680mg/L) Textile dye: Metal complex dye (5.9060mg/L) < Basic dye (41.9660mg/L) <Acidic dye (52.2550mg/L) Organophosphate pesticides: Dichlovos (0.0060mg/L) = Malathion (0.0060mg/L)
Boergesenia forbesii	Four days	Metals: Cu (0.0250mg/L) < Mn (0.0720mg/L) < Co (0.0800mg/L) < Cr (8.9740mg/L)< Fe (23.8000mg/L) < Cd (52.9550mg/L) < Zn (68.3700mg/L). Textile dye: Metal complex dye (6.7860mg/L) < Basic dye (6.9320mg/L) < Acidic dye (20.4970mg/L) < Organophosphate pesticides: Malathion (0.2770mg/L) < Dichlovos (30.6250mg/L)
	Ten days	Metals: Co (4.2900mg/L) < Zn (18.7000mg/L) < Cu (19.8830mg/L) < Fe (25.2340mg/L) < Mn (32.3880mg/L) < Cr (54.0010mg/L) < Cd (54.9170mg/L) Textile dye: Metal complex dye (36.3460mg/L)< Basic dye (67.3270mg/L) < Acidic dye (126.5730mg/L). Organophosphate pesticides: Malathion (0.0050mg/L) < Dichlovos (0.0052mg/L)
Ventricaria ventricosa	Four days	Metals: Cd (5.9800mg/L) < Cu (6.2100mg/L) < Mn (10.4500mg/L) < Co (13.1800mg/L) < Cr (16.1500mg/L) < Fe (52.9700mg/L) < Zn (57.4200mg/L) Textile dye: Basic dye (0.0100mg/L) < Acidic dye (8.9200mg/L) < Metal complex dye (26.6800mg/L) Organophosphate pesticides: Dichlovos (16.4300mg/L) < Malathion (100.0000mg/L).
	Ten days	Metals: Zn (8.8100mg/L) < Fe (15.5500mg/L) < Mn (45.6000mg/L) < Co (52.0200mg/L) < Cr (55.9900mg/L) < Cu (56.9900mg/L) < Cd (62.8300mg/L) Textile dye: Basic dye (20.6200mg/L) < Metal complex dye (27.1100mg/L) < Acidic dye (46.1600mg/L) Organophosphate pesticides: Dichlovos (178.1700mg/L) < Malathion (213.2800mg/L).

(f) Manganese (Mn)

Short term exposure: *Chlorella> Boergesenia> Tetraselmis> Ventricaria*

Long term exposure: *Chlorella> Tetraselmis> Boergesenia> Ventricaria*

(g) Zinc (Zn)

Short term exposure: *Chlorella> Tetraselmis> Ventricaria> Boergesenia*

Long term exposure: *Chlorella> Ventricaria> Boergesenia> Tetraselmis*

(ii) Textile dye

(a) Supranol Br. Red 3Bur (Acidic dye)

Short term exposure: *Tetraselmis> Chlorella> Ventricaria> Boergesenia*

Long term exposure: *Chlorella> Ventricaria> Tetraselmis> Boergesenia*

(b) Astrazon Red FBL (Basic dye)

Short term exposure: *Chlorella> Tetraselmis> Ventricaria> Boergesenia*

Long term exposure: *Chlorella> Ventricaria> Tetraselmis> Boergesenia*

(c) Lanaset Red 2GA (Metal complex dye)

Short term exposure: *Chlorella> Tetraselmis> Boergesenia> Ventricaria*

Long term exposure: *Chlorella> Tetraselmis> Ventricaria> Boergesenia*

(iii) Organophosphate Pesticide

(a) Dichlovos

Short term exposure: *Tetraselmis> Chlorella> Ventricaria> Boergesenia*

Long term exposure: *Boergesenia> Tetraselmis> Chlorella> Ventricaria*

(b) Malathion

Short term exposure: *Tetraselmis> Chlorella> Boergesenia> Ventricaria*

Long term exposure: *Boergesenia> Tetraselmis> Chlorella> Ventricaria*

Of the end-points, Genomic template stability was the most sensitive for *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa. Chlorella vulgaris,* appears to be the most useful bioassay organism because it was the most sensitive to toxicants. Finding from this study will contribute towards the development of bioassays for the detection and monitoring of metal, textile dye and organophosphate pesticide contamination based on DNA damage detection, growth, biochemical composition and stress enzyme response in tropical marine microalgae and macroalgae.

The Research Questions addressed in this thesis are:

Q1: Do selected chemical contaminants (metals, textile dye and organophosphate pesticides) produce effects on the growth, biochemical composition, DNA and Superoxide dismutase enzyme activity in the Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii *and* Ventricaria ventricosa *cultures after exposure for short term (four days) and long term (ten days) duration?*

A1: Yes. Selected chemical contaminants (metals, textile dye and organophosphate pesticides) produce effects on the growth, biochemical composition, DNA and Superoxide dismutase enzyme activity in the *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa* cultures after exposure for short term (four days) and long term (ten days) duration.

Q2: Are there differences in the effects of the selected chemical contaminants (metals, textile dye and organophosphate pesticides) on growth, biochemical composition, DNA and Superoxide dismutase enzyme activity in the Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii *and* Ventricaria ventricosa *cultures after exposure for short term (four days) and long term (ten days) duration?*

A2: Yes. There are differences in the effects of the selected chemical contaminants (metals, textile dye and organophosphate pesticides) on growth, biochemical composition, DNA and Superoxide dismutase enzyme activity in the *Chlorella vulgaris, Tetraselmis tetrahele, Boergesenia forbesii* and *Ventricaria ventricosa* cultures after exposure for short term (four days) and long term (ten days) duration.

Q3: What is the possible mechanism (s) of the effect of these contaminants on the selected algae?

A3: The possible mechanisms (s) of the effect of these contaminants on the selected algae were discussed in Section 5.3, Section 5.4, Section 5.5, Section 5.6 and Section 5.7.

Q4: Can the bioassays based on the four end-points be ranked in terms of sensitivity to the selected chemical contaminants (metals, textile dye & organophosphate pesticides)?

A4: Yes. The bioassays based on the four end-points be ranked in terms of sensitivity to the selected chemical contaminants (metals, textile dye & organophosphate pesticides) as discussed in Section 5.9.

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APPENDIX 1

National Water Quality Standards For Malaysia (2010)

PARAMETER	UNIT	CLASS				
		I	IIA/IIB	III	IV	V
Al	mg/L	↑ NATURAL LEVELS OR ABSENT ↓	-	(0.06)	0.5	↑ LEVEL ABOVE IV ↓
As	mg/L		0.05	4 (0.05)	0.1	
Ba	mg/L		1	-	-	
Cd	mg/L		0.01	0.01*(0.001)	0.01	
Cr (IV)	mg/L		0.05	1.4 (0.05)	0.1	
Cr (III)	mg/L		-	2.5	-	
Cu	mg/L		0.02	-	0.2	
Hardness	mg/L		250	-	-	
Ca	mg/L		-	-	-	
Mg	mg/L		-	-	-	
Na	mg/L		-	-	3SAR	
K	mg/L		-	-	-	
Fe	mg/L		1	1	1(Left) 5(Others)	
Pb	mg/L		0.05	0.02*(0.01)	5	
Mn	mg/L		0.1	0.1	0.2	
Hg	mg/L		0.001	0.004 (0.0001)	0.002	
Ni	mg/L		0.05	0.9*	0.2	
Se	mg/L		0.01	0.25 (0.04)	0.02	
Ag	mg/L		0.05	0.0002	-	
Sn	mg/L		-	0.004	-	
U	mg/L		-	-	-	
Zn	mg/L		5	0.4*	2	
B	mg/L		1	(3.4)	0.8	
Cl	mg/L		200	-	80	
Cl2	mg/L		-	(0.02)	-	
CN	mg/L		0.02	0.06 (0.02)	-	
F	mg/L		1.5	10	1	
NO2	mg/L		0.4	0.4 (0.03)	-	
NO3	mg/L		7	-	5	
P	mg/L		0.2	0.1	-	
Silica	mg/L		50	-	-	
SO4	mg/L		250	-	-	
S	mg/L		0.05	(0.001)	-	
CO2	mg/L		-	-	-	
Gross-α	Bq/L		0.1	-	-	
Gross-β	Bq/L		1	-	-	
Ra-226	Bq/L		<0.1	-	-	
Sr-90	Bq/L		<1	-	-	
CCE	μg/L		500		-	-
MBAS/BAS	μg/L		500	5000 (200)	-	-
O & G (Mineral)	μg/L		400; N	N	-	-
O & G (Emulsified Edible)	μg/L		7000; N	N	-	-
PCB	μg/L		0.1	6 (0.05)	-	-
Phenol	μg/L		10	-	-	-
Aldrin/Dieldrin	μg/L		0.02	0.2 (0.01)	-	-
BHC	μg/L		2	9 (0.1)	-	-
Chlordane	μg/L		0.08	2 (0.02)	-	-
t-DDT	μg/L		0.1	(1)	-	-
Endosulfan	μg/L		10	-	-	-
Heptachlor/Epoxide	μg/L		0.05	0.9 (0.06)	-	-
Lindane	μg/L		2	3 (0.4)	-	-
2,4-D	μg/L		70	450	-	-
2,4,5-T	μg/L		10	160	-	-
2,4,5-TP	μg/L		4	850	-	-
Paraquat	μg/L		10	1800	-	-

Notes :
** = At hardness 50 mg/l $CaCO_3$*
 # = Maximum (unbracketed) and 24-hour average (bracketed) concentrations
N = Free from visible film sheen, discolouration and deposits

APPENDIX 1 (continued)

National Water Quality Standards For Malaysia (2010)

PARAMETER	UNIT	CLASS					
		I	IIA	IIB	III	IV	V
Ammoniacal Nitrogen	mg/L	0.1	0.3	0.3	0.9	2.7	>2.7
Biochemical Oxygen Demand	mg/L	1	3	3	6	12	>12
Chemical Oxygen Demand	mg/L	10	25	25	50	100	>100
Dissolved Oxygen	mg/L	7	5-7	5-7	3-5	<3	<1
pH		6.5-8.5	6-9	6-9	5-9	5-9	-
Colour	TCU	15	150	150	-	-	-
Electrical Conductivity*	μS/cm	1000	1000	-	6000	-	-
Floatables		N	N	N	-	-	-
Odour		N	N	N	-	-	-
Salinity	%	0.5	1	-	-	2	-
Taste		N	N	N	-	-	-
Total Dissolved Solid	mg/L	500	1000	-	-	4000	-
Total Suspended Solid	mg/L	25	50	50	150	300	300
Temperature	°C	-	Normal + 2°C	-	Normal + 2°C	-	-
Turbidity	NTU	5	50	50	-	-	-
Faecal Coliform**	count/100 mL	10	100	400	5000 (20000)[a]	5000 (20000)[a]	-
Total Coliform	count/100 mL	100	5000	5000	50000	50000	>500000

Notes :
N : No visible floatable materials or debris, no objectional odour or no objectional taste
** : Related parameters, only one recommended for use*
*** : Geometric mean*
a : Maximum not to be exceeded

Water Classes And Uses

CLASS	USES
Class I	Conservation of natural environment. Water Supply I – Practically no treatment necessary. Fishery I – Very sensitive aquatic species.
Class IIA	Water Supply II – Conventional treatment required. Fishery II – Sensitive aquatic species.
Class IIB	Recreational use with body contact
Class III	Water Supply III – Extensive treatment required. Fishery III – Common, of economic value and tolerant species; livestock drinking.
Class IV	Irrigation
Class V	None of the above.

DOE Water Quality Classification Based On Water Quality Index

PARAMETER	INDEX RANGE		
	CLEAN	SLIGHTLY POLLUTED	POLLUTED
Biochemical Oxygen Demand (BOD)	91-100	80-90	0-79
Ammoniacal Nitrogen (NH_3-N)	92-100	71-91	0-70
Suspended Solids (SS)	76-100	70-75	0-69
Water Quality Index (WQI)	81-100	60-80	0-59

DOE Water Quality Index Classification

PARAMETER	CLASS	I	II	III	IV	V
Ammoniacal Nitrogen (NH_3-N)	mg/L	<0.1	0.1–0.3	0.3-0.9	0.9-2.7	>2.7
Biochemical Oxygen Demand (BOD)	mg/L	<1	1 – 3	3-6	6-12	>12
Chemical Oxygen Demand (COD)	mg/L	<10	10-25	25-50	50-100	>100
Dissilved Oxygen (DO)	mg/L	>7	5-7	3-5	1-3	<1
PH		>7	6-7	5.6	<5	>5
Suspended Solids (SS)	mg/L	<25	25-50	50-150	150-300	>300
Water Quality Index (WQI)		>92.7	76.5-92.7	51.9-76.5	31.0-51.9	<31.0

APPENDIX 2

Malaysia: Marine Water Quality Criteria and Standards (2010)

	PARAMETER	CLASS 1	CLASS 2	CLASS 3	CLASS E
	BENEFICIAL USES	Preservation, marine protected areas, Marine Parks	Marine Life, Fisheries, Coral Reefs, Recreational and Mariculture	Ports, Oil & Gas Fields	Mangroves, Estuarine & River-mouth water
1	Temperature (°C)	≤2 °C increase over maximum ambient	≤2 °C increase over maximum ambient	≤2 °C increase over maximum ambient	≤2 °C increase over maximum ambient
2	Dissolved Oxygen (mg/L)	>80% saturation	5.0	4.0	3.0
3	Total Suspended Solid (mg/L)	25 mg/L or ≤10% increase in seasonal average, whichever is lower	50 mg/L (25 mg/L) or ≤10% increase in seasonal average, whichever is lower	100 mg/L or ≤10% increase in seasonal average, whichever is lower	100 mg/L or ≤30% increase in seasonal average, whichever is lower
4	Oil and Grease(mg/L)	0.01	0.14	5	0.14
5	Mercury* (µg/L)	0.04	0.16 (0.04)	50	0.5
6	Cadmium* (µg/L)	0.5	2(3)	10	2
7	Chromium (VI)(µg/L)	5	10	48	10
8	Copper (µg/L)	1.3	2.9	10	2.9
9	Arsenic (III)* (µg/L)	3	20(3)	50	20(3)
10	Lead (µg/L)	4.4	8.5	50	8.5
11	Zinc (µg/L)	15	50	100	50
12	Cyanide (µg/L)	2.0	7.0	20	7
13	Ammonia (unionized) (µg/L)	35	70	320	70
14	Nitrite (NO2) (µg/L)	10	55	1000	55
15	Nitrate (NO3) (µg/L)	10	60	1000	60
16	Phosphate (µg/L)	5	75	670	75
17	Phenol (µg/L)	1	10	100	10
18	Tributyltin (TBT) (µg/L)	0.001	0.01	0.05	0.01
19	Faecal Coliform (Human health protection for seafood consumption) -(MPN)	70 faecal coliform/100ml *70 E.coli/100 ml*	100 faecal coliform/100ml (70 faecal coliform/100 ml) *100 E.coli/100ml (70 E.coli/100ml)*	200 faecal coliform/100ml *200 E.coli/100ml*	100 faecal coliform/100ml (70 faecal coliform/100 ml) *100 E.coli/100ml (70 E.coli/100ml)*
20	Polycyclic Aromatic Hydrocarbon (PAHs) ng/g	100	200	1000	1000

** MWQCS in parentheses are for coastal and marine water areas where seafood for human consumption is applicable*

APPENDIX 3

THIRD SCHEDULE

ENVIRONMENTAL QUALITY ACT 1974

ENVIRONMENTAL QUALITY (SEWAGE AND INDUSTRIAL EFFLUENTS) REGULATIONS 1979

(REGULATIONS 8(1), 8(2), 8(3)

PARAMETER LIMITS OF EFFLUENTS OF STANDARDS A AND B

Parameter		Unit	Standard	
			A	B
(i)	Temperature	°C	40	40
(ii)	pH value	-	6.0 - 9.0	5.5 - 9.0
(iii)	BOD at 20°C	mg/ l	20	50
(iv)	COD	mg/ l	50	100
(v)	Suspended Solids	mg/ l	50	100
(vi)	Mercury	mg/ l	0.005	0.05
(vii)	Cadmium	mg/ l	0.01	0.02
(viii)	Chromium, Hexavalent	mg/ l	0.05	0.05
(ix)	Arsenic	mg/ l	0.05	0.10
(x)	Cyanide	mg/ l	0.05	0.10
(xi)	Lead	mg/ l	0.10	0.5
(xii)	Chromium Trivalent	mg/ l	0.20	1.0
(xiii)	Copper	mg/ l	0.20	1.0
(xiv)	Manganese	mg/ l	0.20	1.0
(xv)	Nickel	mg/ l	0.20	1.0
(xvi)	Tin	mg/ l	0.20	1.0
(xvii)	Zinc	mg/ l	2.0	2.0
(xviii)	Boron	mg/ l	1.0	4.0
(xix)	Iron (Fe)	mg/ l	1.0	5.0
(xx)	Phenol	mg/ l	0.001	1.0
(xxi)	Free Chlorine	mg/ l	1.0	2.0
(xxii)	Sulphide	mg/ l	0.50	0.50
(xxiii)	Oil and Grease	mg/ l	Not Detectable	10.0

APPENDIX 4

Provasoli 50 (Prov50) Medium

Synthethic seawater

Dissolved 30 g of sea-salt (Marine Environment, Aquacraft Inc, USA) in 1 litre of deionised water, stir for 2 hours and filter (Whatman No. 4, diameter 90 mm)

Prov Medium

1. $NaNO_3$ 75.0 g/L
2. $NaH_2PO_4.H_2O$ 5.0 g/L
3. f/2 trace metal solution
   - $Na_2EDTA.2H_2O$ 4.36 g
   - $FeCl_3.6H_2O$ 3.15 g
   - $CoCl_2.6H_2O$ 1.0 ml (from 10.0 g/L)
   - $ZnSO_4.7H_2O$ 1.0 ml (from 22.0 g/L)
   - $MnCl_2.4H_2O$ 1.0 ml (from 180.0 g/L)
   - $CuSO_4.5H_2O$ 1.0 ml (from 9.8 g/L)
   - $Na_2MoO_4.2H_2O$ 0.7 mg

   Make up to 1 litre with deionised water
4. Vitamin solution
   - Vitamin B12 1.0 ml (from 1.0 g/L)
   - Biotin 10.0 ml (from 0.1 g/L)
   - Thiamine HCl 100.0 mg
5. Alkaline soil extract
   - Add 2 parts by volume distilled water to 1 part of garden soil (free of fertilizers and pesticides) and to this mixture, add 2 – 3 g/L NaOH. Autoclave for 2 hours and filter after cooling. Dilute this extrac to 1: 50 in distilled water for the final working stock.

Medium
To 950 mL filtered seawater add:
- $NaNO_3$ stock solution 1.0 ml
- $NaH_2PO_4.H_2O$ stock solution 1.0 ml
- Alkaline soil extract 15.0 ml
- F/2 trace metal solution 1.0 ml
- F/2 vitamin solution 0.5 ml

Make final volume up to 1 litre with filtered seawater.

Prov 50 Medium

Medium
To 950 mL filtered seawater add:
- $NaNO_3$ stock solution 1.0 ml
- $NaH_2PO_4.H_2O$ stock solution 1.0 ml
- 50 mM NH_4Cl stock solution 1.0 ml (2.68 g/L)
- Alkaline soil extract 10.0 ml
- F/2 trace metal solution 1.0 ml
- F/2 vitamin solution 0.5 ml

Make final volume up to 1 litre with filtered seawater.

APPENDIX 7: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD)

Cadmium-Day 4		Intensity (%)						
OPA13	BP	0	0.01	0.1	1	10	100	500
	2936.53	120±0.01	96.00±1.00	97.66±12.01	71.67±15.01	63.67+3.06	22.00±2.65	25.33±2.52
	2644.6		29.67±6.03					
	1948.63	26.33±2.89		34.67±9.07	36±6.08	43.00±5.29	57.00±1.73	71.33±5.13
	1833.17	26.33±3.06				37.67±4.51	34.67±4.04	45.00±3.00
	1665.4	68.00±6.08	28.33±2.31	60.67±5.51	58.33±10.21	46.33±3.51	36.33±3.51	37.00±2.65
	1362.57	25.67±2.52	27.67±0.58	33.33±3.79				
	1327.37	25.67±3.32	28.33±2.31					
	1185.03							
	828.63							
	772.76	45.67±4.57	31.67±2.08	55.67±5.51	81.67±6.66	57.67±5.03	67.00+12.17	72.33±4.51
	660.44		86.67±6.03	66.67±7.10	46.67±5.13	119.67±11.02	99.00+13.12	103.00±6.93
	594.78		34.67±2.52	40.33±5.03	23.67±3.51	30.00±4.36		
	486.00					27.67±1.53		

Cadmium-Day 10		Intensity (%)						
OPA13	BP	0	0.01	0.1	1	10	100	500
	2936.53	92.33±9.50	53.00±7.81	68.67±5.51	58.67±4.16	26.67±2.52		
	1833.17	51.67±4.51	32.00±1.00	36.67±1.53	32.33±2.08	32.33±3.21		32.00±6.24
	1665.4	68.67±1.15	37.33±5.51	63.33±2.89	58.00±3.46	48.67±9.07		
	1539.62	73.67±14.19						
	1185.03	32.00±2.00					47.00±2.65	45.00±2.00
	772.76	84.67±4.04	33.00±4.58	39.67±3.21	48.67±1.53	47.00±7.00	38.33±2.31	44.00±6.93
	708.19		31.00±4.36	40.67±2.52	40.00±3.61	40.00±1.00	28.67±3.21	
	660.44		90.67±11.15	113±20.07	110.33±2.31	111±8.72	91.33±13.87	62.67+13.61
	615.91	31.67±4.16	23.00±2.65	26.00±1.73	22.67±2.31			

APPENDIX 12: *Tetraselmis tetrahele* UMACC 144: DNA Damage (RAPD)

Malathion-Day 4		Intensity (%)						
S87	BP	0	0.01	0.1	1	10	100	500
	2825.97	40.67±3.06						
	2200.84	49.67±7.51	78.67±3.79	92.00±1.00	101.00±7.94	112.00±2.65	69.33±1.53	45.67±3.51
	1862.95	91.33±5.86					51.67±1.53	
	1772.09	81.67±7.02	60.00±1.00	58.00±1.00	76.67±6.66	85.00±2.00	57.67±2.08	27.67±2.08
	1538			27.33±3.06	24.00±1.00			
	1415.02	46.33±5.03						
	1357.27		37.33±1.15	37.00±1.00	47.00±2.00	38.33±0.58	32.00±2.65	27.00±1.00
	1291.07	28.67±2.31						
	1207.8			21.00±2.65	25.67±2.08	25.33±0.58		
	1139.36	21.33±3.06					25.00±3.61	25.67±2.52
	925.07		21.00±1.73	21.00±1.73	23.33±2.52	19.67±1.15	21.67±2.08	
	865.41	31.00±2.65	33.00±6.00	23.33±3.06	29.00±3.61	36.67±1.53	22.00±2.00	
	809.59		27.33±4.73	20.33±3.06	20.33±1.16	27.33±2.08		
	751.09			26.00±1.00	23.00±2.00	31.67±2.08	23.33±1.53	21.00±2.65
	720.44				22.67±0.58	25.33±1.15	24.67±0.58	
	657.33		26.67±1.15	27.33±3.51	28.33±2.08	32.67±1.53	27.00±1.73	27.00±1.00
	599.75							22.67±2.08
	507.67	21.33±0.58	17.33±1.53	22.33±1.53	21.33±2.52	24.67±0.58	33.67±2.89	42.00±6.56
	448.02	14.67±0.58	17.33±1.15					
	419.12						17.00±7.00	

Malathion-Day 10		Intensity (%)						
S87	BP	0	0.01	0.1	1	10	100	500
	3539.08	26.00±2.00	29.00±3.61	27.00±1.73		24.00±3.61		
	2825.97	53.67±5.77						
	2200.84	77.67±5.13	125.00±9.64	129.67±0.58	63.33±0.58	137.33±10.21	80.00±8.00	58.33±4.73
	1862.95	91.33±3.21	35.00±3.00				25.00±3.46	
	1772.09	116.00±6.56	37.67±2.52	39.67±1.53	33.33±3.22	45.00±2.65	23.67±3.06	
	1538		29.67±1.53	25.00±2.65				
	1415.02	44.00±2.65	27.00±2.00					
	1357.27		44.00±1.00	45.00±1.00	44.67±2.89	54.67±3.79	19.00±1.00	
	1291.07	35.00±2.65						
	1139.36	29.33±1.53					25.67±3.51	
	925.07		21.00±1.00	23.00±2.65		19.67±0.58	21.67±1.53	
	865.41	27.67±2.08	31.67±2.08	27.67±2.52	29.67±3.51	26.00±2.65		
	809.59						21.67±2.08	
	751.09		28.33±1.53	34.33±4.51	43.33±3.06	42.67±2.52		16.33±0.58
	720.44		26.33±2.08	25.33±3.79				
	657.33		39.00±1.00	25.00±1.00	25.67±2.89	26.33±2.31	21.33±0.58	23.00±1.73
	507.67	24.00±1.73	21.33±1.15				38.00±1.73	65.33±3.79
	448.02	22.00±1.00					26.33±3.06	
	419.12		24.33±2.52	21.33±3.51		24.00±2.00	23.33±2.52	

APPENDIX 17: *Boergesenia forbesii* : DNA Damage (RAPD)

Cadmium-Day 4		Intensity (%)						
OPK14	BP	0	0.01	0.1	1	10	100	500
	2080.16	34.00±4.58						
	1612.3	22.33±0.58						
	1388.62	34.67±3.21						
	1206.52	20.33±1.53						34.33±2.52
	1134.55	28.33±3.06	20.67±1.53	24.67±2.89	32.00±1.00	27.33±1.53	31.00±2.65	37.33±3.21
	1039.13	22.33±2.08						
	918.87	28.33±3.06	88.00±3.61	56.67±3.06	88.00±6.08	74.67±3.51	146.67±2.52	134.00±1.73
	819.69					40.67±1.15	74.00±2.00	
	750.75		31.00±1.73	31.33±1.53	34.67±3.06	36.67±1.53	35.00±1.73	46.67±3.06
	669.72		44.00±2.65	40.00±2.65	32.67±1.15	50.00±4.00	134.67±2.08	61.67±0.58
	613.39	30.33±2.08						
	597.44		33.67±3.79	34.00±2.65	30.67±2.08	45.67±0.58	38.00±3.46	64.67±3.51
	514.55	17.33±1.53	23.33±0.58	35.33±2.52	37.33±2.89	28.33±2.52	25.33±1.53	43.33±3.06
	371.75		36.33±2.89	27.67±4.62	21.33±0.58	26.00±1.00	35.33±3.06	31.33±2.31

Cadmium-Day 10		Intensity (%)						
OPK14	BP	0	0.01	0.1	1	10	100	500
	2080.16	79.67±4.51						
	1612.3	48.67±1.15						
	1388.62	63.67±2.89						
	1206.52	36.67±3.06						
	1134.55	59.67±1.53	33.00±1.00	56.00±4.36		39.00±1.00	35.67±0.58	35.33±4.04
	1039.13			41.67±3.79	37.33±4.51	54.67±2.31		
	918.87	59.67±1.53	85.67±7.37	85.67±2.31	94.33±3.06	122.33±3.21	85.67±3.79	41.33±1.53
	819.69		43.67±2.08	43.67±2.08	35.00±1.73			
	777.6							34.67±4.73
	750.75		60.67±3.21	34.00±1.00	36.00±2.65	43.00±1.73	34.00±1.00	34.67±4.62
	669.72		64.33±2.31	43.33±2.08	33.67±2.52	61.33±0.58	61.33±0.58	41.33±0.58
	613.39	59.67±3.79						
	597.44		67.00±1.73		43.00±2.00	51.67±1.53		40.67±6.35
	514.55	31.67±1.53	44.67±1.53	31.67±2.08	35.00±2.00	55.33±3.51		30.33±3.51
	479.63		37.67±1.53					
	371.75		46.67±1.15		30.00±1.00	24.33±3.06	38.67±2.52	31.00±2.00

APPENDIX 22: *Ventricaria ventricosa* : DNA Damage (RAPD)

Acidic Dye-Day 4		Intensity (%)						
S17	BP	0	0.01	0.1	1	10	100	500
	3501.00						18.00±1.00	
	2579.65	28.33±3.51						
	2329.85	25.67±0.58						
	2169.53	28.33±3.51						
	2040.92	13.33±2.62				11.67±1.53	35.33±2.89	
	1862.16	8.67±1.53		17.00±2.00	21.67±0.58	14.33±0.58	25.00±3.46	
	1664.8	11.33±2.31			37.67±2.31		13.00±2.65	
	1582.14	14.67±2.08						
	1488.35						15.00±1.73	
	1428.94		16.33±2.31	14.00±1.00	18.33±2.08	18.33±1.53	18.00±1.00	
	1177.53	10.67±1.53			17.33±1.15	14.67±1.53		
	1096.5	15.00±3.46		22.00±3.46		17.33±1.53	25.00±1.00	
	1042.06	13.33±0.58		25.67±4.04	33.67±1.15	24.33±1.15		
	931.62	16.67±2.52						
	885.36		12.33±1.53	12.33±1.53	14.00±1.73	20.00±3.00		
	783.51	10.00±1.73						
	737.06	10.00±2.00						
	686.34		21.33±0.58			15.33±1.53	17.00±1.00	
	601.23		19.00±1.00		47.33±2.31			
	571.38		12.67±0.58	15.33±1.15	24.67±1.53	18.00±1.00	26.00±2.65	
	510.82		14.67±1.15	23.67±0.58	16.00±1.00	22.67±2.89	20.33±1.53	
	466.08			16.00±3.46				
	416.68	8.67±1.15	18.33±3.21		26.00±3.00	17.00±2.65	25.00±3.61	
	388.01			19.67±2.08				
	346.88						21.00±3.61	

Acidic Dye-Day 10		Intensity (%)						
S17	BP	0	0.01	0.1	1	10	100	500
	3501.52				17.33±1.53			
	2915		19.33±2.08	16.33±0.58	16.67±0.58			
	2579.65	63.33±2.52			20.33±3.21	29.00±1.73		
	2329.85	14.00±2.00						
	2169.53	14.67±2.31						
	2040.92	22.33±2.89						
	1862.16	22.33±1.53						
	1582.14	28.67±0.58						
	1428.94			16.00±3.61	16.33±0.58			
	1177.53	23.00±2.65			15.00±1.00			
	1096.5	30.33±4.93				15.00±3.61		
	1042.06	33.33±4.16		24.00±5.00				
	931.62	37.33±7.09						
	885.36			26.67±3.06	43.33±2.31	36.00±1.73	61.67±2.08	
	783.51	22.33±1.53						
	737.06	20.33±0.58						
	686.34			22.33±4.04		14.33±0.58		
	601.23		60.00±7.81	46.33±2.31	29.67±3.79	21.33±0.58	19.67±3.79	
	571.38			20.67±1.53		15.33±1.53		
	510.82		20.33±2.52					
	466.08					24.67±3.21		
	416.68	15.33±1.53	18.67±1.15	29.00±6.24	22.00±1.73	19.67±2.08	30.67±2.52	
	388.01			23.00±3.61	34.00±2.00		31.67±3.06	
	368.74		29.00±1.00					
	346.88					22.67±3.79		

APPENDIX 25: ANOVA: *Chlorella vulgaris* UMACC 245: Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-CHL

Newman-Keuls test; CD_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L 1.002000	{0.1 mg/L} .8330000	{1 mg/L} .8740000	{10 mg/L} .6000000	{100 mg/L} .5200000	{500 mg/L} .0240000
A	{0}		*0.000254	*0.000196	*0.000181	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000254		*0.007413	*0.015961	*0.000196	*0.000150	*0.000158
C	{0.1}	*0.000196	*0.007413		0.39434	*0.000351	*0.000198	*0.000196
D	{1}	*0.000181	*0.015961	0.39434		*0.000276	*0.000202	*0.000150
E	{10}	*0.000150	*0.000196	*0.000351	*0.000276		0.108441	*0.000180
F	{100}	*0.000158	*0.000150	*0.000198	*0.000202	0.108441		*0.000176
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; CO_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L .8740000	{0.1 mg/L} .8740000	{1 mg/L} .9780000	{10 mg/L} .8330000	{100 mg/L} .7080000	{500 mg/L} .4480000
A	{0}		*0.000202	*0.000183	*0.000237	*0.000153	*0.000158	*0.000174
B	{0.01	*0.000202		1	0.129753	0.425705	*0.013177	*0.000197
C	{0.1}	*0.000183	1		0.056305	0.696797	*0.023110	*0.000152
D	{1}	*0.000237	0.129753	0.056305		0.050434	*0.000868	*0.000158
E	{10}	*0.000153	0.425705	0.696797	0.050434		*0.025493	*0.000183
F	{100}	*0.000158	*0.013177	*0.023110	*0.000868	*0.025493		*0.000292
G	{500}	*0.000174	*0.000197	*0.000152	*0.000158	*0.000183	*0.000292	

Newman-Keuls test; CR_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L .9090000	{0.1 mg/L} .8740000	{1 mg/L} .9730000	{10 mg/L} .9160000	{100 mg/L} .7610000	{500 mg/L} .0460000
A	{0}		*0.000222	*0.000170	*0.000228	*0.000197	*0.000158	*0.000174
B	{0.01	*0.000222		0.496776	0.430814	0.891102	*0.026771	*0.000196
C	{0.1}	*0.000170	0.496776		0.243573	0.686733	*0.040913	*0.000180
D	{1}	*0.000228	0.430814	0.243573		0.27486	*0.006431	*0.000158
E	{10}	*0.000197	0.891102	0.686733	0.27486		*0.035642	*0.000150
F	{100}	*0.000158	*0.026771	*0.040913	*0.006431	*0.035642		*0.000176
G	{500}	*0.000174	*0.000196	*0.000180	*0.000158	*0.000150	*0.000176	

Newman-Keuls test; CU_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L 1.032000	{0.1 mg/L} .9520000	{1 mg/L} .8820000	{10 mg/L} .8420000	{100 mg/L} 0.000000	{500 mg/L} 0.000000
A	{0}		*0.000380	*0.000198	*0.000197	*0.000151	*0.000174	*0.000158
B	{0.01	*0.000380		0.098273	*0.013191	*0.004410	*0.000158	*0.000150
C	{0.1}	*0.000198	0.098273		0.143468	0.069725	*0.000150	*0.000196
D	{1}	*0.000197	*0.013191	0.143468		0.390756	*0.000196	*0.000180
E	{10}	*0.000151	*0.004410	0.069724	0.390756		*0.000180	*0.000176
F	{100}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180		1
G	{500}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	1	

Newman-Keuls test; CD_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L .5950000	{0.1 mg/L} .5860000	{1 mg/L} .5850000	{10 mg/L} .5610000	{100 mg/L} .4430000	{500 mg/L} .2130000
A	{0}		0.180527	0.289418	0.414793	0.304534	*0.010090	*0.000179
B	{0.01	0.180527		0.873217	0.982256	0.925814	0.096291	*0.000227
C	{0.1}	0.289418	0.873217		0.985941	0.89444	0.089278	*0.000215
D	{1}	0.414793	0.982256	0.985941		0.67117	0.055014	*0.000231
E	{10}	0.304534	0.925814	0.89444	0.67117		0.051239	*.000220
F	{100}	*0.010090	0.096291	0.089278	0.055013	0.051239		*0.001106
G	{500}	*0.000179	*0.000227	*0.000215	*0.000231	*0.000220	*0.001106	

Newman-Keuls test; CO_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L .5620000	{0.1 mg/L} .5710000	{1 mg/L} .5640000	{10 mg/L} .5390000	{100 mg/L} .3890000	{500 mg/L} .0790000
A	{0}		0.178054	0.065531	0.118308	0.118581	*0.000904	*0.000174
B	{0.01	0.178054		0.983096	0.96943	0.659203	*0.011585	*0.000196
C	{0.1}	0.065531	0.983096		0.89297	0.921683	*0.021889	*0.000158
D	{1}	0.118308	0.96943	0.89297		0.87725	*0.018908	*0.000151
E	{10}	0.118581	0.659203	0.921683	0.87725		*0.010919	*0.000180
F	{100}	*0.000904	*0.011585	*0.021889	*0.018908	*0.010919		*0.000197
G	{500}	*0.000174	*0.000196	*0.000158	*0.000151	*0.000180	*0.000197	

Newman-Keuls test; CR_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L .5480000	{0.1 mg/L} .5670000	{1 mg/L} .5880000	{10 mg/L} .5490000	{100 mg/L} .4390000	{500 mg/L} .2920000
A	{0}		0.215027	0.170891	0.146867	0.160005	*0.008845	*0.000263
B	{0.01	0.215027		0.937425	0.886357	0.985941	0.069052	*0.001196
C	{0.1}	0.170891	0.937425		0.710076	0.749852	0.14202	*0.001720
D	{1}	0.146867	0.886357	0.710076		0.764663	0.105727	*0.001262
E	{10}	0.160005	0.985941	0.749852	0.764663		0.151762	*0.002027
F	{100}	*0.008845	0.069052	0.14202	0.105727	0.151762		*0.018920
G	{500}	*0.000263	*0.001196	*0.001720	*0.001262	*0.002027	*0.018920	

Newman-Keuls test; CU_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L .6250000	{0.1 mg/L} .6030000	{1 mg/L} .5280000	{10 mg/L} .3760000	{100 mg/L} .2570000	{500 mg/L} .2280000
A	{0}		0.382518	0.410253	0.069639	*0.000673	*0.000169	*0.000179
B	{0.01	0.382518		0.685716	0.19838	*0.001914	*0.000199	*0.000180
C	{0.1}	0.410253	0.685716		0.180695	*0.002247	*0.000250	*0.000190
D	{1}	0.069639	0.19838	0.180695		*0.012852	*0.000594	*0.000492
E	{10}	*0.000673	*0.001914	*0.002247	*0.012852		*0.042273	*0.036935
F	{100}	*0.000169	*0.000199	*0.000250	*0.000594	*0.042273		0.594508
G	{500}	*0.000179	*0.000180	*0.000190	*0.000492	*0.036935	0.594508	

APPENDIX 25: ANOVA: *Chlorella vulgaris* UMACC 245: Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-CHL

Newman-Keuls test; FE_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L} .8740000	{0.1 mg/L} .8740000	{1 mg/L} .7730000	{10 mg/L} .6000000	{100 mg/L} .5200000	{500 mg/L} 0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000180		1	*0.020979	*0.000189	*0.000196	*0.000150
C	{0.1}	*0.000176	1		0.051406	*0.000214	*0.000151	*0.000158
D	{1}	*0.000196	*0.020979	0.051406		*0.000684	*0.000205	*0.000196
E	{10}	*0.000150	*0.000189	*0.000214	*0.000684		0.058377	*0.000180
F	{100}	*0.000158	*0.000196	*0.000151	*0.000205	0.058377		*0.000176
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; MN_C_C4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L} .9090000	{0.1 mg/L} .9090000	{1 mg/L} .9780000	{10 mg/L} .8740000	{100 mg/L} .7730000	{500 mg/L} 0.000000
A	{0}		*0.000200	*0.000182	*0.000191	*0.000152	*0.000158	*0.000174
B	{0.01	*0.000200		1	0.29563	0.44277	*0.021352	*0.000196
C	{0.1}	*0.000182	1		0.141748	0.714995	*0.037039	*0.000150
D	{1}	*0.000191	0.295635	0.141748		0.134115	*0.003125	*0.000158
E	{10}	*0.000152	0.442771	0.714995	0.134115		*0.038902	*0.000180
F	{100}	*0.000158	*0.021352	*0.037039	*0.003125	*0.038902		*0.000176
G	{500}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000180	*0.000176	

Newman-Keuls test; ZN_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L} .8740000	{0.1 mg/L} .8330000	{1 mg/L} .7840000	{10 mg/L} .6210000	{100 mg/L} .5200000	{500 mg/L} .2400000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.325643	0.099436	*0.000278	*0.000152	*0.000158
C	{0.1}	*0.000180	0.325643		0.243556	*0.000476	*0.000200	*0.000150
D	{1}	*0.000196	0.099436	0.243556		*0.001336	*0.000204	*0.000196
E	{10}	*0.000150	*0.000278	*0.000476	*0.001336		*0.025118	*0.000180
F	{100}	*0.000158	*0.000152	*0.000200	*0.000204	*0.025118		
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000180	

Newman-Keuls test; AD_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L} 1.131000	{0.1 mg/L} .8330000	{1 mg/L} .6210000	{10 mg/L} .4480000	{100 mg/L} 0.000000	{500 mg/L} 0.000000
A	{0}		*0.014409	*0.000180	*0.000196	*0.000150	*0.000174	*0.000158
B	{0.01	*0.014409		*0.000181	*0.000180	*0.000196	*0.000158	*0.000150
C	{0.1}	*0.000180	*0.000181		*0.000423	*0.000181	*0.000150	*0.000196
D	{1}	*0.000196	*0.000180	*0.000423		*0.001638	*0.000196	*0.000180
E	{10}	*0.000150	*0.000196	*0.000181	*0.001638		*0.000180	*0.000176
F	{100}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180		1
G	{500}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	1	

Newman-Keuls test; FE_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 0.673	{0.01 mg/L} .5390000	{0.1 mg/L} .6020000	{1 mg/L} .5880000	{10 mg/L} .5690000	{100 mg/L} .5310000	{500 mg/L} .1160000
A	{0}		0.179457	0.229026	0.317975	0.295359	0.184272	*0.000174
B	{0.01	0.179457		0.685876	0.668277	0.603454	0.889407	*0.000184
C	{0.1}	0.22902	0.685876		0.807799	0.830436	0.719318	*0.000160
D	{1}	0.317975	0.668277	0.807799		0.741479	0.746376	*0.000153
E	{10}	0.295359	0.603454	0.830436	0.741479		0.782497	*0.000198
F	{100}	0.184272	0.889407	0.719318	0.746376	0.782497		*0.000178
G	{500}	*.000174	*0.000184	*0.000160	*0.000153	*0.000198	*0.000178	

Newman-Keuls test; MN_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L} .5880000	{0.1 mg/L} .5510000	{1 mg/L} .5590000	{10 mg/L} .5160000	{100 mg/L} .4080000	{500 mg/L} .1960000
A	{0}		0.140421	0.15933	0.126527	0.075348	*0.002793	*0.000176
B	{0.01	0.140421		0.778482	0.602255	0.563562	*0.035118	*0.000194
C	{0.1}	0.15933	0.778482		0.885242	0.530292	*0.048943	*0.000247
D	{1}	0.126527	0.602255	0.885242		0.714563	0.063332	*0.000223
E	{10}	0.075348	0.563562	0.530292	0.714563		0.067046	*0.000274
F	{100}	*0.002793	*0.035118	*0.048943	0.063332	0.067046		*0.001744
G	{500}	*0.000176	*0.000194	*0.000247	*0.000223	*0.000274	*0.001744	

Newman-Keuls test; ZN_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L} .5950000	{0.1 mg/L} .6070000	{1 mg/L} .6100000	{10 mg/L} .5110000	{100 mg/L} .4930000	{500 mg/L} .3560000
A	{0}		0.456696	0.429114	0.242228	*0.047894	*0.034280	*0.000528
B	{0.01	0.456696		0.819531	0.954702	0.125858	0.154507	*0.002069
C	{0.1}	0.429114	0.819531		0.954547	0.186649	0.168362	*0.002054
D	{1}	0.242228	0.954702	0.954547		0.264383	0.212116	*0.002562
E	{10}	*0.047894	0.125858	0.186649	0.264383		0.732444	*0.024183
F	{100}	*0.034280	0.154507	0.168362	0.212116	0.732444		*0.018961
G	{500}	*0.000528	*0.002069	*0.002054	*0.002562	*0.024183	*0.018961	

Newman-Keuls test; AD_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L} .5940000	{0.1 mg/L} .5490000	{1 mg/L} .5000000	{10 mg/L} .4870000	{100 mg/L} .3260000	{500 mg/L} 0.000000
A	{0}		0.179726	0.102845	*0.035482	*0.034145	*0.000397	*0.000174
B	{0.01	0.179726		0.434623	0.24679	0.266701	*0.002336	*0.000158
C	{0.1}	0.102845	0.434623		0.395887	0.524636	*0.006642	*0.000151
D	{1}	*0.035482	0.24679	0.395887		0.819666	*0.019744	*0.000196
E	{10}	*0.034145	0.266701	0.524636	0.819666		*0.012295	*0.000181
F	{100}	*0.000397	*0.002336	*0.006642	*0.019744	*0.012295		*0.000210
G	{500}	*0.000174	*0.000158	*0.000151	*0.000196	*0.000181	*0.000210	

APPENDIX 25: ANOVA: *Chlorella vulgaris* UMACC 245: Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-CHL

Newman-Keuls test; BD_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L} .8740000	{0.1 mg/L} .8740000	{1 mg/L} .7840000	{10 mg/L} .7080000	{100 mg/L} .3270000	{500 mg/L} 0.000000
A	{0}		*0.000181	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01}	*0.000181		1	0.060614	*0.005660	*0.000196	*0.000150
C	{0.1}	*0.000176	1		0.138823	*0.010050	*0.000150	*0.000158
D	{1}	*0.000196	0.060614	0.138823		0.106797	*0.000180	*0.000196
E	{10}	*0.000150	*0.005660	*0.010050	0.106797		*0.000176	*0.000180
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176		*0.000177
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000177	

Newman-Keuls test; BD_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L} .5590000	{0.1 mg/L} .5610000	{1 mg/L} .5550000	{10 mg/L} .5410000	{100 mg/L} .4920000	{500 mg/L} 0.000000
A	{0}		0.099154	*0.045309	0.141271	0.125678	*0.030500	*0.000174
B	{0.01}	0.099154		0.969374	0.938614	0.933878	0.568723	*0.000150
C	{0.1}	*0.045309	0.969374		0.992453	0.978715	0.663957	*0.000158
D	{1}	0.141271	0.938614	0.992453		0.787546	0.452092	*0.000196
E	{10}	0.125678	0.933878	0.978715	0.787546		0.352474	*0.000180
F	{100}	*0.030500	0.568723	0.663957	0.452092	0.352474		*0.000176
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; MC_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L} .8740000	{0.1 mg/L} .8330000	{1 mg/L} .8590000	{10 mg/L} .7730000	{100 mg/L} .3130000	{500 mg/L} 0.000000
A	{0}		*0.000176	*0.000196	*0.000180	*0.000150	*0.000158	*0.000174
B	{0.01}	*0.000176		0.61521	0.731781	0.132368	*0.000150	*0.000158
C	{0.1}	*0.000196	0.615217		0.55408	0.183568	*0.000180	*0.000196
D	{1}	*0.000180	0.731781	0.55408		0.147405	*0.000196	*0.000150
E	{10}	*0.000150	0.132368	0.183568	0.147405		*0.000176	*0.000180
F	{100}	*0.000158	*0.000150	*0.000180	*0.000196	*0.000176		*0.000178
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000180	*0.000178	

Newman-Keuls test; MC_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L} .5610000	{0.1 mg/L} .5160000	{1 mg/L} .5130000	{10 mg/L} .4440000	{100 mg/L} .3390000	{500 mg/L} 0.000000
A	{0}		*0.049096	*0.023405	*0.036391	*0.004632	*0.000315	*0.000174
B	{0.01}	*0.049096		0.401151	0.634713	0.157198	*0.005918	*0.000158
C	{0.1}	*0.023405	0.401151		0.954869	0.374462	*0.019731	*0.000151
D	{1}	*0.036391	0.634713	0.954869		0.20547	*0.012531	*0.000196
E	{10}	*0.004632	0.157198	0.374462	0.205472		0.06294	*0.000181
F	{100}	*0.000315	*0.005918	*0.019731	*0.012531	0.06294		*0.000184
G	{500}	*0.000174	*0.000158	*0.000151	*0.000196	*0.000181	*0.000184	

Newman-Keuls test; DD_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L} 1.044000	{0.1 mg/L} 1.024000	{1 mg/L} 1.024000	{10 mg/L} .9970000	{100 mg/L} .9460000	{500 mg/L} .7490000
A	{0}		*0.000667	*0.001321	*0.000776	*0.000775	*0.000281	*0.000174
B	{0.01}	*0.000667		0.905392	0.676437	0.750836	0.277524	*0.000359
C	{0.1}	*0.001321	0.905392		1	0.574139	0.253511	*0.000390
D	{1}	*0.000776	0.676437	1		0.835175	0.377996	*0.000458
E	{10}	*0.000775	0.750836	0.574139	0.835175		0.295389	*0.000466
F	{100}	*0.000281	0.277524	0.253511	0.377996	0.295389		*0.001030
G	{500}	*0.000174	*0.000359	*0.000390	*0.000458	*0.000466	*0.001030	

Newman-Keuls test; DD_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L} .4820000	{0.1 mg/L} .5430000	{1 mg/L} .5450000	{10 mg/L} .4960000	{100 mg/L} .4800000	{500 mg/L} .3260000
A	{0}		*0.030386	0.087877	*0.039545	*0.032486	*0.038563	*0.000520
B	{0.01}	*0.030386		0.539922	0.684943	0.807529	0.972318	*0.037826
C	{0.1}	0.087877	0.539922		0.972318	0.418421	0.684943	*0.012917
D	{1}	*0.039545	0.684943	0.972318		0.667525	0.776372	*0.016590
E	{10}	*0.032486	0.807529	0.418421	0.667525		0.956762	*0.040899
F	{100}	*0.038563	0.972318	0.684943	0.776372	0.956762		*0.016329
G	{500}	*0.000520	*0.037826	*0.012917	*0.016590	*0.040899	*0.016329	

Newman-Keuls test; MA_C_D4 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.254000	{0.01 mg/L} 1.308000	{0.1 mg/L} 1.083000	{1 mg/L} .9680000	{10 mg/L} .9680000	{100 mg/L} .8820000	{500 mg/L} .6670000
A	{0}		0.269037	*0.002798	*0.00031	*0.000245	*0.000157	*0.000158
B	{0.01}	0.269037		*0.000911	*0.000180	*0.000209	*0.000159	*0.000174
C	{0.1}	*0.002798	*0.000911		0.067825	*0.028087	*0.003836	*0.000152
D	{1}	*0.000313	*0.000180	0.067825		1	0.088199	*0.000211
E	{10}	*0.000245	*0.000209	*0.028087	1		0.195032	*0.000260
F	{100}	*0.000157	*0.000159	*0.003836	0.088199	0.195032		
G	{500}	*0.000158	*0.000174	*0.000152	*0.000211	*0.000260	*0.000573	

Newman-Keuls test; MA_C_D10 (anova-chl-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .6730000	{0.01 mg/L} .5550000	{0.1 mg/L} .5380000	{1 mg/L} .5450000	{10 mg/L} .5150000	{100 mg/L} .4960000	{500 mg/L} .3260000
A	{0}		*0.033416	0.072692	0.055631	*0.046176	*0.031226	*0.000255
B	{0.01}	*0.033416		0.938596	0.84444	0.85325	0.762154	*0.004681
C	{0.1}	0.072692	0.938596		0.890765	0.652641	0.685166	*0.004156
D	{1}	0.055631	0.84444	0.890765		0.82238	0.76277	*0.004864
E	{10}	*0.046176	0.853254	0.652641	0.82238		0.709707	*0.005503
F	{100}	*0.031226	0.762154	0.685166	0.76277	0.709707		*0.004459
G	{500}	*0.000255	*0.004681	*0.004156	*0.004864	*0.005503	*0.004459	

APPENDIX 26: ANOVA: *Chlorella vulgaris* UMACC 245: Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-CHL

Newman-Keuls test; CD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	1.897800	2.361100	1.390730	.6162000	.4664000	.1196800
A	{0}		*0.000183	*0.000179	*0.000197	*0.000151	*0.000158	*0.000174
B	{0.01	*0.000183		0.500011	0.461125	0.170867	0.188409	0.112255
C	{0.1}	*0.000179	0.500011		0.343269	0.085593	0.083001	*0.044071
D	{1}	*0.000197	0.461125	0.343269		0.266376	0.376445	0.271854
E	{10}	*0.000151	0.170867	0.085593	0.266376		0.826141	0.743101
F	{100}	*0.000158	0.188409	0.083001	0.376445	0.826141		0.612435
G	{500}	*0.000174	0.112255	*0.044071	0.271854	0.743101	0.612435	

Newman-Keuls test; CD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	20.44070	21.24100	24.10710	25.86210	18.95600	3.775740
A	{0}		0.265974	0.350351	0.179968	*0.031348	0.054265	*0.000150
B	{0.01	0.265974		0.517579	*0.039016	*0.003933	0.23835	*0.000180
C	{0.1}	0.350351	0.517579		0.077413	*0.008840	0.176475	*0.000196
D	{1}	0.179968	*0.039016	0.077413		0.167503	*0.005908	*0.000158
E	{10}	*0.031348	*0.003933	*0.008840	0.167503		*0.000714	*0.000174
F	{100}	0.054265	0.23835	0.176475	*0.005908	*0.000714		*0.000176
G	{500}	*0.000150	*0.000180	*0.000196	*0.000158	*0.000174	*0.000176	

Newman-Keuls test; CO_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	2.482900	3.240500	3.394000	3.369600	1.325800	.1891400
A	{0}		*0.000274	*0.000727	*0.000320	*0.000566	*0.000166	*0.000174
B	{0.01	*0.000274		0.308454	0.594684	0.451881	0.128832	*0.016646
C	{0.1}	*0.000727	0.308454		0.97515	0.859733	*0.045313	*0.004030
D	{1}	*0.000320	0.594684	0.97515		0.973459	0.075566	*0.005693
E	{10}	*0.000566	0.451881	0.859733	0.973459		0.055316	*0.004388
F	{100}	*0.000166	0.128832	*0.045313	0.075566	0.055316		0.135169
G	{500}	*0.000174	*0.016646	*0.004030	*0.005693	*0.00438	0.135169	

Newman-Keuls test; CO_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	28.95680	30.87350	26.26310	21.82330	18.03380	.1119880
A	{0}		*0.000432	*0.000220	*0.007207	0.641551	*0.008159	*0.000196
B	{0.01	*0.000432		0.139676	*0.045087	*0.000401	*0.000151	*0.000158
C	{0.1}	*0.000220	0.139676		*0.005636	*0.000175	*0.000158	*0.000174
D	{1}	*0.007207	*0.045087	*0.005636		*0.007342	*0.000231	*0.000150
E	{10}	0.641551	*0.000401	*0.000175	*0.007342		*0.008041	*0.000180
F	{100}	*0.008159	*0.000151	*0.000158	*0.000231	*0.008041		*0.000176
G	{500}	*0.000196	*0.000158	*0.000174	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; CR_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	1.726700	1.542200	.8234000	.6060000	.1310000	.0174800
A	{0}		*0.000176	*0.000181	*0.000196	*0.000151	*0.000158	*0.000174
B	{0.01	*0.000176		0.775919	0.357066	0.330072	0.143691	0.139174
C	{0.1}	*0.000181	0.775919		0.277079	0.332797	0.165414	0.17239
D	{1}	*0.000196	0.357066	0.277079		0.73746	0.535703	0.596468
E	{10}	*0.000151	0.330072	0.332797	0.73746		0.46726	0.633354
F	{100}	*0.000158	0.143691	0.165414	0.535703	0.46726		0.86089
G	{500}	*0.000174	0.139174	0.17239	0.596468	0.633354	0.86089	

Newman-Keuls test; CR_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	26.49830	22.07420	18.05680	18.68670	12.58760	4.223900
A	{0}		*0.000733	0.726168	*0.001878	*0.003569	*0.000150	*0.000158
B	{0.01	*0.000733		*0.000949	*0.000151	*0.000197	*0.000158	*0.000174
C	{0.1}	0.726168	*0.000949		*0.001990	*0.002759	*0.000196(	*0.000150
D	{1}	*0.001878	*0.000151	*0.001990		0.508168	*0.000205	*0.000180
E	{10}	*0.003569	*0.000197	*0.002759	0.508168		*0.000203	*0.000196
F	{100}	*0.000150	*0.000158	*0.000196	*0.000205	*0.000203		*0.000176
G	{500}	*0.000158	*0.000174	*0.000150	*0.000180	*0.000196	*0.000176	

Newman-Keuls test; CU_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	.7948000	1.796900	.7075000	.5620000	.1730770	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000180		0.084843	0.874022	0.903413	0.66561	0.595507
C	{0.1}	*0.000176	0.084843		0.144693	0.148582	0.060988	*0.046025
D	{1}	*0.000196	0.874022	0.144693		0.791678	0.595229	0.571983
E	{10}	*0.000150	0.903413	0.148582	0.791678		0.483488	0.564721
F	{100}	*0.000158	0.66561	0.060988	0.595229	0.48348		0.753487
G	{500}	*0.000174	0.595507	*0.046025	0.571983	0.564721	0.753487	

Newman-Keuls test; CU_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	26.00880	19.40170	13.31540	12.77290	9.442200	2.159900
A	{0}		*0.003244	*0.010222	*0.000181	*0.000196	*0.000150	*0.000158
B	{0.01	*0.003244		*0.000205	*0.000196	*0.000150	*0.000158	*0.000174
C	{0.1}	*0.010222	*0.000205		*0.000199	*0.000204	*0.000196	*0.000150
D	{1}	*0.000181	*0.000196	*0.000199		0.599812	*0.004975	*0.000196
E	{10}	*0.000196	*0.000150	*0.000204	0.599812		*0.005441	*0.000180
F	{100}	*0.000150	*0.000158	*0.000196	*0.004975	*0.005441		*0.000178
G	{500}	*0.000158	*0.000174	*0.000150	*0.000196	*0.000180	*0.000178	

APPENDIX 26: ANOVA: *Chlorella vulgaris* UMACC 245: Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-CHL

Newman-Keuls test; FE_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	3.378400	3.507200	2.171900	1.607140	.1861700	0.000000
A	{0}		*0.000824	*0.000495	*0.000254	*0.000182	*0.000159	*0.000174
B	{0.01	*0.000824		0.866935	0.132049	0.081563	*0.004214	*0.004068
C	{0.1}	*0.000495	0.866935		0.215133	0.099997	*0.004664	*0.004132
D	{1}	*0.000254	0.132049	0.215133		0.466434	*0.048650	0.052535
E	{10}	*0.000182	0.081563	0.099997	0.466434		0.080557	0.119293
F	{100}	*0.000159	*0.004214	*0.004664	*0.048650	0.080557		0.808703
G	{500}	*0.000174	*0.004068	*0.004132	0.052535	0.119293	0.808703	

Newman-Keuls test; FE_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	22.19250	17.55320	19.54240	13.60250	13.82940	1.910920
A	{0}		0.841693	*0.002065	*0.040283	*0.000161	*0.000154	*0.000174
B	{0.01	0.841693		*0.001677	*0.024124	*0.000154	*0.000199	*0.000158
C	{0.1}	*0.002065	*0.001677		0.078372	*0.005583	*0.003307	*0.000196
D	{1}	*0.040283	*0.024124	0.078372		*0.000474	*0.000387	*0.000150
E	{10}	*0.000161	*0.000154	*0.005583	*0.0004742		0.831676	*0.000176
F	{100}	*0.000154	*0.000199	*0.003307	*0.000387	0.831676		*0.000180
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000176	*0.000180	

Newman-Keuls test; MN_C_C4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	2.145500	1.367200	.1786000	.1576600	.0534200	.0147850
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.171674	*0.007161	*0.011793	*0.012383	*0.014890
C	{0.1}	*0.000180	0.171674		*0.045156	0.098927	0.116197	0.145401
D	{1}	*0.000196	*0.007161	*0.045156		0.96976	0.970958	0.989949
E	{10}	*0.000150	*0.011793	0.098927	0.96976		0.849838	0.96235
F	{100}	*0.000158	*0.012383	0.116197	0.970958	0.849838		0.944074
G	{500}	*0.000174	*0.014890	0.145401	0.989949	0.96235	0.944074	

Newman-Keuls test; MN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	27.31990	28.28660	23.42900	19.88790	12.35750	4.507740
A	{0}		*0.002517	*0.001087	0.396505	*0.049365	*0.000181	*0.000196
B	{0.01	*0.002517		0.422483	*0.005135	*0.000268	*0.000150	*0.000158
C	{0.1}	*0.001087	0.42248		*0.002747	*0.000183	*0.000158	*0.000174
D	{1}	0.396505	*0.005135	*0.002747		*0.023145	*0.000196	*0.000150
E	{10}	*0.049365	*0.000268	*0.000183	*0.023145		*0.000186	*0.000180
F	{100}	*0.000181	*0.000150	*0.000158	*0.000196	*0.000186		*0.000182
G	{500}	*0.000196	*0.000158	*0.000174	*0.000150	*0.000180	*0.000182	

Newman-Keuls test; ZN_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	1.193300	1.209700	1.036600	.6477000	.5849000	.0096900
A	{0}		*0.000180	*0.000176	*0.000196	*0.000151	*0.000158	*0.000174
B	{0.01	*0.000180		0.97998	0.809852	0.676669	0.777602	0.384032
C	{0.1}	*0.000176	0.97998		0.960508	0.815146	0.860553	0.452254
D	{1}	*0.000196	0.809852	0.960508		0.552432	0.763302	0.405773
E	{10}	*0.000151	0.676669	0.815146	0.552432		0.923173	0.589477
F	{100}	*0.000158	0.777602	0.860553	0.763302	0.923173		0.383145
G	{500}	*0.000174	0.384032	0.452254	0.405773	0.589477	0.383145	

Newman-Keuls test; ZN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	24.40240	20.41480	19.40020	11.26100	7.328800	1.039580
A	{0}		*0.049228	*0.049803	*0.015357	*0.000196	*0.000150	*0.000158
B	{0.01	*0.049228		*0.002104	*0.000649	*0.000150	*0.000158	*0.000174
C	{0.1}	*0.049803	*0.002104		0.292212	*0.000180	*0.000196	*0.000150
D	{1}	*0.015357	*0.000649	0.292212		*0.000176	*0.000180	*0.000196
E	{10}	*0.000196	*0.000150	*0.000180	*0.000176		*0.000959	*0.000180
F	{100}	*0.000150	*0.000158	*0.000196	*0.000180	*0.000959		*0.000181
G	{500}	*0.000158	*0.000174	*0.000150	*0.000196	*0.000180	*0.000181	

Newman-Keuls test; AD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	.3348000	.3099000	.2586000	.2388000	.0147060	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.964086	0.989232	0.997988	0.974244	0.987794
C	{0.1}	*0.000180	0.964086		0.925966	0.990632	0.946373	0.977098
D	{1}	*0.000196	0.989232	0.925965		0.971476	0.895053	0.962849
E	{10}	*0.000150	0.997987	0.990632	0.971476		0.685337	0.89913
F	{100}	*0.000158	0.974244	0.946372	0.895053	0.685337		0.978825
G	{500}	*0.000174	0.987794	0.977098	0.962849	0.899131	0.978825	

Newman-Keuls test; AD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	33.23030	35.83470	30.06810	28.99480	0.000000	0.000000
A	{0}		*0.000198	*0.000151	*0.000299	*0.000368	*0.000180	*0.000176
B	{0.01	*0.000198		0.070865	*0.032525	*0.017294	*0.000158	*0.000150
C	{0.1}	*0.000151	0.070865		*0.001997	*0.000925	*0.000174	*0.000158
D	{1}	*0.000299	*0.032525	*0.001997		0.433863	*0.000150	*0.000196
E	{10}	*0.000368	*0.017294	*0.000925	0.433863		*0.000196	*0.00018
F	{100}	*0.000180	*0.000158	*0.000174	*0.000150	*0.000196		1
G	{500}	*0.000176	*0.000150	*0.000158	*0.000196	*0.000180	1	

APPENDIX 26: ANOVA: *Chlorella vulgaris* UMACC 245: Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-CHL

Newman-Keuls test; BD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	.8930000	.4845000	.2697000	.0795450	0.000000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000174	*0.000158
B	{0.01}	*0.000176		0.462269	0.498663	0.460228	0.58053	0.490804
C	{0.1}	*0.000180	0.462269		0.697058	0.738881	0.893443	0.80671
D	{1}	*0.000196	0.498663	0.697058		0.730231	0.957976	0.872923
E	{10}	*0.000150	0.460228	0.738881	0.730231		0.988223	0.885169
F	{100}	*0.000174	0.58053	0.893443	0.957976	0.988223		1
G	{500}	*0.000158	0.490804	0.806711	0.872924	0.88517	1	

Newman-Keuls test; BD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	20.83330	26.68900	19.35730	2.828210	0.000000	0.000000
A	{0}		0.084012	*0.000327	*0.007642	*0.000196	*0.000158	*0.000150
B	{0.01}	0.084012		*0.000191	0.102681	*0.000180	*0.000150	*0.000196
C	{0.1}	*0.000327	*0.000191986560821		*0.000196	*0.000150	*0.000174	*0.000158
D	{1}	*0.007642	0.102681	*0.000196814537		*0.000176	*0.000196	*0.000180
E	{10}	*0.000196	*0.000180	*0.000150	*0.000176681671142		*0.012584	*0.004941
F	{100}	*0.000158	*0.000150	*0.000174	*0.000196	*0.012584478607178		1
G	{500}	*0.000150	*0.000196	*0.000158	*0.000180	*0.004941	1	

Newman-Keuls test; MC_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	2.146500	3.070200	4.985500	2.765600	.5067600	0.000000
A	{0}		*0.000192	*0.000312	*0.010634	*0.000305	*0.000158	*0.000174
B	{0.01}	*0.000192		0.40563	*0.005702	0.38994	*0.034039	*0.021069
C	{0.1}	*0.000312	0.40563		*0.015922	0.669129	*0.011918	*0.004687
D	{1}	*0.010634	*0.005702	*0.015922665596008		*0.017236	*0.000262	*0.000198
E	{10}	*0.000305	0.38994	0.669129	*0.017236709594726		*0.015492	*0.006937
F	{100}	*0.000158	*0.034039	*0.011918	*0.000262	*0.015492677685898		0.479655
G	{500}	*0.000174	*0.021069	*0.004687	*0.000198	*0.006937	0.479655	

Newman-Keuls test; MC_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	29.24840	42.72150	23.53990	18.23240	0.000000	0.000000
A	{0}		*0.000279	*0.000196	0.348394	*0.003217	*0.000196	*0.000180
B	{0.01}	*0.000279843807220		*0.000176	*0.000394	*0.000196	*0.000158	*0.000150
C	{0.1}	*0.000196	*0.000176668167114		*0.000180	*0.000150	*0.000174	*0.000158
D	{1}	0.348394	*0.000394	*0.000180900096893		*0.001380	*0.000150	*0.000196
E	{10}	*0.003217	*0.000196	*0.000150	*0.001380205154418		*0.000180	*0.000176
F	{100}	*0.000196	*0.000158	*0.000174	*0.000150	*0.000180900096893		1
G	{500}	*0.000180	*0.000150	*0.000158	*0.000196	*0.000176	1	

Newman-Keuls test; DD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	1.360900	1.539200	1.556400	1.432800	.5231000	.2678600
A	{0}		*0.000151	*0.000181	*0.000176	*0.000196	*0.000158	*0.000174
B	{0.01}	*0.000151515007019		0.95525	0.988563	0.908998	0.196092	0.214878
C	{0.1}	*0.000181	0.95525		0.978268	0.865669	0.385977	0.289259
D	{1}	*0.000176	0.988563	0.978268		0.978244	0.478603	0.346181
E	{10}	*0.000196	0.908998	0.865669	0.978244		0.332314	0.276718
F	{100}	*0.000158	0.196092	0.385977	0.478603	0.332314		0.685492
G	{500}	*0.000174	0.214879	0.289259	0.346182	0.276719	0.685492	

Newman-Keuls test; DD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	3.864200	7.018000	8.125900	4.306000	3.527000	.3578000
A	{0}		*0.000150	*0.000180	*0.000176	*0.000196	*0.000158	*0.000174
B	{0.01}	*0.000150978565216		*0.000285	*0.000199	0.428321	0.54356	*0.000208
C	{0.1}	*0.000180	*0.000285327434539		0.060106	*0.000346	*0.000256	*0.000150
D	{1}	*0.000176	*0.000199	0.060106		*0.000189	*0.000153	*0.000158
E	{10}	*0.000196	0.428321	*0.000346	*0.000189602375030		0.348871	*0.000207
F	{100}	*0.000158	0.54356	*0.000256	*0.000153	0.348871		*0.000208
G	{500}	*0.000174	*0.000208	*0.000150	*0.000158	*0.000207	*0.000208	

Newman-Keuls test; MA_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		7.045500	3.286900	2.221310	1.625000	1.556400	.8502000	.2272700
A	{0}		*0.000192	*0.000182	*0.000196	*0.000151	*0.000158	*0.000174
B	{0.01}	*0.000192940235137		0.101235	*0.040103	0.055511	*0.009574	*0.002105
C	{0.1}	*0.000182	0.101235		0.342851	0.532555	0.155657	*0.036876
D	{1}	*0.000196	*0.040103	0.342851		0.91177	0.430952	0.144792
E	{10}	*0.000151	0.055511	0.532555	0.91177		0.264356	0.10802
F	{100}	*0.000158	*0.009574	0.155657	0.430952	0.264356		0.32243
G	{500}	*0.000174	*0.002105	*0.036876	0.144793	0.108021	0.322439	

Newman-Keuls test; MA_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		22.40570	4.916700	5.421000	10.01890	4.079500	3.465300	.3170300
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000158	*0.000174
B	{0.01}	*0.000196039676666		0.368874	*0.000180	0.145495	*0.045184	*0.000197
C	{0.1}	*0.000180	0.368874		*0.000176	0.065507	*0.013666	*0.000151
D	{1}	*0.000176	*0.000180	*0.000176846981048		*0.000196	*0.000150	*0.000158
E	{10}	*0.000150	0.145495	0.065507	*0.000196039676666		0.277053	*0.000191
F	{100}	*0.000158	*0.045184	*0.013666	*0.000150	0.277053		*0.000212
G	{500}	*0.000174	*0.000197	*0.000151	*0.000158	*0.000191	*0.000212	

APPENDIX 26: ANOVA: *Chlorella vulgaris* UMACC 245: Biochemical composition (Protein)

* = Significant difference : p<0.05

ANOVA-PRO-CHL

Newman-Keuls test; CD_C_D4 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	26.23300	26.37900	31.69000	33.54200	25.76600	5.090600
A	{0}		0.379844	0.203926	0.846734	0.749906	0.458079	*0.0001730918884277
B	{0.01	0.379844		0.967162	0.42449	0.270983	0.895022	*0.0002458095550537
C	{0.1}	0.203926	0.967162		0.307495	0.2125	0.983054	*0.0003058314323425
D	{1}	0.846734	0.42449	0.307495		0.602147	0.461007	*0.0001733303070068
E	{10}	0.749906	0.270983	0.2125	0.602147		0.280089	*0.000181376934
F	{100}	0.458079	0.895022	0.983054	0.461007	0.280089		*0.0002025961875915
G	{500}	*0.000173(	*0.0002456	*0.000305(	*0.000173:	*0.000181:	*0.00020259618759155	

Newman-Keuls test; CD_C_D10 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	59.00800	55.91800	45.34400	32.13300	20.35800	.1103480
A	{0}		0.448159	0.37998	*0.019699-	*0.000415:	*0.000163:	*0.000174045562744141
B	{0.01	0.448159		0.562031	*0.049202(	*0.000885(	*0.000174(	*0.0001581907272
C	{0.1}	0.37998	0.562031		0.061576	*0.001308:	*0.000224:	*0.000150978565216064
D	{1}	*0.019699-	*0.049202(	0.061576		*0.023703:	*0.000901:	*0.00019681453704834
E	{10}	*0.000415:	*0.000885(	*0.001308:	*0.023703336715698:		*0.040098-	*0.00023883581161499
F	{100}	*0.000163:	*0.000174(	*0.000224:	*0.000901:	*0.040098428726196:		*0.00177007913589478
G	{500}	*0.000174(	*0.000158:	*0.000150:	*0.000196(	*0.000238(	*0.00177007913589478	

Newman-Keuls test; CO_C_D4 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	32.52200	36.30200	32.86700	31.35300	25.69000	10.99400
A	{0}		0.932741	0.72634	0.970978	0.936214	0.231785	*0.0010511875152587
B	{0.01	0.932741		0.655773	0.936581	0.787493	0.40637	*0.0014760494232177
C	{0.1}	0.72634	0.655773		0.432817	0.658048	0.190283	*0.0006718039512634
D	{1}	0.970978	0.936581	0.432817		0.932911	0.471254	*0.0017658472061157
E	{10}	0.936214	0.787493	0.658048	0.932911		0.401666	*0.0015937685966491
F	{100}	0.231785	0.40637	0.190283	0.471254	0.401666		*0.0040110945701599
G	{500}	*0.001051:	*0.001476(	*0.000671(	*0.001765(	*0.001593:	*0.00401109457015991	

Newman-Keuls test; CO_C_D10 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	56.09900	62.10600	52.68900	49.07600	36.46400	4.139800
A	{0}		0.613482	0.896679	0.503332	0.348976	*0.025055-	*0.000182867050170898
B	{0.01	0.613482		0.422408	0.646203	0.608995	0.072461	*0.0001848340034484 86
C	{0.1}	0.896679	0.422408		0.420331	0.316786	*0.023469:	*0.000166416168212891
D	{1}	0.503332	0.646203	0.420331		0.626852	0.100057	*0.000234901905059814
E	{10}	0.348976	0.608995	0.316786	0.626852		0.104663	*0.000236153602600098
F	{100}	*0.025055-	0.072461	*0.023469:	0.100057	0.104663		*0.000695705413818359
G	{500}	*0.000182:	*0.000184:	*0.000166-	*0.000234:	*0.000236:	*0.000695705413818359	

Newman-Keuls test; CR_C_D4 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	27.77800	31.16900	28.60900	29.04600	20.83100	11.10200
A	{0}		0.733125	0.959222	0.728279	0.540441	*0.038634:	*0.0003312230110168
B	{0.01	0.733125		0.810774	0.794131	0.913735	*0.043171:	*0.0004387497901916
C	{0.1}	0.959222	0.810774		0.8442	0.77883	*0.047376(	*0.0003862977027893
D	{1}	0.728279	0.794131	0.8442		0.890826	0.063166	*0.0005082488059997
E	{10}	0.540441	0.913735	0.77883	0.890826		0.082303	*0.0005344748497009
F	{100}	*0.038634:	*0.043171:	*0.047376(	0.063166	0.082303		*0.0077421665191650
G	{500}	*0.000331:	*0.000438:	*0.000386:	*0.000508:	*0.000534-	*0.00774216651916504	

Newman-Keuls test; CR_C_D10 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	58.20700	54.18200	48.80700	43.48100	31.01800	.1339000
A	{0}		0.387499	0.265754	0.084246	*0.020760-	*0.000578(	*0.000174045562744141
B	{0.01	0.387499		0.472384	0.230684	0.072375	*0.001664(	*0.000158190727233887
C	{0.1}	0.265754	0.472384		0.340702	0.157895	*0.004074(	*0.00015103816986084
D	{1}	0.084246	0.230684	0.340702		0.344977	*0.014714:	*0.000196456909179687
E	{10}	*0.020760-	0.072375	0.157895	0.344977		*0.038355(	*0.000182271003723145
F	{100}	*0.000578(	*0.001664(	*0.004074(	*0.014714:	*0.038355827331543		*0.000222682952880859
G	{500}	*0.000174(	*0.000158:	*0.000151(	*0.000196-	*0.000182:	*0.000222682952880859	

Newman-Keuls test; CU_C_D4 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	27.36000	29.63000	29.81600	40.58200	26.69520	6.949900
A	{0}		0.762091	0.927625	0.753568	*0.022049:	0.77271	*0.0003029108047485
B	{0.01	0.762091		0.551088	0.789322	*0.022198(	0.860657	*0.0003736615180969
C	{0.1}	0.927625	0.551088		0.960895	*0.046400:	0.715073	*0.0003119111061096
D	{1}	0.753568	0.789322	0.960895		*0.029620(	0.834663	*0.0003300309181213
E	{10}	*0.022049:	*0.022198(	*0.046400:	*0.029620051383972:		*0.021777:	*0.0001754164695739
F	{100}	0.77271	0.860657	0.715073	0.834663	*0.021777212619781		*0.0002694725990295
G	{500}	*0.000302:	*0.000373(	*0.000311:	*0.000330(	*0.000175-	*0.00026947259902954 1	

Newman-Keuls test; CU_C_D10 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	67.53500	64.42000	42.14500	31.33000	13.06250	.3808200
A	{0}		0.719171	0.816202	*0.002685:	*0.000350:	*0.000196:	*0.000150978565216064
B	{0.01	0.719171		0.593734	*0.002849:	*0.000278:	*0.000158-	*0.000174045562744141
C	{0.1}	0.816202	0.593734		*0.004352:	*0.000412-	*0.000151:	*0.000158190727233887
D	{1}	0.002685	0.00285	0.004352		0.07892	*0.000590:	*0.000207245349884033
E	{10}	*0.000350:	*0.000278:	*0.000412-	0.07892		*0.006547(	*0.000404240036010 74
F	{100}	*0.000196:	*0.000158-	*0.000151:	*0.000590:	*0.006547033786773(		*0.0433059334754944
G	{500}	*0.000150:	*0.000174(	*0.000158:	*0.000207:	*0.000400-	*0.0433059334754944	

APPENDIX 26: ANOVA: *Chlorella vulgaris* UMACC 245: Biochemical composition (Protein)

* = Significant difference : p<0.05

ANOVA-PRO-CHL

Newman-Keuls test; FE_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	32.03200	34.31900	29.67600	27.56600	25.37400	2.120200
A	{0}		0.773656	0.620592	0.709541	0.598709	0.404514	*0.0001542568206787
B	{0.01}	0.773656		0.523875	0.782312	0.591328	0.359362	*0.0001608133316040
C	{0.1}	0.620592	0.523875		0.561571	0.346487	0.17249	*0.0001750588417053
D	{1}	0.709541	0.782312	0.561571		0.556057	0.455801	*0.0001997351646423
E	{10}	0.598709	0.591328	0.346487	0.556057		0.541015	*0.0001865625381469
F	{100}	0.404514	0.359362	0.17249	0.455801	0.541015		*0.0001828670501708
G	{500}	*0.0001542	*0.0001608	*0.0001750	*0.0001997	*0.0001865	*0.0001828670501708	

Newman-Keuls test; FE_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	56.01100	55.55600	50.77100	43.86000	25.49400	2.779900
A	{0}		0.274844	0.46692	0.241189	0.0521	*0.0004698	*0.000174462795257568
B	{0.01}	0.274844		0.942705	0.682843	0.249863	*0.0018875	*0.000160574913024902
C	{0.1}	0.46692	0.942705		0.453803	0.179928	*0.0014584	*0.000153064727783203
D	{1}	0.241189	0.682843	0.453803		0.284472	*0.0031906	*0.000200927257537842
E	{10}	0.0521	0.249863	0.179928	0.284472		*0.0105288	*0.000201582908630371
F	{100}	*0.0004698	*0.0018875	*0.0014584	*0.0031906	*0.0105288624763485		*0.00273096561431885
G	{500}	*0.0001744	*0.0001605	*0.0001536	*0.0002005	*0.0002015	*0.00273096561431885	

Newman-Keuls test; MN_C_C4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	35.43400	37.09500	34.02100	32.77600	20.58200	5.097600
A	{0}		0.726256	0.615007	0.760505	0.682333	*0.0275202	*0.0002426505088806
B	{0.01}	0.726256		0.700825	0.743622	0.80755	*0.0243892	*0.0001970529556274
C	{0.1}	0.615007	0.700825		0.752495	0.740715	*0.0161585	*0.0001994371414184
D	{1}	0.760505	0.743622	0.752495		0.773126	*0.0305095	*0.0002068281173706
E	{10}	0.682333	0.80755	0.740715	0.773126		*0.0306225	*0.0002470612525939
F	{100}	*0.0275202	*0.0243892	*0.0161585	*0.0305095	*0.0306229591369625		*0.0027364492416381
G	{500}	*0.0002426	*0.0001970	*0.0001994	*0.0002068	*0.0002470	*0.00273644924163818	

Newman-Keuls test; MN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	62.31600	56.11400	56.73900	53.55800	35.21500	1.073420
A	{0}		0.914829	0.747305	0.638456	0.649801	*0.0125423	*0.000175595283508301
B	{0.01}	0.914829		0.64957	0.432373	0.595508	*0.0111622	*0.000159561634063721
C	{0.1}	0.747305	0.64957		0.929178	0.716574	*0.0230334	*0.000199079513549805
D	{1}	0.638456	0.432373	0.929178		0.890289	*0.0337075	*0.000155568122863777
E	{10}	0.649801	0.595508	0.716574	0.890289		*0.0188178	*0.000183761119842529
F	{100}	*0.0125423	*0.0111622	*0.0230334	*0.0337075	*0.0188178420066833		*0.000367820262908936
G	{500}	*0.0001755	*0.0001595	*0.0001996	*0.0001555	*0.0001837	*0.000367820262908936	

Newman-Keuls test; ZN_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	24.43700	25.44800	23.23400	24.60200	21.78300	18.31800
A	{0}		0.155388	0.076753	0.109505	0.105533	0.060714	*0.0090376138687133
B	{0.01}	0.155388		0.935918	0.685462	0.955651	0.64147	0.19931
C	{0.1}	0.076753	0.935918		0.87036	0.775477	0.718016	0.203762
D	{1}	0.109505	0.685462	0.87036		0.886163	0.625642	0.243163
E	{10}	0.105533	0.955651	0.775477	0.886163		0.768579	0.249673
F	{100}	0.060714	0.64147	0.718016	0.625642	0.768579		0.253318
G	{500}	*0.0090376	0.19931	0.203762	0.243163	0.249673	0.253318	

Newman-Keuls test; ZN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	53.04700	46.96700	46.73100	40.32400	23.20500	2.460500
A	{0}		0.089448	*0.0277141	*0.0440748	*0.0075082	*0.0001906	*0.000174105167388916
B	{0.01}	0.089448		0.286808	0.500432	0.141035	*0.0008295	*0.000158846378326416
C	{0.1}	*0.0277141	0.286808		0.966427	0.466844	*0.0035451	*0.000155329704284668
D	{1}	*0.0440748	0.500432	0.966427		0.262744	*0.0021677	*0.000198602676391602
E	{10}	*0.0075082	0.141035	0.466844	0.262744		*0.0076995	*0.000192761421203613
F	{100}	*0.0001906	*0.0008295	*0.0035451	*0.0021677	*0.0076995491981506		*0.00218969583511353
G	{500}	*0.0001741	*0.0001588	*0.0001555	*0.0001986	*0.0001927	*0.00218969583511353	

Newman-Keuls test; AD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	42.59300	39.18000	32.64400	30.86800	4.002500	0.000000
A	{0}		*0.0440299	0.12585	0.68041	0.972348	*0.0001919	*0.000199079513549
B	{0.01}	*0.0440299510955811		0.395555	0.056108	0.060366	*0.0001583	*0.000174105167388
C	{0.1}	0.12585	0.395555		0.115421	0.1898	*0.0001516	*0.000158309936523
D	{1}	0.68041	0.056108	0.115421		0.892507	*0.0002064	*0.000153601169586
E	{10}	0.972348	0.060366	0.1898	0.892507		*0.0001803	*0.000182271003723
F	{100}	*0.0001919	*0.0001583	*0.0001516	*0.0002064	*0.0001803636550903		0.321383
G	{500}	*0.0001996	*0.0001741	*0.0001583	*0.0001536	*0.0001822	0.321383	

Newman-Keuls test; AD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	51.74500	51.69000	46.09100	25.24300	0.000000	0.000000
A	{0}		*0.0012943	*0.0031124	*0.0003205	*0.0001505	*0.0001746	*0.000158190727233887
B	{0.01}	*0.0012943148612976		0.984631	0.141576	*0.0001961	*0.0001583	*0.000150978565216064
C	{0.1}	*0.0031124	0.984631		0.0642	*0.0001805	*0.0001505	*0.00019603967666626
D	{1}	*0.0003205	0.141576	0.0642		*0.0001778	*0.0001961	*0.000180900096893311
E	{10}	*0.0001505	*0.0001961	*0.0001805	*0.0001778006553645		*0.0001805	*0.000176787376403809
F	{100}	*0.0001746	*0.0001583	*0.0001505	*0.0001961	*0.0001805959701538		1
G	{500}	*0.0001583	*0.0001505	*0.0001961	*0.0001805	*0.0001767	1	

APPENDIX 26: ANOVA: *Chlorella vulgaris* UMACC 245: Biochemical composition (Protein)

* = Significant difference : p<0.05

ANOVA-PRO-CHL

Newman-Keuls test; BD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	33.70400	31.76700	28.64200	18.68700	3.317500	0.000000
A	{0}		0.676969	0.813296	0.466909	*0.004403	*0.000196	*0.0001511573791503
B	{0.01}	0.676969		0.549835	0.408884	*0.002503	*0.000158	*0.0001741051673889
C	{0.1}	0.813296	0.549835		0.595518	*0.005020	*0.000151	*0.0001583099365234
D	{1}	0.466909	0.408884	0.595518		*0.007238	*0.000182	*0.0001964569091796
E	{10}	*0.004403	*0.002503	*0.005020	*0.007238388061523		*0.000403	*0.0002697110176086
F	{100}	*0.000196	*0.000158	*0.000151	*0.000182	*0.000403583049774		0.311665
G	{500}	*0.000151	*0.000174	*0.000158	*0.000196	*0.000269	0.311665	

Newman-Keuls test; BD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	57.92300	51.87500	52.10700	31.07400	0.000000	0.000000
A	{0}		0.321517	0.161313	0.107837	*0.000268	*0.000174	*0.000158190727233887
B	{0.01}	0.321517		0.467847	0.264767	*0.000676	*0.000158	*0.000150978565216064
C	{0.1}	0.161313	0.467847		0.963803	*0.001108	*0.000196	*0.000180900096893311
D	{1}	0.107837	0.264767	0.963803		*0.002516	*0.000150	*0.00019603967666626
E	{10}	*0.000268	*0.000676	*0.001108	*0.002516031265258		*0.000233	*0.000192344188690186
F	{100}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000233948230743		1
G	{500}	*0.000158	*0.000150	*0.000180	*0.000196	*0.000192	1	

Newman-Keuls test; MC_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	24.35500	27.72400	27.23200	19.58300	3.228200	0.000000
A	{0}		0.130167	0.262676	0.396324	*0.008713	*0.000158	*0.0001741051673889
B	{0.01}	0.130167		0.473462	0.323632	0.111865	*0.000184	*0.0001968145370483
C	{0.1}	0.262676	0.473462		0.863707	0.051084	*0.000152	*0.0001583099365234
D	{1}	0.396324	0.323632	0.863707		*0.041349	*0.000197	*0.0001511573791503
E	{10}	*0.008713	0.111865	0.051084	*0.041349232196807		*0.000210	*0.0001913309097296
F	{100}	*0.000158	*0.000184	*0.000152	*0.000197	*0.000210881233215		0.270236
G	{500}	*0.000174	*0.000196	*0.000158	*0.000151	*0.000191	0.270236	

Newman-Keuls test; MC_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	46.71500	52.19900	42.59700	20.74200	0.000000	0.000000
A	{0}		*0.012448	*0.042881	*0.004503	*0.000152	*0.000174	*0.000158190727233887
B	{0.01}	*0.012448668479919		0.279883	0.412864	*0.000446	*0.000151	*0.000196158885955811
C	{0.1}	*0.042881	0.279883		0.156752	*0.000256	*0.000158	*0.000150978565216064
D	{1}	*0.004503	0.412864	0.156752		*0.000663	*0.000196	*0.000181198120117187
E	{10}	*0.000152	*0.000446	*0.000256	*0.000663697719573		*0.002299	*0.000944674015045166
F	{100}	*0.000174	*0.000151	*0.000158	*0.000196	*0.002299249172210		1
G	{500}	*0.000158	*0.000196	*0.000150	*0.000181	*0.000944	1	

Newman-Keuls test; DD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	15.38200	14.46500	15.14100	15.35200	5.987000	2.430700
A	{0}		*0.000177	*0.000157	*0.000203	*0.000184	*0.000158	*0.0001740455627441
B	{0.01}	*0.000177919864654		0.971174	0.992837	0.988918	*0.004116	*0.0004131793975830
C	{0.1}	*0.000157	0.971174		0.752411	0.907066	*0.001368	*0.0003051161766052
D	{1}	*0.000203	0.992837	0.752411		0.92151	*0.001900	*0.0003364086151123
E	{10}	*0.000184	0.988918	0.907066	0.92151		*0.002807	*0.0003308057785034
F	{100}	*0.000158	*0.004116	*0.001368	*0.001900	*0.002807557582855		0.112616
G	{500}	*0.000174	*0.000413	*0.000305	*0.000336	*0.000330	0.112616	

Newman-Keuls test; DD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	20.02900	21.01200	21.35300	13.51600	2.126000	.2285700
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000196039676666		0.751729	0.901811	0.050671	*0.000275	*0.000250577926635742
C	{0.1}	*0.000180	0.751729		0.912543	0.066611	*0.000292	*0.000207364559173584
D	{1}	*0.000176	0.901811	0.912543		0.091026	*0.000286	*0.000223815441131592
E	{10}	*0.000150	0.050671	0.066611	0.091026		*0.002349	*0.00188523530960083
F	{100}	*0.000158	*0.000275	*0.000292	*0.000286	*0.002349376678466		0.543365
G	{500}	*0.000174	*0.000250	*0.000207	*0.000223	*0.001885	0.543365	

Newman-Keuls test; MA_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		31.00600	15.87700	13.13900	13.37400	12.95900	4.440000	2.326400
A	{0}		*0.002194	*0.002796	*0.001760	*0.003877	*0.000268	*0.0002278685569763
B	{0.01}	*0.002194344997406		0.776689	0.542144	0.884067	0.079673	*0.041545569896698
C	{0.1}	*0.002796	0.776689		0.954129	0.964901	0.111191	0.072765
D	{1}	*0.001760	0.542144	0.954129		0.994183	0.162738	0.094423
E	{10}	*0.003877	0.884067	0.964901	0.994183		0.051769	*0.0467370748519897
F	{100}	*0.000268	0.079673	0.111191	0.162738	0.051769		0.606044
G	{500}	*0.000227	*0.041545	0.072765	0.094423	*0.046737	0.606044	

Newman-Keuls test; MA_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		63.06800	18.93800	18.81200	21.00500	15.81300	2.521700	.2260400
A	{0}		*0.000180	*0.000196	*0.000176	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000180900096893		0.973159	0.58123	0.676881	*0.002678	*0.00136035680770874
C	{0.1}	*0.000196	0.973159		0.822821	0.426358	*0.001608	*0.00100725889205933
D	{1}	*0.000176	0.58123	0.822821		0.508805	*0.001505	*0.000768423080444336
E	{10}	*0.000150	0.676881	0.426358	0.508805		*0.002869	*0.00227111577987671
F	{100}	*0.000158	*0.002678	*0.001608	*0.001505	*0.002869427204132		0.540664
G	{500}	*0.000174	*0.001360	*0.001007	*0.000768	*0.002271	0.540664	

APPENDIX 26: ANOVA: *Chlorella vulgaris* UMACC 245: Biochemical composition (Lipid)

* = Significant difference : p<0.05

ANOVA-LIP-CHL

Newman-Keuls test; CD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	5.505560	2.637900	1.619700	.3029850	.2414890	0.000000
A	{0}		*0.004266(	*0.000813(	*0.000188	*0.000196(	*0.000150	*0.0001581907272338
B	{0.01	*0.0042666196832126		*0.000182	*0.000196(	*0.000150	*0.000158	*0.0001740455627441
C	{0.1}	*0.000813(	*0.000182867050170		*0.015518	*0.000217	*0.000251	*0.0001851320266723
D	{1}	*0.000188	*0.000196(	*0.015518367290496		*0.003236	*0.006004	*0.0031819343566894
E	{10}	*0.000196(	*0.000150	*0.000217	*0.003236293792724		0.870151	0.696635
F	{100}	*0.000150	*0.000158	*0.000251	*0.006004	0.870151		0.523619
G	{500}	*0.000158	*0.000174(	*0.000185	*0.003181	0.696635	0.523619	

Newman-Keuls test; CD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	7.220340	7.826980	3.011900	1.310340	.2610100	0.000000
A	{0}		*0.000183	*0.000181	*0.000196(	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.0001835823059082		0.401757	*0.000200	*0.000181	*0.000196(	*0.000150978565216064
C	{0.1}	*0.000181	0.401757		*0.000193	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000196(	*0.000200	*0.000193595886230		*0.029477	*0.004203	*0.00376862287521362
E	{10}	*0.000150	*0.000181	*0.000196	*0.029477894306182		0.156896	0.184443
F	{100}	*0.000158	*0.000196(	*0.000150	*0.004203	0.156896		0.715439
G	{500}	*0.000174(	*0.000150	*0.000158	*0.003768	0.184443	0.715439	

Newman-Keuls test; CO_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	.8778600	.7947000	1.295650	3.454550	.2170000	0.000000
A	{0}		*0.000231(	*0.000200	*0.000272	0.137379	*0.000165	*0.0001781582832336
B	{0.01	*0.0002310872077941		0.870269	0.417132	*0.000545(	0.40625	0.33293
C	{0.1}	*0.000200	0.870269		0.58717	*0.000712	0.266927	0.281803
D	{1}	*0.000272	0.417132	0.58717		*0.000845(	0.182648	0.125202
E	{10}	0.137379	*0.000545(	*0.000712	*0.000845074653625		*0.000251	*0.0002261400222778
F	{100}	*0.000165	0.40625	0.266927	0.182648	*0.000251770019531		0.670722
G	{500}	*0.000178	0.33293	0.281803	0.125202	*0.000226	0.670722	

Newman-Keuls test; CO_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	2.359700	2.819550	1.946800	.6198300	0.000000	0.000000
A	{0}		*0.000180	*0.000176(	*0.000196(	*0.000150	*0.000174(	*0.000158190727233887
B	{0.01	*0.0001809000968933		0.572473	0.611915	0.108304	0.065477	*0.0448424816131592
C	{0.1}	*0.000176(	0.572473		0.531261	0.064675	*0.030992	*0.0227940678596497
D	{1}	*0.000196(	0.611915	0.531261		0.117566	0.113241	0.068324
E	{10}	*0.000150	0.108304	0.064675	0.117566		0.721413	0.448932
F	{100}	*0.000174(	0.065477	*0.030992	0.113241	0.721413		1
G	{500}	*0.000158	*0.044842	*0.022794(	0.068324	0.448932	1	

Newman-Keuls test; CR_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	4.733770	.6586800	.6484800	.2183400	0.000000	0.000000
A	{0}		0.212683	*0.000176	*0.000180	*0.000196(	*0.000158	*0.0001509785652160
B	{0.01	0.212683		*0.000180	*0.000196(	*0.000150	*0.000174(	*0.0001581907272338
C	{0.1}	*0.000176	*0.0001809000968933		0.978882	0.489687	0.437907	0.336656
D	{1}	*0.000180	*0.000196(	0.978882		0.27257	0.34929	0.231798
E	{10}	*0.000196(	*0.000150	0.489687	0.27257		0.832973	0.57134
F	{100}	*0.000158	*0.000174(	0.437907	0.34929	0.832973		1
G	{500}	*0.000150	*0.000158	0.336656	0.231798	0.57134	1	

Newman-Keuls test; CR_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	1.609320	.7941500	.7878800	.1013500	.0053729	0.000000
A	{0}		*0.000176(	*0.000180	*0.000196(	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.0001766681671142		*0.044428	0.101212	*0.005525	*0.005172	*0.00695407390594482
C	{0.1}	*0.000180	*0.044428706169128		0.986794	0.181725	0.189023	0.252914
D	{1}	*0.000196(	0.101212	0.986794		0.084024	0.121424	0.189755
E	{10}	*0.000150	*0.005525	0.181725	0.084024		0.798662	0.959463
F	{100}	*0.000158	*0.005172	0.189023	0.121424	0.798662		0.988702
G	{500}	*0.000174(	*0.006954(	0.252914	0.189755	0.959463	0.988702	

Newman-Keuls test; CU_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	1.200000	1.465410	2.273500	.8000000	.5588240	0.000000
A	{0}		*0.000198	*0.000185	*0.000291	*0.000151	*0.000158	*0.0001741051673889
B	{0.01	*0.0001987218856811		0.49419	*0.033025	0.307925	0.241042	*0.0304783582687378
C	{0.1}	*0.000185	0.49419		0.05074	0.218554	0.123083	*0.0122989416122437
D	{1}	*0.000291	*0.033025	0.05074		*0.007842	*0.003673	*0.000490844249725
E	{10}	*0.000151	0.307925	0.218554	*0.007842481136322		0.53383	0.122301
F	{100}	*0.000158	0.241042	0.123083	*0.003673	0.53383		0.16152
G	{500}	*0.000174	*0.030478	*0.012298	*0.000490	0.122301	0.16152	

Newman-Keuls test; CU_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	3.657000	3.732230	3.549960	.7350160	.0202100	0.000000
A	{0}		*0.000180	*0.000176(	*0.000196(	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.0001809000968933		0.869348	0.815009	*0.000206	*0.000198	*0.000155031681060791
C	{0.1}	*0.000176(	0.869348		0.913664	*0.000234	*0.000154	*0.000162303447723389
D	{1}	*0.000196(	0.815009	0.913664		*0.000190	*0.000182	*0.00019955635070800
E	{10}	*0.000150	*0.000206	*0.000234	*0.000190436840057		0.133562	0.262946
F	{100}	*0.000158	*0.000198	*0.000154	*0.000182	0.133562		0.964824
G	{500}	*0.000174(	*0.000155	*0.000162	*0.000199	0.262946	0.964824	

APPENDIX 26: ANOVA: *Chlorella vulgaris* UMACC 245: Biochemical composition (Lipid)

* = Significant difference : p<0.05

ANOVA-LIP-CHL

Newman-Keuls test; FE_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	1.338130	1.338130	.3043750	.1328000	.0627660	0.000000
A	{0}		*0.000181(	*0.000176	*0.000196(	*0.000150	*0.000158	*0.0001740455627441
B	{0.01	*0.0001810789108276		1	*0.006718	*0.006192	*0.007340	*0.0077273845672607
C	{0.1}	*0.000176	1		*0.017024	*0.010988	*0.011069	*0.0106489062309265
D	{1}	*0.000196(	*0.006718	*0.017024		0.605034	0.741341	0.78488
E	{10}	*0.000150	*0.006192	*0.010988	0.605034		0.83218	0.912285
F	{100}	*0.000158	*0.007340	*0.011069	0.741341	0.83218		0.849366
G	{500}	*0.000174(	*0.007727	*0.010648	0.78488	0.912285	0.849366	

Newman-Keuls test; FE_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	2.919790	6.680850	5.958060	.5590100	.0784710	0.000000
A	{0}		*0.000196(	*0.000176	*0.000180	*0.000150	*0.000158	*0.00017405562744141
B	{0.01	*0.0001960396766662		*0.000182	*0.000186	*0.000343	*0.000251	*0.00029110908508300
C	{0.1}	*0.000176	*0.000182151794335		0.146675	*0.000196(	*0.000150	*0.00015819072723388
D	{1}	*0.000180	*0.000186	0.146675		*0.000180	*0.000196(	*0.00015097856521606
E	{10}	*0.000150	*0.000343	*0.000196	*0.000180900096893		0.324242	0.478758
F	{100}	*0.000158	*0.000251	*0.000150	*0.000196(	0.324242		0.869961
G	{500}	*0.000174(	*0.000291	*0.000158	*0.000150	0.478758	0.869961	

Newman-Keuls test; MN_C_C4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	2.242190	1.978570	4.207210	1.166239	.2290320	0.000000
A	{0}		*0.005014	*0.003507	0.947915	*0.000444	*0.000172	*0.0001825690269470
B	{0.01	*0.0050141215324401		0.62164	*0.002253	0.134507	*0.008501	*0.0056992769241333
C	{0.1}	*0.003507	0.62164		*0.002237	0.142227	*0.012501	*0.0096217989921569
D	{1}	0.947915	*0.002253	*0.002237021923065		*0.000403	*0.000169	*0.0001651644706726
E	{10}	*0.000444	0.134507	0.142227	*0.0004039406776428		0.094415	0.099984
F	{100}	*0.000172	*0.008501	*0.012501	*0.000169	0.094415		0.667758
G	{500}	*0.000182	*0.005699	*0.009621	*0.000165	0.099984	0.667758	

Newman-Keuls test; MN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	4.529900	8.426720	3.770300	.8468200	.2465330	0.000000
A	{0}		*0.000180	*0.000205	*0.000196(	*0.000150	*0.000158	*0.00017405562744141
B	{0.01	*0.0001809000968933		*0.000237	0.299513	*0.000501	*0.000324	*0.00026184320449829
C	{0.1}	*0.000205	*0.000237345695495		*0.000202	*0.000196(	*0.000150	*0.00015819072723388
D	{1}	*0.000196(	0.299513	*0.000202119350433		*0.001124	*0.000675	*0.00069040060043335
E	{10}	*0.000150	*0.000501	*0.000196(	*0.001124680042266		0.408913	0.471734
F	{100}	*0.000158	*0.000324	*0.000150	*0.000675	0.408913		0.731851
G	{500}	*0.000174(	*0.000261	*0.000158	*0.000690	0.471734	0.731851	

Newman-Keuls test; ZN_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	3.670100	2.808000	1.212100	.8271000	0.000000	0.000000
A	{0}		0.10785	*0.002075(	*0.000196	*0.000150	*0.000174(	*0.0001581907272338
B	{0.01	0.10785		*0.021512	*0.000185	*0.000197	*0.000158	*0.0001509785652160
C	{0.1}	*0.002075(	0.021512		*0.000436	*0.000263	*0.000153	*0.0001972317695617
D	{1}	*0.000196	0.000185	*0.000436842441558		0.266791	*0.012674	*0.0071564316749572
E	{10}	*0.000150	0.000197	*0.000263	0.266791		0.06381	*0.0263329744338985
F	{100}	*0.000174(	0.000158	*0.000153	*0.012674	0.06381		1
G	{500}	*0.000158	0.000151	*0.000197	*0.007156	*0.026332	1	

Newman-Keuls test; ZN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	5.876500	6.987000	2.333211	.5474100	.0704142	0.000000
A	{0}		*0.000180	*0.000176	*0.000196(	*0.000150	*0.000158	*0.00017405562744141
B	{0.01	*0.0001809000968933		0.088424	*0.000208	*0.000181	*0.000196	*0.00015115737915039
C	{0.1}	*0.000176	0.088424		*0.000183	*0.000196(	*0.000150	*0.00015819072723388
D	{1}	*0.000196(	*0.000208	*0.000183403491973		*0.010784	*0.006022	*0.00860202312469482
E	{10}	*0.000150	*0.000181	*0.000196(	*0.010784924030304		0.444606	0.647313
F	{100}	*0.000158	*0.000196	*0.000150	*0.006022	0.444606		0.909286
G	{500}	*0.000174(	*0.000151	*0.000158	*0.008602	0.647313	0.909286	

Newman-Keuls test; AD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	4.991070	4.131150	3.104000	.2333000	0.000000	0.000000
A	{0}		0.134309	0.817518	0.072418	*0.000197	*0.000159	*0.0001517534255981
B	{0.01	0.134309		0.197589	*0.006426	*0.000151	*0.000174	*0.0001581907272338
C	{0.1}	0.817518	0.197589		*0.046929	*0.000181	*0.000152	*0.0001967549324035
D	{1}	0.072418	*0.006426	*0.046929299831390		*0.000196	*0.000242	*0.0002028942108154
E	{10}	*0.000197	*0.000151	*0.000181	*0.000196397304534		0.874809	0.628279
F	{100}	*0.000159	*0.000174	*0.000152	*0.000242	0.874809		1
G	{500}	*0.000151	*0.000158	*0.000196	*0.000202	0.628279	1	

Newman-Keuls test; AD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	5.556410	1.367622	.4901700	.1228770	0.000000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196(	*0.000150	*0.000174	*0.00015819072723387
B	{0.01	*0.0001766681671142		*0.000180	*0.000181	*0.000196	*0.000158	*0.00015151507019043
C	{0.1}	*0.000180	*0.000180065631866		0.168546	0.134539	0.21356	0.154209
D	{1}	*0.000196(	*0.000181	0.168546		0.553073	0.848137	0.702385
E	{10}	*0.000150	*0.000196	0.134539	0.553073		0.97757	0.841864
F	{100}	*0.000174(	*0.000158	0.21356	0.848137	0.97757		1
G	{500}	*0.000158	*0.000151	0.154209	0.702385	0.841864	1	

APPENDIX 26: ANOVA: *Chlorella vulgaris* UMACC 245: Biochemical composition (Lipid)

* = Significant difference : p<0.05

ANOVA-LIP-CHL

Newman-Keuls test; BD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	4.464200	2.116280	.8352900	.2076000	0.000000	0.000000
A	{0}		0.543299	0.000202	*0.000180	*0.000196	*0.000158	*0.000150978565216
B	{0.01}	0.543299		0.000203	*0.000196	*0.000150	*0.000174	*0.000158190727233
C	{0.1}	*0.000202	*0.000203013420104		*0.003088	*0.000432	*0.000421	*0.000367105007171
D	{1}	*0.000180	*0.000196	*0.003088474273681		0.100258	0.135263	0.082464
E	{10}	*0.000196	*0.000150	*0.000432	0.100258		0.831734	0.569774
F	{100}	*0.000158	*0.000174	*0.000421	0.135263	0.831734		1
G	{500}	*0.000150	*0.000158	*0.000367	0.082464	0.569774	1	

Newman-Keuls test; BD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	5.513700	5.396500	5.115380	4.245810	0.000000	0.000000
A	{0}		*0.000176	*0.000181	*0.000196	*0.000150	*0.000174	*0.000158190727233887
B	{0.01}	*0.000176846981048		0.885835	0.873735	0.418503	*0.000229	*0.000202059745788574
C	{0.1}	*0.000181	0.885835		0.730868	0.349664	*0.000215	*0.000230669975280762
D	{1}	*0.000196	0.873735	0.730868		0.295918	*0.000263	*0.000213503837585449
E	{10}	*0.000150	0.418503	0.349664	0.295918		*0.000457	*0.000271737575531006
F	{100}	*0.000174	*0.000229	*0.000215	*0.000263	*0.000457882881164		1
G	{500}	*0.000158	*0.000202	*0.000230	*0.000213	*0.000271	1	

Newman-Keuls test; MC_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	4.373740	4.320000	4.393100	.2594000	0.000000	0.000000
A	{0}		0.926263	0.82805	0.972586	*0.000176	*0.000196	*0.000180900096893
B	{0.01}	0.926263		0.881021	0.957049	*0.000196	*0.000158	*0.000150978565216
C	{0.1}	0.82805	0.881021		0.976657	*0.000180	*0.000150	*0.000196039676666
D	{1}	0.972586	0.957049	0.976657		*0.000150	*0.000174	*0.000158190727233
E	{10}	*0.000176	*0.000196	*0.000180	*0.000150978565216		0.746489	0.473758
F	{100}	*0.000196	*0.000158	*0.000150	*0.000174	0.746489		1
G	{500}	*0.000180	*0.000150	*0.000196	*0.000158	0.473758	1	

Newman-Keuls test; MC_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	3.385600	3.382982	3.480330	2.258590	0.000000	0.000000
A	{0}		*0.000180	*0.000196	*0.000176	*0.000150	*0.000174	*0.000158190727233887
B	{0.01}	*0.000180959701538		0.997922	0.920843	0.46942	*0.019777	*0.0131790041923523
C	{0.1}	*0.000196	0.997922		0.994127	0.249129	*0.013248	*0.0074838399887085
D	{1}	*0.000176	0.920843	0.994127		0.573766	*0.022397	*0.0163934826850891
E	{10}	*0.000150	0.46942	0.249129	0.573766		0.072347	*0.0300703644752502
F	{100}	*0.000174	*0.019777	*0.013248	*0.022397	0.072347		1
G	{500}	*0.000158	*0.013179	*0.007483	*0.016393	*0.030070	1	

Newman-Keuls test; DD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	2.938310	1.944030	.6731500	.3913000	.3010000	.2467500
A	{0}		*0.000310	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744
B	{0.01}	*0.000310420989990		*0.001701	*0.000181	*0.000196	*0.000150	*0.000158190727233
C	{0.1}	*0.000180	*0.001701593399047		*0.000349	*0.000243	*0.000254	*0.000222802162170
D	{1}	*0.000196	*0.000181	*0.000349223613739		0.28612	0.336805	0.370484
E	{10}	*0.000150	*0.000196	*0.000243	0.28612		0.727716	0.838638
F	{100}	*0.000158	*0.000150	*0.000254	0.336805	0.727716		0.834118
G	{500}	*0.000174	*0.000158	*0.000222	0.370484	0.838638	0.834118	

Newman-Keuls test; DD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	1.854800	2.108050	2.461960	.4350000	.1725230	0.000000
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000196039676666		0.545098	0.326411	*0.003842	*0.002914	*0.00242114067077637
C	{0.1}	*0.000180	0.545098		0.400731	*0.003036	*0.001722	*0.00125402212142944
D	{1}	*0.000176	0.326411	0.400731		*0.001198	*0.000640	*0.000481605529785156
E	{10}	*0.000150	*0.003842	*0.003036	*0.001198172569274		0.530754	0.549834
F	{100}	*0.000158	*0.002914	*0.001722	*0.000640	0.530754		0.679101
G	{500}	*0.000174	*0.002421	*0.001254	*0.000481	0.549834	0.679101	

Newman-Keuls test; MA_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.242000	.4581700	.5967200	.5300000	.3330000	.1234660	0.000000
A	{0}		*0.000196	*0.000176	*0.000180	*0.000150	*0.000158	*0.000174045562744
B	{0.01}	*0.000196039676666		0.747775	0.709283	0.518027	0.214132	0.117083
C	{0.1}	*0.000176	0.747775		0.729043	0.520981	0.144456	0.06176
D	{1}	*0.000180	0.709283	0.729043		0.562642	0.18411	0.08654
E	{10}	*0.000150	0.518027	0.520981	0.562642		0.285627	0.217156
F	{100}	*0.000158	0.214132	0.144456	0.18411	0.285627		0.523655
G	{500}	*0.000174	0.117083	0.06176	0.08654	0.217156	0.523655	

Newman-Keuls test; MA_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		12.58255	1.710000	.9275000	.9860000	.1205000	.0147685	0.000000
A	{0}		*0.000176	*0.000196	*0.000180	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176668167114		0.160706	0.092347	*0.006894	*0.006380	*0.00823068618774414
C	{0.1}	*0.000196	0.160706		0.886091	0.063688	0.092379	0.141576
D	{1}	*0.000180	0.092347	0.886091		0.113429	0.117774	0.155922
E	{10}	*0.000150	*0.006894	0.063688	0.113429		0.795774	0.951603
F	{100}	*0.000158	*0.006380	0.092379	0.117774	0.795774		0.971244
G	{500}	*0.000174	*0.008230	0.141576	0.155922	0.951603	0.971244	

APPENDIX 27: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD-Appearance of new band)

* = Significant difference : p<0.05

ANOVA-APPEARANCE-CHL

Newman-Keuls test; CD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	31.55000	36.11000	29.17000	23.33000	22.92000	16.67000
A	{0}		0.051933	*0.028390	0.060198	0.120681	0.078842	0.107143
B	{0.01}	0.051933		0.644949	0.80946	0.679727	0.809329	0.557011
C	{0.1}	*0.028390	0.644949		0.757747	0.565842	0.659297	0.384775
D	{1}	0.060198	0.80946	0.757747		0.55605	0.797823	0.582842
E	{10}	0.120681	0.679727	0.565842	0.55605		0.96693	0.774223
F	{100}	0.078842	0.809329	0.659297	0.797823	0.96693		0.529043
G	{500}	0.107144	0.557012	0.384775	0.582842	0.774224	0.529043	

Newman-Keuls test; CD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	41.96000	35.24000	36.01000	26.19000	21.43000	14.28000
A	{0}		*0.011135	*0.021855	*0.025795	0.079364	0.111711	0.170401
B	{0.01}	*0.011135		0.778597	0.556694	0.411713	0.28192	0.115334
C	{0.1}	*0.021855	0.778597		0.939068	0.375246	0.368483	0.193874
D	{1}	*0.025795	0.556694	0.939068		0.59241	0.476579	0.235481
E	{10}	0.079364	0.411713	0.375246	0.59241		0.637467	0.469303
F	{100}	0.111711	0.28192	0.368483	0.476579	0.637467		0.48123
G	{500}	0.170401	0.115335	0.193874	0.235482	0.469304	0.48123	

Newman-Keuls test; FE_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	33.97000	35.77000	29.10000	21.69000	21.69000	16.93000
A	{0}		*0.000158	*0.000174	*0.000150	*0.000183	*0.000202	*0.000203
B	{0.01}	*0.000158		0.538215	0.109872	*0.003692	*0.002085	*0.000403
C	{0.1}	*0.000174	0.538215		0.083026	*0.001819	*0.001252	*0.000273
D	{1}	*0.000150	0.109872	0.083026		0.051891	*0.021186	*0.003959
E	{10}	*0.000183	*0.003692	*0.001819	0.051891		1	0.117396
F	{100}	*0.000202	*0.002085	*0.001252	*0.021186	1		0.251136
G	{500}	*0.000203	*0.000403	*0.000273	*0.003959	0.117397	0.251136	

Newman-Keuls test; FE_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	51.92000	52.21000	46.70000	38.09000	23.59000	12.60000
A	{0}		*0.001625	*0.002023	*0.002889	0.009231	0.079911	0.2281
B	{0.01}	*0.001625		0.977379	0.609747	0.375485	0.057073	*0.011068
C	{0.1}	*0.002023	0.977379		0.847543	0.512242	0.078587	*0.014401
D	{1}	*0.002889	0.609747	0.847543		0.403631	0.08703	*0.019545
E	{10}	0.009231	0.375485	0.512242	0.403631		0.16897	0.056659
F	{100}	0.079911	0.057073	0.078587	0.08703	0.16897		0.290134
G	{500}	0.228101	*0.011068	*0.014401	*0.019545	0.05666	0.290134	

Newman-Keuls test; MN_C_C4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	54.21000	52.42000	52.43000	52.42000	47.66000	45.57000
A	{0}		*0.008129	*0.003896	*0.008107	*0.005884	*0.004491	*0.002449
B	{0.01}	*0.008129		0.998885	0.886734	0.988414	0.982191	0.978281
C	{0.1}	*0.003896	0.998885		1	1	0.703741	0.84376
D	{1}	*0.008107	0.886734	1		0.999464	0.979269	0.978898
E	{10}	*0.005884	0.988414	1	0.999464		0.920771	0.942634
F	{100}	*0.004491	0.982191	0.703741	0.979269	0.920771		0.86717
G	{500}	*0.002449	0.978282	0.843767	0.978898	0.942634	0.86717	

Newman-Keuls test; MN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	27.97000	28.83000	28.20000	27.69000	24.89000	20.99000
A	{0}		*0.001038	*0.001413	*0.001306	*0.000807	*0.001057	*0.001548
B	{0.01}	*0.001038		0.985646	0.966062	0.958671	0.83188	0.566696
C	{0.1}	*0.001413	0.985646		0.907049	0.996368	0.942134	0.68056
D	{1}	*0.001306	0.966062	0.907049		0.994977	0.922243	0.659504
E	{10}	*0.000807	0.958671	0.996368	0.994977		0.605174	0.436325
F	{100}	*0.001057	0.83188	0.942134	0.922243	0.605174		0.47349
G	{500}	*0.001548	0.566696	0.680561	0.659505	0.436325	0.47349	

Newman-Keuls test; AD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	105.6700	104.0000	105.6700	102.2200	85.00000	12.50000
A	{0}		*0.000158	*0.000151	*0.000174	*0.000196	*0.000184	0.288326
B	{0.01}	*0.000158		0.88497	1	0.950374	0.302742	*0.000154
C	{0.1}	*0.000151	0.88497		0.988182	0.877441	0.247826	*0.000198
D	{1}	*0.000174	1	0.988182		0.989815	0.398065	*0.000163
E	{10}	*0.000196	0.950374	0.877441	0.989815		0.15069	*0.000182
F	{100}	*0.000184	0.302742	0.247826	0.398065	0.15069		*0.000187
G	{500}	0.288326	*0.000154	*0.000198	*0.000163	*0.000182	*0.000187	

Newman-Keuls test; AD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	34.11000	38.93000	37.62000	28.57000	15.72000	0.000000
A	{0}		*0.029044	*0.021045	*0.020713	0.054572	0.28909	1
B	{0.01}	*0.029044		0.880734	0.730726	0.588136	0.192849	*0.019481
C	{0.1}	*0.021045	0.880734		0.89768	0.731396	0.19474	*0.016290
D	{1}	*0.020713	0.730726	0.89768		0.645691	0.173396	*0.015142
E	{10}	0.054572	0.588136	0.731396	0.645691		0.219387	*0.031864
F	{100}	0.289091	0.192849	0.19474	0.173396	0.219387		0.138107
G	{500}	1	*0.019481	*0.016290	*0.015142	*0.031864	0.138107	

APPENDIX 27: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD-Appearance of new band)

* = Significant difference : p<0.05

ANOVA-APPEARANCE-CHL

Newman-Keuls test; BD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	125.0000	145.8300	160.7100	141.6700	116.6700	0.000000
A	{0}		*0.000196	*0.000158	*0.000174	*0.000151	*0.000181	1
B	{0.01}	*0.000196		0.34058	0.104025	0.263354	0.569648	*0.000181
C	{0.1}	*0.000158	0.34058		0.315875	0.77553	0.22074	*0.000150
D	{1}	*0.000174	0.104025	0.315875		0.401962	0.053492	*0.000158
E	{10}	*0.000151	0.263354	0.77553	0.401962		0.222814	*0.000196
F	{100}	*0.000181	0.569648	0.22074	0.053492	0.222814		*0.000176
G	{500}	1	*0.000181	*0.000150	*0.000158	*0.000196	*0.000176	

Newman-Keuls test; BD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	52.75000	67.63000	59.30000	51.12000	29.17000	0.000000
A	{0}		*0.000682	*0.000229	*0.000369	*0.000647	*0.020934	1
B	{0.01}	*0.000682		0.289853	0.50056	0.865905	0.063243	*0.000528
C	{0.1}	*0.000229	0.289853		0.394019	0.339302	*0.008733	*0.000198
D	{1}	*0.000369	0.50056	0.394019		0.67093	*0.030081	*0.000298
E	{10}	*0.000647	0.865905	0.339302	0.67093		*0.036209	*0.000411
F	{100}	*0.020934	0.063243	*0.008733	*0.030081	*0.036209		*0.008304
G	{500}	1	*0.000528	*0.000198	*0.000298	*0.000411	*0.008304	

Newman-Keuls test; MC_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	160.7100	170.2400	157.1400	147.6200	123.6100	0.000000
A	{0}		*0.000158	*0.000174	*0.000150	*0.000196	*0.000180	1
B	{0.01}	*0.000158		0.189839	0.61381	0.177277	*0.000675	*0.000150
C	{0.1}	*0.000174	0.189839		0.176864	*0.025447	*0.000215	*0.000158
D	{1}	*0.000150	0.61381	0.176864		0.190275	*0.000841	*0.000196
E	{10}	*0.000196	0.177277	*0.025447	0.190275		*0.003883	*0.000180
F	{100}	*0.000180	*0.000675	*0.000215	*0.000841	*0.003883		*0.000176
G	{500}	1	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; MC_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	91.48000	88.26000	85.42000	60.54000	24.56000	0.000000
A	{0}		*0.000177	*0.000162	*0.000156	*0.000478	0.089348	1
B	{0.01}	*0.000177		0.767798	0.839659	0.0512	*0.000299	*0.000160
C	{0.1}	*0.000162	0.767798		0.794476	0.052401	*0.000360	*0.000154
D	{1}	*0.000156	0.839659	0.794476		*0.035576	*0.000313	*0.000199
E	{10}	*0.000478	0.0512	0.052401	*0.035576		*0.004767	*0.000321
F	{100}	*0.089348	*0.000299	*0.000360	*0.000313	*0.004767		*0.037647
G	{500}	1	*0.000160	*0.000154	*0.000199	*0.000321	*0.037647	

Newman-Keuls test; DD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	31.61000	27.59000	23.55000	14.00000	12.67000	10.37000
A	{0}		*0.000216	*0.000335	*0.000838	*0.027621	*0.028359	*0.031545
B	{0.01}	*0.000216		0.369899	0.187489	*0.005809	*0.004991	*0.002685
C	{0.1}	*0.000335	0.369899		0.367593	*0.018937	*0.018547	*0.010362
D	{1}	*0.000838	0.187489	0.367593		*0.045072	0.061192	*0.039277
E	{10}	*0.027621	*0.005809	*0.018937	*0.045072		0.763763	0.68713
F	{100}	*0.028359	*0.004991	*0.018547	0.061192	0.763763		0.604353
G	{500}	*0.031545	*0.002685	*0.010362	*0.039277	0.687131	0.604354	

Newman-Keuls test; DD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	17.69000	22.21000	19.95000	17.65000	13.65000	13.65000
A	{0}		*0.006102	*0.001667	*0.003174	*0.004111	*0.005574	*0.014185
B	{0.01}	*0.006102		0.536924	0.595258	0.992559	0.767172	0.605672
C	{0.1}	*0.001667	0.536924		0.595258	0.696965	0.359762	0.289616
D	{1}	*0.003174	0.595258	0.595258		0.846495	0.569515	0.454632
E	{10}	*0.004111	0.992559	0.696965	0.846495		0.611478	0.35227
F	{100}	*0.005574	0.767172	0.359762	0.569515	0.611478		1
G	{500}	*0.014185	0.605673	0.289616	0.454632	0.35227	1	

Newman-Keuls test; MA_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	16.97000	21.40000	19.35000	15.89000	16.40000	13.40000
A	{0}		*0.035868	*0.013050	*0.020839	*0.020597	*0.029731	*0.020917
B	{0.01}	*0.035868		0.67253	0.650828	0.976109	0.913454	0.897724
C	{0.1}	*0.013050	0.67253		0.696371	0.818009	0.767201	0.637685
D	{1}	*0.020839	0.650828	0.696371		0.905741	0.836261	0.774653
E	{10}	*0.020597	0.976109	0.818009	0.905741		0.922542	0.635954
F	{100}	*0.029731	0.913454	0.767201	0.836261	0.922542		0.831235
G	{500}	*0.020917	0.897725	0.637685	0.774654	0.635955	0.831235	

Newman-Keuls test; MA_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	22.80000	38.30000	25.30000	18.15000	18.62000	17.01000
A	{0}		*0.025886	*0.000782	*0.017497	*0.037909	0.056653	*0.021396
B	{0.01}	*0.025886		0.07953	0.708935	0.762372	0.534366	0.813789
C	{0.1}	*0.000782	0.07953		0.067586	0.054206	*0.042151	0.053224
D	{1}	*0.017497	0.708935	0.067586		0.70108	0.577881	0.716076
E	{10}	*0.037909	0.762372	0.054206	0.70108		0.943981	0.864626
F	{100}	0.056653	0.534366	*0.042151	0.577881	0.943981		0.967496
G	{500}	*0.021396	0.81379	0.053224	0.716077	0.864626	0.967496	

APPENDIX 27: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD-Disappearance of band)

* = Significant difference : p<0.05

ANOVA-DISAPPEARANCE-CHL

Newman-Keuls test; CD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	30.46000	32.95000	38.10000	40.01000	42.96000	48.13000
A	{0}		*0.022793	*0.037719	*0.028941	*0.031881	*0.027461	*0.016052544116973
B	{0.01}	*0.022793		0.837327	0.799737	0.852068	0.827802	0.678289
C	{0.1}	*0.037719	0.837327		0.671797	0.825885	0.834009	0.709201
D	{1}	*0.028941	0.799737	0.671797		0.874853	0.912743	0.833205
E	{10}	*0.031881	0.852068	0.825885	0.874853		0.807876	0.777326
F	{100}	*0.027461	0.827802	0.834009	0.912743	0.807876		0.670605
G	{500}	*0.016052	0.678289	0.709201	0.833205	0.777326	0.670605	

Newman-Keuls test; CD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	45.64000	48.58000	50.01000	62.06000	64.88000	62.93000
A	{0}		*0.002219	*0.003543	*0.005082	*0.001327	*0.001621	*0.00161105394363403
B	{0.01}	*0.002219		0.811684	0.931073	0.544493	0.617376	0.620594
C	{0.1}	*0.003543	0.811684		0.907723	0.521535	0.668435	0.645165
D	{1}	*0.005082	0.931073	0.907723		0.336371	0.619801	0.548621
E	{10}	*0.001327	0.544493	0.521535	0.336371		0.970647	0.943793
F	{100}	*0.001621	0.617376	0.668435	0.619801	0.970647		0.874398
G	{500}	*0.001611	0.620594	0.645165	0.548621	0.943793	0.874398	

Newman-Keuls test; FE_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	43.63000	47.73000	58.76000	66.57000	69.78000	64.33000
A	{0}		*0.001190	*0.001478	*0.000546	*0.000363	*0.000336	*0.0003663301467895
B	{0.01}	*0.001190		0.704967	0.354598	0.248905	0.199426	0.251846
C	{0.1}	*0.001478	0.704967		0.316027	0.324252	0.281562	0.29241
D	{1}	*0.000546	0.354598	0.316027		0.746489	0.730094	0.607764
E	{10}	*0.000363	0.248905	0.324252	0.746489		0.766696	0.83586
F	{100}	*0.000336	0.199426	0.281562	0.730094	0.766696		0.865918
G	{500}	*0.000366	0.251846	0.29241	0.607764	0.83586	0.865918	

Newman-Keuls test; FE_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	42.04000	55.56000	56.10000	57.64000	60.24000	64.06000
A	{0}		*0.006528	*0.002376	*0.003904	*0.004762	*0.004599	*0.00361257791519165
B	{0.01}	*0.006528		0.320432	0.546383	0.643406	0.645126	0.565816
C	{0.1}	*0.002376	0.320432		0.96788	0.986354	0.983866	0.964298
D	{1}	*0.003904	0.546383	0.96788		0.908355	0.946903	0.928358
E	{10}	*0.004762	0.643406	0.986354	0.908355		0.845898	0.877567
F	{100}	*0.004599	0.645126	0.983866	0.946903	0.845898		0.775352
G	{500}	*0.003612	0.565816	0.964298	0.928358	0.877567	0.775352	

Newman-Keuls test; MN_C_C4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	45.12000	47.23000	47.23000	47.23000	49.73000	39.42000
A	{0}		*0.005918	*0.007577	*0.011424	*0.015672	*0.013924	*0.0057381391525266
B	{0.01}	*0.005918		0.863703	0.983357	0.998065	0.994981	0.64381
C	{0.1}	*0.007577	0.863703		1	1	0.996755	0.796706
D	{1}	*0.011424	0.983357	1		1	0.9767	0.914661
E	{10}	*0.015672	0.998065	1	1		0.83883	0.964302
F	{100}	*0.013924	0.994981	0.996755	0.9767	0.83883		0.951253
G	{500}	*0.005738	0.64381	0.796706	0.914661	0.964302	0.951253	

Newman-Keuls test; MN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	45.91000	46.82000	48.94000	55.37000	55.25000	55.11000
A	{0}		*0.001534	*0.003319	*0.004214	*0.004238	*0.003293	*0.00244009494781494
B	{0.01}	*0.001534		0.938472	0.963027	0.959239	0.923907	0.855223
C	{0.1}	*0.003319	0.938472		0.857243	0.943416	0.883841	0.757692
D	{1}	*0.004214	0.963027	0.857243		0.943364	0.8503	0.602028
E	{10}	*0.004238	0.959239	0.943416	0.943364		0.991974	0.999754
F	{100}	*0.003293	0.923907	0.883841	0.8503	0.991974		0.990637
G	{500}	*0.002440	0.855223	0.757692	0.602028	0.999754	0.990637	

Newman-Keuls test; AD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	66.39000	70.63000	71.95000	73.00000	80.00000	91.67000
A	{0}		*0.000226	*0.000258	*0.000313	*0.000319	*0.000243	*0.0001910328865051
B	{0.01}	*0.000226		0.724758	0.885778	0.942209	0.776229	0.321305
C	{0.1}	*0.000258	0.724758		0.91261	0.978108	0.855912	0.419809
D	{1}	*0.000313	0.885778	0.91261		0.93044	0.777422	0.373654
E	{10}	*0.000319	0.942209	0.978108	0.93044		0.56251	0.285211
F	{100}	*0.000243	0.776229	0.855912	0.777422	0.56251		0.33944
G	{500}	*0.000191	0.321305	0.419809	0.373654	0.285211	0.33944	

Newman-Keuls test; AD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	29.35000	34.26000	36.15000	57.86000	93.93000	100.0000
A	{0}		*0.004304	*0.003700	*0.004386	*0.000215	*0.000158	*0.000174045562744141
B	{0.01}	*0.004304		0.576508	0.71378	*0.023178	*0.000171	*0.000163137912750244
C	{0.1}	*0.003700	0.576508		0.829018	*0.039187	*0.000219	*0.000168502330780029
D	{1}	*0.004386	0.71378	0.829018		*0.024207	*0.000197	*0.000204980373382568
E	{10}	*0.000215	*0.023178	*0.039187	*0.024207		*0.001026	*0.000769376754760742
F	{100}	*0.000158	*0.000171	*0.000219	*0.000197	*0.001026		0.491229
G	{500}	*0.000174	*0.000163	*0.000168	*0.000204	*0.000769	0.491229	

APPENDIX 27: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD-Disappearance of band)

* = Significant difference : p<0.05

ANOVA-DISAPPEARANCE-CHL

Newman-Keuls test; BD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	68.75000	66.67000	66.67000	71.43000	71.43000	100.0000
A	{0}		*0.000301	*0.000202	*0.000261	*0.000268	*0.000324	*0.000175595283508
B	{0.01	*0.000301	0.301479	0.981176	0.855057	0.813972	0.968914	0.061012
C	{0.1}	*0.000202	0.981176		1	0.973095	0.992381	0.084455
D	{1}	*0.000261	0.855057	1		0.905479	0.973095	0.063482
E	{10}	*0.000268	0.813972	0.973095	0.905479		1	0.055899
F	{100}	*0.000324	0.968914	0.992381	0.973095	1		*0.0229069590568542
G	{500}	*0.000175	0.061012	0.084455	0.063482	0.055899	*0.0229069590568542	

Newman-Keuls test; BD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	48.59000	50.89000	53.72000	58.33000	69.57000	100.0000
A	{0}		*0.000521	*0.000810	*0.000914	*0.000667	*0.000262	*0.000174522399902344
B	{0.01	*0.000521	0.600246	0.828944	0.828944	0.876608	0.78809	0.311046
C	{0.1}	*0.000810	0.828944		0.790429	0.760295	0.318673	*0.00272643566131592
D	{1}	*0.000914	0.876608	0.790429		0.665706	0.31275	*0.00294488668441772
E	{10}	*0.000667	0.78809	0.760295	0.665706		0.300006	*0.00370258092880249
F	{100}	*0.000262	0.311046	0.318673	0.31275	0.300006		*0.0114687085151672
G	{500}	*0.000174	*0.002564	*0.002726	*0.002944	*0.003702	*0.0114687085151672	

Newman-Keuls test; MC_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	68.75000	75.00000	91.67000	88.10000	88.89000	100.0000
A	{0}		*0.000176	*0.000180	*0.000158	*0.000196	*0.000150	*0.000174045562744
B	{0.01	*0.000176	0.469810	0.462137	0.092001	0.082633	0.115273	*0.020094156265258
C	{0.1}	*0.000180	0.462137		0.228188	0.135354	0.24677	0.058954
D	{1}	*0.000158	0.092001	0.228188		0.902989	0.741672	0.330662
E	{10}	*0.000196	0.082633	0.135354	0.902989		0.925304	0.496755
F	{100}	*0.000150	0.115273	0.24677	0.741672	0.925304		0.395243
G	{500}	*0.000174	*0.020094	0.058954	0.330662	0.496755	0.395243	

Newman-Keuls test; MC_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	67.10000	65.83000	67.10000	68.00000	79.69000	100.0000
A	{0}		*0.000188	*0.000179	*0.000212	*0.000181	*0.000161	*0.000174105167388916
B	{0.01	*0.000188	0.350677	0.894702	0.894702	1	0.995053	0.555641
C	{0.1}	*0.000179	0.894702		0.990095	0.995535	0.594791	*0.0264964699745178
D	{1}	*0.000212	1	0.990095		0.925279	0.398645	*0.0166902542114258
E	{10}	*0.000181	0.995053	0.995535	0.925279		0.234679	*0.0113589763641357
F	{100}	*0.000161	0.555641	0.594791	0.398645	0.234679		*0.048891007900238
G	{500}	*0.000174	*0.024953	*0.026496	*0.016690	*0.011358	*0.048891007900238	

Newman-Keuls test; DD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	36.60000	47.18000	47.95000	50.62000	55.26000	59.62000
A	{0}		*0.009136	*0.004329	*0.006852	*0.006858	*0.004715	*0.003281056880950
B	{0.01	*0.009136	0.979743	0.395828	0.624983	0.659674	0.552249	0.437325
C	{0.1}	*0.004329	0.395828		0.95014	0.956468	0.906993	0.837356
D	{1}	*0.006852	0.624983	0.95014		0.828286	0.819562	0.770153
E	{10}	*0.006858	0.659674	0.956468	0.828286		0.706644	0.741282
F	{100}	*0.004715	0.552249	0.906993	0.819562	0.706644		0.723516
G	{500}	*0.003281	0.437325	0.837356	0.770153	0.741282	0.723516	

Newman-Keuls test; DD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	40.17000	40.75000	45.08000	51.42000	55.02000	62.65000
A	{0}		*0.001033	*0.002273	*0.001812	*0.000908	*0.000695	*0.000345877304077
B	{0.01	*0.001033	0.677032	0.952618	0.952618	0.866291	0.651119	0.548489
C	{0.1}	*0.002273	0.952618		0.657947	0.520859	0.468081	0.205431
D	{1}	*0.001812	0.866291	0.657947		0.518489	0.565713	0.29816
E	{10}	*0.000908	0.651119	0.520859	0.518489		0.712495	0.487314
F	{100}	*0.000695	0.548489	0.468081	0.565713	0.712495		0.438672
G	{500}	*0.000345	0.238383	0.205431	0.29816	0.487314	0.438672	

Newman-Keuls test; MA_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	57.70000	61.04000	61.79000	66.90000	71.46000	84.76000
A	{0}		*0.000377	*0.000511	*0.000762	*0.000559	*0.000445	*0.000221073627471
B	{0.01	*0.000377	0.833432	0.779875	0.93542	0.859957	0.765249	0.252949
C	{0.1}	*0.000511	0.779875		0.949953	0.872459	0.810451	0.304448
D	{1}	*0.000762	0.93542	0.949953		0.669499	0.694032	0.248629
E	{10}	*0.000559	0.859957	0.872459	0.669499		0.703096	0.309943
F	{100}	*0.000445	0.765249	0.810451	0.694032	0.703096		0.275464
G	{500}	*0.000221	0.252949	0.304448	0.248629	0.309943	0.275464	

Newman-Keuls test; MA_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	47.02000	51.15000	57.50000	77.21000	63.50000	54.93000
A	{0}		*0.000594	*0.000716	*0.000680	*0.000203	*0.000418	*0.000713050365447998
B	{0.01	*0.000594	0.437122	0.695097	0.743185	0.093356	0.522327	0.728845
C	{0.1}	*0.000716	0.695097		0.814216	0.14025	0.638573	0.719672
D	{1}	*0.000680	0.743185	0.814216		0.172425	0.570237	0.807026
E	{10}	*0.000203	0.093356	0.14025	0.172425		0.205248	0.182625
F	{100}	*0.000418	0.522327	0.638573	0.570237	0.205248		0.690869
G	{500}	*0.000713	0.728845	0.719672	0.807026	0.182625	0.690869	

APPENDIX 27: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD- Similarity of Band)

* = Significant difference : p<0.05

ANOVA-SIMILIAR-CHL

Newman-Keuls test; CD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	73.89000	69.35000	69.64000	69.64000	59.81000	56.10000
A	{0}		*0.043141(	0.121417	0.088856	0.052957	*0.038546(	*0.027852416038513
B	{0.01	*0.043141007423400		0.979606	0.930672	0.722725	0.751522	0.660489
C	{0.1}	0.121417	0.979606		0.980733	0.999703	0.429964	0.512715
D	{1}	0.088856	0.930672	0.980733		1	0.686625	0.663946
E	{10}	0.052957	0.722725	0.999703	1		0.835776	0.776127
F	{100}	*0.038546(	0.751522	0.429964	0.686625	0.835776		0.756652
G	{500}	*0.027852	0.660489	0.512715	0.663946	0.776127	0.756652	

Newman-Keuls test; CD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	62.65000	60.26000	57.73000	50.59000	44.45000	44.94000
A	{0}		*0.005577	*0.009483	*0.011018	*0.005198(	*0.003596	*0.00295543670654297
B	{0.01	*0.005577266216278(		0.836683	0.902737	0.718051	0.611307	0.545445
C	{0.1}	*0.009483	0.836683		0.827279	0.679104	0.643264	0.55022
D	{1}	*0.011018	0.902737	0.827279		0.540363	0.655854	0.515362
E	{10}	*0.005198(	0.718051	0.679104	0.540363		0.853252	0.627161
F	{100}	*0.003596	0.611307	0.643264	0.655854	0.853252		0.966354
G	{500}	*0.002954	0.545445	0.55022	0.515362	0.627161	0.966354	

Newman-Keuls test; FE_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	56.89000	50.23000	41.24000	33.43000	30.22000	30.50000
A	{0}		*0.000772(	*0.000624	*0.000357(	*0.000213	*0.000235	*0.000205636024475(
B	{0.01	*0.0007728338241577		0.50986	0.282285	0.126366	0.134792	0.108028
C	{0.1}	*0.000624	0.50986		0.376744	0.237372	0.300972	0.232768
D	{1}	*0.000357(	0.282285	0.376744		0.440948	0.684074	0.534915
E	{10}	*0.000213	0.126366	0.237372	0.440948		0.943394	0.770471
F	{100}	*0.000235	0.134792	0.300972	0.684074	0.943394		0.977826
G	{500}	*0.000205(	0.108028	0.232768	0.534915	0.770471	0.977826	

Newman-Keuls test; FE_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	51.11000	39.33000	37.88000	36.09000	35.42000	28.89000
A	{0}		*0.000204(	*0.000185	*0.000203	*0.000159(	*0.000168	*0.000172046089172360
B	{0.01	*0.000204086303710		0.175094	0.276224	0.304325	0.359709	0.137971
C	{0.1}	*0.000185	0.175094		0.863028	0.918944	0.963579	0.714703
D	{1}	*0.000203	0.276224	0.863028		0.83136	0.952363	0.700871
E	{10}	*0.000159(	0.304325	0.918944	0.83136		0.936483	0.665242
F	{100}	*0.000168	0.359709	0.963579	0.952363	0.936483		0.441692
G	{500}	*0.000177	0.137971	0.714703	0.700871	0.665242	0.441692	

Newman-Keuls test; MN_C_C4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	63.99000	58.14000	55.55000	52.77000	50.27000	46.73000
A	{0}		*0.005883(	*0.005455	*0.006258(	*0.005942	*0.005442(	*0.0040395855903625
B	{0.01	*0.0058839917182922		0.605207	0.730866	0.743822	0.729056	0.63442
C	{0.1}	*0.005455	0.605207		0.818325	0.879252	0.890863	0.836671
D	{1}	*0.006258(	0.730866	0.818325		0.805285	0.882985	0.854415
E	{10}	*0.005942	0.743822	0.879252	0.805285		0.824522	0.850111
F	{100}	*0.005442(	0.729056	0.890863	0.882985	0.824522		0.753721
G	{500}	*0.004039	0.63442	0.836671	0.854415	0.850111	0.753721	

Newman-Keuls test; MN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	49.83000	53.18000	49.43000	44.63000	41.10000	40.33000
A	{0}		*0.002360(	*0.001579	*0.003929(	*0.002854(	*0.002347(	*0.00275415182113647
B	{0.01	*0.0023600459098815		0.781449	0.973661	0.899906	0.880465	0.925593
C	{0.1}	*0.001579	0.781449		0.946488	0.886661	0.841906	0.879258
D	{1}	*0.003929(	0.973661	0.946488		0.691387	0.765383	0.867221
E	{10}	*0.002854(	0.899906	0.886661	0.691387		0.770063	0.930292
F	{100}	*0.002347(	0.880465	0.841906	0.765383	0.770063		0.94915
G	{500}	*0.002754	0.925593	0.879258	0.867221	0.930292	0.94915	

Newman-Keuls test; AD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	50.42000	47.00000	47.00000	38.33000	31.43000	8.330000
A	{0}		*0.000433	*0.000929	*0.000563(	*0.000404	*0.000266	*0.0001758933067321
B	{0.01	*0.0004332065582275		0.941633	0.745469	0.653864	0.391015	*0.011690616607666
C	{0.1}	*0.000929	0.941633		1	0.415286	0.3171	*0.0104366540908813
D	{1}	*0.000563(	0.745469	1		0.685387	0.45885	*0.0156965851783752
E	{10}	*0.000404	0.653864	0.415286	0.685387		0.514917	*0.0291840434074402
F	{100}	*0.000266	0.391015	0.3171	0.45885	0.514917		*0.042152106761932
G	{500}	*0.000175	*0.011690(	*0.010436	*0.015696	*0.029184(	*0.042152106761932	

Newman-Keuls test; AD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	70.66000	65.74000	68.25000	42.14000	6.070000	0.000000
A	{0}		*0.006769(	*0.010970	*0.010395	*0.000294	*0.000158	*0.000174105167388916
B	{0.01	*0.0067698359489440		0.856114	0.797543	*0.035313(	*0.000192	*0.000172972679138184
C	{0.1}	*0.010970	0.856114		0.789349	*0.022731	*0.000208	*0.00021207332611084
D	{1}	*0.010395	0.797543	0.789349		*0.033409	*0.000230(	*0.000174820423126221
E	{10}	*0.000294	*0.035313(	*0.022731	*0.033409297466278		*0.001694(	*0.00130540132522583
F	{100}	*0.000158	*0.000192	*0.000208	*0.000230(	*0.001694(		0.520771
G	{500}	*0.000174	*0.000172	*0.000212(	*0.000174	*0.001305	0.520771	

APPENDIX 27: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD- Similarity of Band)

* = Significant difference : p<0.05

ANOVA-SIMILIAR-CHL

Newman-Keuls test; BD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	31.25000	33.33000	33.33000	28.57000	20.83000	0.000000
A	{0}		*0.000199(	*0.000183	*0.000177	*0.000154(	*0.000158	*0.00017404556274441
B	{0.01	*0.000199079513549		0.813247	0.96867	0.760966	0.468705	*0.0132334232330322
C	{0.1}	*0.000183	0.813247		1	0.847565	0.492438	*0.0126945972442627
D	{1}	*0.000177	0.96867	1		0.944722	0.609492	*0.0174023509025574
E	{10}	*0.000154(	0.760966	0.847565	0.944722		0.385347	*0.0135369300842285
F	{100}	*0.000158	0.468705	0.492438	0.609492	0.385347		*0.0302701592445374
G	{500}	*0.000174(	*0.013233	*0.012694	*0.017402	*0.013536	*0.0302701592445374	

Newman-Keuls test; BD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	44.47000	41.84000	38.60000	38.89000	20.49000	0.000000
A	{0}		*0.000177	*0.000181	*0.000153(	*0.000197	*0.000158	*0.00017404556274414
B	{0.01	*0.000177323818206		0.720854	0.846856	0.72459	*0.034148(	*0.00041300058364868
C	{0.1}	*0.000181	0.720854		0.895536	0.688752	*0.045310(	*0.00049871206283569
D	{1}	*0.000153(	0.846856	0.895536		0.968631	*0.025034	*0.00043231248855590
E	{10}	*0.000197	0.72459	0.688752	0.968631		0.056514	*0.00065189599908447
F	{100}	*0.000158	*0.034148(	*0.045310(	*0.025034	0.056514		*0.0132139921188354
G	{500}	0.000174	*0.000413(	*0.000498	*0.000432	*0.000651	*0.0132139921188354	

Newman-Keuls test; MC_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	83.33000	66.67000	66.67000	50.00000	41.67000	0.000000
A	{0}		0.087522	*0.011929(	*0.006732(	*0.000739	*0.000314	*0.00017410516738
B	{0.01	0.087522		0.194029	0.087693	*0.011929(	*0.003325(	*0.00015884637832
C	{0.1}	*0.011929(	0.194029		1	0.087522	*0.038696	*0.00020658969879
D	{1}	*0.006732(	*0.087693	1		0.193691	0.065787	*0.00017660856246
E	{10}	*0.000739	*0.011929(	*0.087522(	0.193691		0.374162	*0.00036728382110
F	{100}	*0.000314	*0.003325(	*0.038696	*0.065787(	0.374162		*0.00056529045104
G	{500}	*0.000174	*0.000158	*0.000206	*0.000176(	*0.000367	*0.00056529045104	

Newman-Keuls test; MC_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	38.39000	29.90000	28.79000	30.45000	20.31000	0.000000
A	{0}		*0.000176(	*0.000196(	*0.000150	*0.000180	*0.000158	*0.00017404556274414
B	{0.01	*0.000176668167114		0.080891	0.077983	*0.045126(	*0.001605	*0.00015819072723388
C	{0.1}	*0.000196(	0.080891		0.762958	0.881102	*0.046438	*0.00019758939743042
D	{1}	*0.000150	0.077983	0.762958		0.890767	*0.034036(	*0.00018215179443359
E	{10}	*0.000180	*0.045126(	0.881102	0.890767		0.059579	*0.00015342235565185
F	{100}	*0.000158	*0.001605(	*0.046438	*0.034036(	0.059579		*0.00022631883621215
G	{500}	*0.000174	*0.000158	*0.000197	*0.000182	*0.000153	*0.00022631883621215	

Newman-Keuls test; DD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	63.40000	52.81000	52.05000	49.38000	44.73000	40.38000
A	{0}		*0.009125	*0.004316	*0.006836	*0.006847(	*0.004700	*0.00327533483505
B	{0.01	*0.009125232696533		0.39529	0.624863	0.659531	0.551579	0.437112
C	{0.1}	*0.004316	0.39529		0.950777	0.956697	0.906942	0.837638
D	{1}	*0.006836	0.624863	0.950777		0.828251	0.819055	0.770044
E	{10}	*0.006847(	0.659531	0.956697	0.828251		0.705985	0.741191
F	{100}	*0.004700	0.551579	0.906942	0.819055	0.705985		0.724066
G	{500}	*0.003275	0.437112	0.837638	0.770044	0.741191	0.724066	

Newman-Keuls test; DD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	59.83000	64.34000	48.06000	48.58000	48.48000	41.54000
A	{0}		*0.007003	*0.006033	*0.003631	*0.001920	*0.002811	*0.00174051523208618
B	{0.01	*0.007003188133239		0.687989	0.712288	0.323655	0.569581	0.484823
C	{0.1}	*0.006033	0.687989		0.590094	0.351403	0.495351	0.353059
D	{1}	*0.003631	0.712288	0.590094		0.998837	0.970205	0.562749
E	{10}	*0.001920	0.323655	0.351403	0.998837		0.992983	0.917227
F	{100}	*0.002811	0.569581	0.495351	0.970205	0.992983		0.805728
G	{500}	*0.001740	0.484823	0.353059	0.562749	0.917227	0.805728	

Newman-Keuls test; MA_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	33.51000	38.96000	38.22000	37.82000	34.35000	32.38000
A	{0}		*0.000158	*0.000176(	*0.000180	*0.000196(	*0.000150	*0.00017404556274414
B	{0.01	*0.000158190727233		0.841401	0.81374	0.704481	0.877254	0.83542
C	{0.1}	*0.000176(	0.841401		0.891792	0.975268	0.823042	0.813956
D	{1}	*0.000180	0.81374	0.891792		0.941391	0.752925	0.806476
E	{10}	*0.000196(	0.704481	0.975268	0.941391		0.526098	0.741027
F	{100}	*0.000150	0.877254	0.823042	0.752925	0.526098		0.928054
G	{500}	*0.000174(	0.83542	0.813956	0.806476	0.741027	0.928054	

Newman-Keuls test; MA_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	38.43000	48.85000	51.03000	33.96000	33.13000	26.67000
A	{0}		*0.000196(	*0.000180	*0.000176	*0.000150	*0.000158	*0.00017404556274414
B	{0.01	*0.000196039676666		0.086843	0.10101	0.4427	0.626827	0.207368
C	{0.1}	*0.000180	0.086843		0.705846	*0.048731	0.06311	*0.01133865111802673
D	{1}	*0.000176	0.10101	0.705846		*0.040814	*0.045847	*0.00768560171127319
E	{10}	*0.000150	0.4427	*0.048731	*0.040814161300659		0.885552	0.42415
F	{100}	*0.000158	0.626827	0.06311	*0.045847	0.885552		0.272687
G	{500}	*0.000174	0.207368	*0.011338	*0.007685	0.42415	0.272687	

APPENDIX 27: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD- Intensity of Band)

* = Significant difference : p<0.05

ANOVA-INTENSITY-CHL

Newman-Keuls test; CD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	59.11000	81.94000	78.60000	75.88000	53.05000	23.70000
A	{0}		*0.003016	0.059375	0.070205	0.067508	*0.001289	*0.0001769661903381
B	{0.01	*0.003016889095306		0.087646	0.103158	0.077463	0.502312	*0.003474891185760
C	{0.1}	*0.059374	0.087646		0.710003	0.773777	*0.036873	*0.0002706050872802
D	{1}	*0.070205	0.103158	0.710003		0.761855	0.050271	*0.0003046989440917
E	{10}	*0.067507	0.077463	0.773777	0.761855		0.052203	*0.0003684163093566
F	{100}	*0.001289	0.502312	*0.036873	0.050271	0.052203		*0.0050446987152095
G	{500}	*0.000176	*0.003474	*0.000270	*0.000304	*0.000368	*0.00504469871520996	

Newman-Keuls test; CD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	53.58000	55.96000	55.38000	34.35000	35.46000	6.370000
A	{0}		*0.001427	*0.000555	*0.001120	*0.000232	*0.000214	*0.000174343585968018
B	{0.01	*0.001427888870239		0.966585	0.853473	0.146038	0.079005	*0.00125116109848022
C	{0.1}	*0.000555	0.966585		0.95258	0.2147	0.187134	*0.00164693593978882
D	{1}	*0.001120	0.853473	0.95258		0.171201	0.129379	*0.00133234262466431
E	{10}	*0.000232	0.146038	0.2147	0.171201		0.909332	*0.0111995339393616
F	{100}	*0.000214	0.079005	0.187134	0.129379	0.909332		*0.0225018858909607
G	{500}	*0.000174	*0.001251	*0.001646	*0.001332	*0.011199	*0.0225018858909607	

Newman-Keuls test; FE_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	90.14000	106.2400	114.8400	94.46000	94.51000	93.86000
A	{0}		0.898479	0.585168	0.403045	0.874321	0.630645	0.945086
B	{0.01	0.898479		0.703024	0.347749	0.92132	0.978909	0.744045
C	{0.1}	0.585168	0.703024		0.454065	0.721061	0.558697	0.799337
D	{1}	0.403045	0.347749	0.454065		0.398106	0.304703	0.452184
E	{10}	0.874321	0.92132	0.721061	0.398106		0.996592	0.95802
F	{100}	0.630645	0.978909	0.558697	0.304703	0.996592		0.998213
G	{500}	0.945086	0.744045	0.799337	0.452184	0.95802	0.998213	

Newman-Keuls test; FE_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	68.83000	76.62000	84.21000	84.21000	76.83000	61.90000
A	{0}		*0.002710	*0.018109	0.064674	*0.026709	*0.012821	*0.000648736953735352
B	{0.01	*0.002710163593292		0.241797	0.120011	0.168529	0.44201	0.295273
C	{0.1}	*0.018109	0.241797		0.477487	0.642068	0.97431	0.087394
D	{1}	0.064674	0.120011	0.477487		1	0.266291	*0.0247138142585754
E	{10}	*0.026709	0.168529	0.642068	1		0.496117	*0.033561646938324
F	{100}	*0.012821	0.44201	0.97431	0.266291	0.496117		0.135213
G	{500}	*0.000648	0.295273	0.087394	*0.024713	*0.033561	0.135213	

Newman-Keuls test; MN_C_C4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	113.0100	105.5700	102.4500	90.00000	74.16000	71.97000
A	{0}		0.833731	0.931304	0.876388	0.5281	0.249924	0.307651
B	{0.01	0.833731		0.637716	0.776826	0.585109	0.184796	0.181167
C	{0.1}	0.931304	0.637716		0.842992	0.747662	0.300883	0.307445
D	{1}	0.876388	0.776826	0.842992		0.705713	0.300408	0.327299
E	{10}	0.5281	0.585109	0.747662	0.705713		0.322767	0.491124
F	{100}	0.249924	0.184796	0.300883	0.300408	0.322767		0.889422
G	{500}	0.307651	0.181167	0.307445	0.327299	0.491124	0.889422	

Newman-Keuls test; MN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	108.9200	112.9600	105.0700	101.9900	98.18000	87.40000
A	{0}		0.949851	0.934273	0.951083	0.907304	0.915196	0.737654
B	{0.01	0.949851		0.813167	0.821786	0.910761	0.965659	0.788827
C	{0.1}	0.934273	0.813167		0.886071	0.91232	0.944891	0.727127
D	{1}	0.951083	0.821786	0.886071		0.856971	0.975735	0.826217
E	{10}	0.907304	0.910761	0.91232	0.856971		0.972056	0.819948
F	{100}	0.915196	0.965659	0.944891	0.975735	0.972056		0.530705
G	{500}	0.737654	0.788827	0.727127	0.826217	0.819948	0.530705	

Newman-Keuls test; AD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	129.6300	125.0200	128.2900	114.1600	54.57000	0.000000
A	{0}		0.161317	0.134426	0.13813	0.263062	*0.002342	*0.0001816749572753
B	{0.01	0.161317		0.9241	0.913793	0.592965	*0.000406	*0.0001741051673889
C	{0.1}	0.134426	0.9241		0.791705	0.38632	*0.000411	*0.0001509785652160
D	{1}	0.13813	0.913793	0.791705		0.492854	*0.000364	*0.0001581907272338
E	{10}	0.263062	0.592965	0.38632	0.492854		*0.000770	*0.0001962184906005
F	{100}	*0.002342	*0.000406	*0.000411	*0.000364	*0.000770270824432		*0.0006499290466308
G	{500}	*0.000181	*0.000174	*0.000150	*0.000158	*0.000196	*0.00064992904663085	

Newman-Keuls test; AD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	81.91000	69.56000	61.13000	49.25000	5.410000	0.000000
A	{0}		0.084056	*0.019024	*0.006516	*0.001148	*0.000158	*0.000174224376678467
B	{0.01	*0.084055781364440		0.224814	0.118084	*0.021596	*0.000157	*0.000161588191986084
C	{0.1}	*0.019024	0.224814		0.400705	0.128217	*0.000241	*0.000184714794158936
D	{1}	*0.006516	0.118084	0.400705		0.242025	*0.000305	*0.00027805566787719
E	{10}	*0.001148	*0.021596	0.128217	0.242025		*0.000636	*0.000616729259490967
F	{100}	*0.000158	*0.000157	*0.000241	*0.000305	*0.000636		0.586827
G	{500}	*0.000174	*0.000161	*0.000184	*0.000278	*0.000616	0.586827	

APPENDIX 27: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD- Intensity of Band)

* = Significant difference : p<0.05

ANOVA-INTENSITY-CHL

Newman-Keuls test; BD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	134.2100	146.7500	175.1200	184.6200	157.3500	0.000000
A	{0}		*0.020122	*0.007941	*0.000522	*0.000298	*0.003115	*0.000177443027496
B	{0.01	*0.020122	30052948	0.352364	*0.032587	*0.012505	0.213502	*0.000180900096893
C	{0.1}	*0.007941	0.352364		0.110277	0.050122	0.429757	*0.000196039676666
D	{1}	*0.000522	*0.032587	0.110277		0.478176	0.194324	*0.000158190727233
E	{10}	*0.000298	*0.012505	0.050122	0.478176		0.127418	&0.000174045562744
F	{100}	*0.003115	0.213502	0.429757	0.194324	0.127418		*0.000150978565216
G	{500}	*0.000177	*0.000180	*0.000196	*0.000158	*0.000174	*0.000150978565216064	

Newman-Keuls test; BD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	96.61000	111.5000	103.7300	75.67000	78.22000	0.000000
A	{0}		0.754166	0.539061	0.730518	0.146895	0.136247	*0.000151336193084717
B	{0.01	0.754166		0.517611	0.78376	0.155447	0.105102	*0.000196456909179687
C	{0.1}	0.539061	0.517611		0.476179	*0.042037	*0.048216	*0.000174164772033691
D	{1}	0.730518	0.78376	0.476179		0.114866	0.121889	*0.000158309936523437
E	{10}	0.146895	0.155447	*0.042037	0.114866		0.813662	*0.000179052352905273
F	{100}	0.136247	0.105102	*0.048216	0.121889	0.813662		*0.000185489654541016
G	{500}	*0.000151	*0.000196	*0.000174	*0.000158	*0.000179	*0.000185489654541016	

Newman-Keuls test; MC_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	69.98000	85.37000	95.08000	119.1200	151.2300	0.000000
A	{0}		*0.020972	0.260507	0.589069	*0.049699	*0.000298	*0.000150978565216
B	{0.01	*0.020972	28729248	0.105718	*0.034229	*0.000728	*0.000159	*0.000177145004272
C	{0.1}	0.260507	0.105718		0.293585	*0.009537	*0.000174	*0.000180959701538
D	{1}	0.589069	*0.034229	0.293585		*0.042780	*0.000274	*0.000196039676666
E	{10}	*0.049699	*0.000728	*0.009537	*0.042780	518531799	*0.002993	*0.000158190727233
F	{100}	*0.000298	*0.000159	*0.000174	*0.000274	*0.002993	583679199	*0.000174045562744
G	{500}	*0.000150	*0.000177	*0.000180	*0.000196	*0.000158	*0.000174045562744141	

Newman-Keuls test; MC_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	97.14000	97.74000	95.26000	111.3000	66.10000	0.000000
A	{0}		0.94613	0.805462	0.951226	0.229833	*0.015072	*0.000158190727233887
B	{0.01	0.94613		0.947872	0.837616	0.423661	*0.010336	*0.000196039676666626
C	{0.1}	0.805462	0.947872		0.9592	0.317625	*0.016061	*0.000150978565216064
D	{1}	0.951226	0.837616	0.9592		0.420318	*0.006065	*0.000180900096893311
E	{10}	0.229833	0.423661	0.317625	0.420318		*0.002161	*0.000174045562744141
F	{100}	*0.015072	*0.010336	*0.016061	*0.006065	*0.002161	502838134	*0.000178158283233643
G	{500}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000174	*0.000178158283233643	

Newman-Keuls test; DD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	94.18000	99.86000	114.9800	132.2800	126.3000	98.86000
A	{0}		0.986938	0.993855	0.407815	0.296758	0.321283	0.997776
B	{0.01	0.986938		0.944195	0.759416	0.367286	0.478902	0.7937
C	{0.1}	0.993855	0.944195		0.672271	0.386946	0.459623	0.955461
D	{1}	0.407815	0.759416	0.672271		0.597369	0.52943	0.795491
E	{10}	0.296758	0.367286	0.386946	0.597369		0.738452	0.438444
F	{100}	0.321283	0.478902	0.459623	0.52943	0.738452		0.541678
G	{500}	0.997776	0.7937	0.955461	0.795491	0.438444	0.541678	

Newman-Keuls test; DD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	93.91000	95.59000	87.81000	73.42000	51.15000	37.76000
A	{0}		0.886576	0.739016	0.784387	0.293909	*0.020662	*0.00417119264602661
B	{0.01	0.886576		0.898903	0.645499	0.286382	*0.024267	*0.00534504652023315
C	{0.1}	0.739016	0.898903		0.822522	0.355401	*0.028377	*0.00583058595657349
D	{1}	0.784387	0.645499	0.822522		0.286014	*0.033903	*0.00844466862487 79
E	{10}	0.293909	0.286382	0.355401	0.286014		0.108117	*0.0391803979873657
F	{100}	*0.020662	*0.024267	*0.028377	*0.033903	0.108117		0.319497
G	{500}	*0.004171	*0.005345	*0.005830	*0.008444	*0.039180	0.319497	

Newman-Keuls test; MA_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	129.7900	153.8000	157.9900	120.0000	65.69000	57.13000
A	{0}		0.186711	*0.021534	*0.019744	0.232133	0.050264	*0.044800877571106
B	{0.01	0.186711		0.156	0.218239	0.550777	*0.006441	*0.003660678863525
C	{0.1}	*0.021534	0.156		0.79746	0.123425	*0.000748	*0.00047737360000061
D	{1}	*0.019744	0.218239	0.79746		0.128548	*0.000680	*0.000444352626800
E	{10}	0.232133	0.550777	0.123425	0.128548		*0.011538	*0.007438004016876
F	{100}	0.050264	*0.006441	*0.000748	*0.000680	*0.011538	3863449097	0.601361
G	{500}	*0.044800	*0.003660	*0.000477	*0.000444	*0.007438	0.601361	

Newman-Keuls test; MA_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	87.16000	90.86000	90.45000	112.6600	88.03000	85.40000
A	{0}		0.825795	0.465251	0.718234	0.315989	0.760975	0.830035
B	{0.01	0.825795		0.989882	0.960717	0.343032	0.944116	0.887201
C	{0.1}	0.465251	0.989882		0.97374	0.208414	0.970775	0.990739
D	{1}	0.718234	0.960717	0.97374		0.303027	0.845365	0.975072
E	{10}	0.315989	0.343032	0.208414	0.303027		0.304828	0.335113
F	{100}	0.760975	0.944116	0.970775	0.845365	0.304828		0.974714
G	{500}	0.830035	0.887201	0.990739	0.975072	0.335113	0.974714	

APPENDIX 27: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD-Genomic Template Stability)

* = Significant difference : p<0.05

ANOVA-GTS-CHL

Newman-Keuls test; CD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	34.35000	28.42000	27.68000	27.68000	25.08000	15.18000
A	{0}		*0.000177	*0.000181	*0.000154	*0.000197	*0.000160	*0.000174522399902
B	{0.01	*0.000177	0.510411	0.510411	0.870981	0.732782	0.82521	0.303343
C	{0.1}	*0.000181	0.510411		0.996177	0.934107	0.980553	0.573801
D	{1}	*0.000154	0.870981	0.996177		1	0.771518	0.355793
E	{10}	*0.000197	0.732782	0.934107	1		0.953036	0.50573
F	{100}	*0.000160	0.82521	0.980553	0.771518	0.953036		0.278392
G	{500}	*0.000174	0.303343	0.573801	0.355793	0.50573	0.278392	

Newman-Keuls test; CD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	36.31000	33.53000	28.28000	24.05000	20.53000	20.41000
A	{0}		*0.000177	*0.000182	*0.000196	*0.000151	*0.000158	*0.000174641609191895
B	{0.01	*0.000177	0.743785	0.743785	0.610868	0.479386	0.364412	0.436734
C	{0.1}	*0.000182	0.743785		0.539032	0.507971	0.431003	0.535642
D	{1}	*0.000196	0.610868	0.539032		0.61981	0.631131	0.782067
E	{10}	*0.000151	0.479386	0.507971	0.61981		0.67931	0.900982
F	{100}	*0.000158	0.364412	0.431003	0.631131	0.67931		0.98883
G	{500}	*0.000174	0.436734	0.535642	0.782067	0.900982	0.98883	

Newman-Keuls test; FE_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	43.26000	50.48000	46.67000	40.00000	40.00000	20.00000
A	{0}		*0.000268	*0.000235	*0.000260	*0.000253	*0.000218	*0.000175595283508
B	{0.01	*0.000268	0.704115	0.704115	0.708431	0.929624	0.720647	0.08621
C	{0.1}	*0.000235	0.704115		0.676257	0.765754	0.652432	*0.039398908615112
D	{1}	*0.000260	0.708431	0.676257		0.876539	0.740431	0.063213
E	{10}	*0.000253	0.929624	0.765754	0.876539		1	*0.041995346546173
F	{100}	*0.000218	0.720647	0.652432	0.740431	1		0.098927
G	{500}	*0.000175	0.08621	*0.039398	0.063213	*0.041995	0.098927	

Newman-Keuls test; FE_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	39.64000	31.11000	23.33000	21.86000	19.84000	17.70000
A	{0}		*0.000177	*0.000181	*0.000196	*0.000151	*0.000158	*0.000174105167388916
B	{0.01	*0.000177	0.290698	0.290698	0.125809	0.147843	0.134866	0.110983
C	{0.1}	*0.000181	0.290698		0.333584	0.477529	0.490514	0.450094
D	{1}	*0.000196	0.125809	0.333584		0.852702	0.895532	0.88558
E	{10}	*0.000151	0.147843	0.477529	0.852702		0.798694	0.855352
F	{100}	*0.000158	0.134866	0.490514	0.895532	0.798694		0.787036
G	{500}	*0.000174	0.110983	0.450094	0.88558	0.855352	0.787036	

Newman-Keuls test; MN_C_C4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	33.93000	26.19000	26.19000	23.21000	21.43000	17.68000
A	{0}		*0.000176	*0.000196	*0.000180	*0.000150	*0.000158	*0.000174045562744
B	{0.01	*0.000176	0.010489	*0.004122	*0.001650	*0.000693	*0.000192	*0.000176681671142
C	{0.1}	*0.000196	*0.010489	1	0.206426	0.122289	*0.009707	*0.010489106178283
D	{1}	*0.000180	*0.004122	1		0.40512	0.195464	*0.014607608318328
E	{10}	*0.000150	*0.001650	0.206426	0.40512		0.441968	0.06691
F	{100}	*0.000158	*0.000693	0.122289	0.195464	0.441968		0.117709
G	{500}	*0.000174	*0.000192	*0.009707	*0.014607	0.06691	0.117709	

Newman-Keuls test; MN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	44.87000	42.73000	37.45000	35.91000	33.74000	33.54000
A	{0}		*0.000183	*0.000194	*0.000204	*0.000168	*0.000167	*0.000186562538146973
B	{0.01	*0.000183	0.802204	0.802204	0.657764	0.712846	0.679099	0.752706
C	{0.1}	*0.000194	0.802204		0.5388	0.70075	0.710799	0.80529
D	{1}	*0.000204	0.657764	0.5388		0.856898	0.898354	0.965183
E	{10}	*0.000168	0.712846	0.70075	0.856898		0.799496	0.957064
F	{100}	*0.000167	0.679099	0.710799	0.898354	0.799496		0.981396
G	{500}	*0.000186	0.752706	0.80529	0.965183	0.957064	0.981396	

Newman-Keuls test; AD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	13.33000	13.33000	13.33000	6.670000	0.000000	0.000000
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000174	*0.000158197272733
B	{0.01	*0.000196		1	1	*0.001835	*0.000200	*0.000182986259460
C	{0.1}	*0.000180	1		1	*0.004625	*0.000159	*0.000200808048248
D	{1}	*0.000176	1	1		*0.008228	*0.000170	*0.000159800052642
E	{10}	*0.000150	*0.001835	*0.004625	*0.008228		*0.004575	*0.001816277273559
F	{100}	*0.000174	*0.000200	*0.000159	*0.000170	*0.004575		1
G	{500}	*0.000158	*0.000182	*0.000200	*0.000159	*0.001816	1	

Newman-Keuls test; AD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	56.79000	45.08000	43.34000	0.000000	0.000000	0.000000
A	{0}		*0.000181	*0.000181	*0.000196	*0.000174	*0.000158	*0.000150978565216064
B	{0.01	*0.000181	0.088508	0.088508	0.125061	*0.000159	*0.000151	*0.000196635723114014
C	{0.1}	*0.000181	0.088508		0.789603	*0.000190	*0.000215	*0.000189661979675293
D	{1}	*0.000196	0.125061	0.789603		*0.000228	*0.000195	*0.00018155574798584
E	{10}	*0.000174	*0.000159	*0.000190	*0.000228		1	1
F	{100}	*0.000158	*0.000151	*0.000215	*0.000195	1		1
G	{500}	*0.000150	*0.000196	*0.000189	*0.000181	1	1	

APPENDIX 27: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (RAPD-Genomic Template Stability)

* = Significant difference : p<0.05

ANOVA-GTS-CHL

Newman-Keuls test; BD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	16.67000	16.67000	8.330000	0.000000	0.000000	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000174	*0.000158	*0.000150978565216
B	{0.01	*0.000180	9000968933	1	0.05239	*0.006299	*0.004170	*0.002355217933654
C	{0.1}	*0.000176	1		0.121444	*0.008692	*0.006299	*0.004170238971710
D	{1}	*0.000196	0.05239	0.121444		0.194995	0.121972	0.052637
E	{10}	*0.000174	*0.006299	*0.008692	0.194995		1	1
F	{100}	*0.000158	*0.004170	*0.006299	0.121972	1		1
G	{500}	*0.000150	*0.002355	*0.004170	0.052637	1	1	

Newman-Keuls test; BD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	21.43000	14.29000	16.67000	9.820000	2.380000	0.000000
A	{0}		*0.000176	*0.000196	*0.000180	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	*0.000555	*0.004223	*0.000197	*0.000150	*0.000158190727233887
C	{0.1}	*0.000196	*0.000555	9325218200	0.108577	*0.006309	*0.000181	*0.000196099281311035
D	{1}	*0.000180	*0.004223	0.108577		*0.000740	*0.000196	*0.000150978565216064
E	{10}	*0.000150	*0.000197	*0.006309	*0.000740	9453392028	*0.000261	*0.000189244747161865
F	{100}	*0.000158	*0.000150	*0.000181	*0.000196	*0.000261	5451812742	0.108577
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000189	0.108577	

Newman-Keuls test; MC_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	22.22000	33.33000	22.22000	22.22000	0.000000	0.000000
A	{0}		*0.000150	*0.000176	*0.000196	*0.000180	*0.000174	*0.000158190727233
B	{0.01	*0.000150	9785652160	0.18066	1	1	*0.001990	*0.000831365585327
C	{0.1}	*0.000176	0.18066		0.112343	*0.048154	*0.000297	*0.000248789787292
D	{1}	*0.000196	1	0.112343		1	*0.003520	*0.001990556716918
E	{10}	*0.000180	1	*0.048154	1		*0.005313	*0.003520727157592
F	{100}	*0.000174	*0.001990	*0.000297	*0.003520	*0.005313754081726		1
G	{500}	*0.000158	*0.000831	*0.000248	*0.001990	*0.003520	1	

Newman-Keuls test; MC_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	11.12000	7.150000	7.150000	6.700000	4.910000	0.000000
A	{0}		*0.000176	*0.000196	*0.000180	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	*0.001729	*0.000733	*0.001300	*0.000200	*0.000158190727233887
C	{0.1}	*0.000196	*0.001729	3691635131	1	0.624803	0.063265	*0.000199258327484131
D	{1}	*0.000180	*0.000733	1		0.872427	0.105265	*0.000156998634338379
E	{10}	*0.000150	*0.001300	0.624803	0.872427		0.066626	*0.000184834003448486
F	{100}	*0.000158	*0.000200	0.063265	0.105265	0.066626		*0.000246524810791016
G	{500}	*0.000174	*0.000158	*0.000199	*0.000156	*0.000184	*0.000246524810791016	

Newman-Keuls test; DD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	46.47000	29.03000	28.25000	24.30000	22.87000	14.72000
A	{0}		*0.000177	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744
B	{0.01	*0.000177	5026321411	*0.027157	0.05371	*0.032606	*0.033185	*0.005460023880004
C	{0.1}	*0.000180	*0.027157	6642990112	0.913745	0.784541	0.819054	0.303798
D	{1}	*0.000196	0.05371	0.913745		0.584939	0.73175	0.265771
E	{10}	*0.000150	*0.032606	0.784541	0.584939		0.842582	0.389194
F	{100}	*0.000158	*0.033185	0.819054	0.73175	0.842582		0.267963
G	{500}	*0.000174	*0.005460	0.303798	0.265771	0.389194	0.267963	

Newman-Keuls test; DD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	38.93000	37.04000	32.40000	29.03000	28.78000	25.14000
A	{0}		*0.000176	*0.000181	*0.000196	*0.000151	*0.000158	*0.000174105167388916
B	{0.01	*0.000176	8469810485	0.791329	0.629645	0.511875	0.608625	0.404756
C	{0.1}	*0.000181	0.791329		0.51856	0.50441	0.649007	0.465163
D	{1}	*0.000196	0.629645	0.51856		0.637973	0.864511	0.731561
E	{10}	*0.000151	0.511875	0.50441	0.637973		0.97216	0.845494
F	{100}	*0.000158	0.608625	0.649007	0.864511	0.97216		0.611515
G	{500}	*0.000174	0.404756	0.465163	0.731561	0.845494	0.611515	

Newman-Keuls test; MA_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	32.72000	40.95000	34.05000	24.32000	21.28000	18.33000
A	{0}		*0.000196	*0.000176	*0.000180	*0.000150	*0.000158	*0.000174045562744
B	{0.01	*0.000196	0992813110	0.438691	0.841493	0.218845	0.220901	0.16949
C	{0.1}	*0.000176	0.438691		0.308203	0.094993	0.060028	*0.035676300525665
D	{1}	*0.000180	0.841493	0.308203		0.324534	0.249758	0.169576
E	{10}	*0.000150	0.218845	0.094993	0.324534		0.648508	0.638161
F	{100}	*0.000158	0.220901	0.060028	0.249758	0.648508		0.658171
G	{500}	*0.000174	0.16949	*0.035676	0.169576	0.638161	0.658171	

Newman-Keuls test; MA_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	27.63000	35.01000	36.78000	30.52000	28.66000	23.94000
A	{0}		*0.000158	*0.000180	*0.000176	*0.000196	*0.000150	*0.000174045562744141
B	{0.01	*0.000158	1907272338	0.546346	0.476557	0.858065	0.852965	0.509664
C	{0.1}	*0.000180	0.546346		0.750348	0.424121	0.492334	0.301865
D	{1}	*0.000176	0.476557	0.750348		0.501747	0.469127	0.236286
E	{10}	*0.000196	0.858065	0.424121	0.501747		0.738158	0.6327
F	{100}	*0.000150	0.852965	0.492334	0.469127	0.738158		0.669765
G	{500}	*0.000174	0.509664	0.301865	0.236286	0.6327	0.669765	

APPENDIX 28: ANOVA: *Chlorella vulgaris* UMACC 245: DNA Damage (AP-Site)

* = Significant difference : p<0.05

ANOVA-AP-SITE-CHL

Newman-Keuls test; FE_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		23.67800	33.66200	36.18000	41.61800	48.03400	28.46900	0.000000
A	{0}		*0.000181(	*0.000196(	*0.000150(	*0.000158(	*0.000874(	*0.000176
B	{0.01	*0.000181078910827(		*0.040392(	*0.000188(	*0.000196(	*0.000516(	*0.000196
C	{0.1}	*0.000196(	*0.040392756462097(		*0.000396(	*0.000180(	*0.000192(	*0.000150
D	{1}	*0.000150(	*0.000188(	*0.000396072864532(		*0.000215(	*0.000196(	*0.000158
E	{10}	*0.000158(	*0.000196(	*0.000180(	*0.000215113162994(		*0.000150(	*0.000174
F	{100}	*0.000874(	*0.000516(	*0.000192(	*0.000196(	*0.000150978565216(		*0.000180
G	{500}	*0.000176(	*0.000196(	*0.000150(	*0.000158(	*0.000174(	*0.000180	

Newman-Keuls test; MN_C_C4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		23.67800	31.71900	31.36700	31.23300	31.75200	32.00300	0.000000
A	{0}		*0.015165(	*0.011559(	*0.005082(	*0.022109(	*0.024575(	*0.000176
B	{0.01	*0.015165746212005(		0.878942	0.97511	0.988708	0.991471	*0.000150
C	{0.1}	*0.011559(	0.878942		0.953797	0.984328	0.992016	*0.000196
D	{1}	*0.005082(	0.97511	0.953797		0.995629	0.996817	*0.000180
E	{10}	*0.022109(	0.988708	0.984328	0.995629		0.91353	*0.000158
F	{100}	*0.024575(	0.991471	0.992016	0.996817	0.91353		*0.000174
G	{500}	*0.000176(	*0.000150(	*0.000196(	*0.000180(	*0.000158(	*0.000174	

Newman-Keuls test; FE_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		42.52300	42.89100	48.13400	45.52100	43.15400	42.79100	42.02200
A	{0}		0.99316	0.547354	0.886163	0.997392	0.936161	0.880931
B	{0.01	0.99316		0.41198	0.708007	0.937349	0.976241	0.993203
C	{0.1}	0.547354	0.41198		0.439633	0.314094	0.505064	0.532333
D	{1}	0.886163	0.708007	0.439633		0.482972	0.837875	0.885525
E	{10}	0.997392	0.937349	0.314094	0.482972		0.993344	0.996569
F	{100}	0.936161	0.976241	0.505064	0.837875	0.993344		0.970237
G	{500}	0.880931	0.993203	0.532333	0.885526	0.99657	0.970237	

Newman-Keuls test; MN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		42.52300	51.25000	52.25500	52.40000	53.17600	51.41700	50.88500
A	{0}		*0.047330(	0.067214	0.082587	0.069454	0.072719	*0.0238
B	{0.01	*0.047330021858215(		0.949424	0.984403	0.974309	0.960111	0.912837
C	{0.1}	0.067214	0.949424		0.965378	0.957341	0.801619	0.97421
D	{1}	0.082587	0.984403	0.965378		0.816009	0.951562	0.989406
E	{10}	0.069454	0.974309	0.957341	0.816009		0.948029	0.978489
F	{100}	0.072719	0.960111	0.801619	0.951562	0.948029		0.985576
G	{500}	*0.0238661	0.912837	0.974211	0.989407	0.97849	0.985576	

APPENDIX 29: ANOVA: *Chlorella vulgaris* UMACC 245: Superoxide dismutase (SOD) activity

* = Significant difference : p<0.05

ANOVA-SOD-CHL

Newman-Keuls test; CD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		53.85800	55.89800	53.55500	52.63500	60.64600	78.83500	112.4520
A	{0}		0.564462	0.931467	0.933682	0.157818	*0.000209	*0.000150
B	{0.01	0.564462		0.779848	0.782028	0.191113	*0.000200	*0.000196
C	{0.1}	0.931467	0.779848		0.794037	0.216274	*0.000177	*0.000158
D	{1}	0.933682	0.782028	0.794037		0.19611	*0.000175	*0.000174
E	{10}	0.157818	0.191113	0.216274	0.19611		*0.000279	*0.000180
F	{100}	0.00021	0.000201	*0.000177	*0.000175	*0.000279247760772		*0.000176
G	{500}	0.000151	0.000196	*0.000158	*0.000174	*0.000180	*0.000176	

Newman-Keuls test; CD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		50.18200	38.65900	36.76900	38.43600	49.56100	77.88000	63.53600
A	{0}		*0.002824	*0.002238	*0.004330	0.826853	*0.000180	*0.000436
B	{0.01	*0.002824664115905		0.779469	0.937404	*0.001695	*0.000150	*0.000196
C	{0.1}	*0.002238	0.779469		0.559081	*0.002219	*0.000174	*0.000158
D	{1}	*0.004330	0.937404	0.559081		*0.003672	*0.000158	*0.000151
E	{10}	0.826853	*0.001695	*0.002219	*0.003672182559967		*0.000196	*0.000657
F	{100}	*0.000180	*0.000150	*0.000174	*0.000158	*0.000196092813110		*0.000305
G	{500}	*0.000436	*0.000196	*0.000158	*0.000151	*0.000657	*0.000305	

Newman-Keuls test; FE_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		53.85800	49.95200	51.51300	61.09300	73.85900	53.21900	45.84900
A	{0}		0.593443	0.730111	*0.033524	*0.000205	0.838078	0.121613
B	{0.01	0.593443		0.618858	*0.019387	*0.000169	0.550137	0.202428
C	{0.1}	0.730111	0.618858		*0.033568	*0.000178	0.586978	0.190996
D	{1}	*0.033524	*0.019387	*0.033568263053894		*0.001097	0.054964	*0.002369
E	{10}	*0.000205	*0.000169	*0.000178	*0.001097559928894		*0.000231	*0.000175
F	{100}	0.838078	0.550137	0.586978	0.054964	*0.000231385231018		0.122233
G	{500}	0.121614	0.202428	0.190997	*0.002369	*0.000175	0.122233	

Newman-Keuls test; FE_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		50.18200	60.52400	44.70000	48.95200	19.65000	2.042000	0.000000
A	{0}		*0.000597	0.072627	0.596739	*0.000196	*0.000150	*0.000158
B	{0.01	*0.000597655773162		*0.000218	*0.000592	*0.000150	*0.000158	*0.000174
C	{0.1}	0.072627	*0.000218331813812		0.082328	*0.000176	*0.000180	*0.000196
D	{1}	0.596739	*0.000592	0.082328		*0.000180	*0.000196	*0.000150
E	{10}	*0.000196	*0.000150	*0.000176	*0.000180900096893		*0.000177	*0.000181
F	{100}	*0.000150	*0.000158	*0.000180	*0.000196	*0.000177204608917		0.38396
G	{500}	*0.000158	*0.000174	*0.000196	*0.000150	*0.000181	0.38396	

Newman-Keuls test; MN_C_C4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		53.85800	51.44700	66.31100	63.24800	74.03200	69.62900	64.59300
A	{0}		0.383501	*0.002015	*0.003650	*0.000177	*0.000445	*0.003592
B	{0.01	0.383501		*0.000703	*0.001753	*0.000178	*0.000242	*0.001313
C	{0.1}	*0.002015	*0.000703454017639		0.504552	*0.030520	0.23596	0.531813
D	{1}	*0.003650	*0.001753	0.504552		*0.009355	0.126619	0.62354
E	{10}	*0.000177	*0.000178	*0.030520	*0.009355783462524		0.12265	*0.015845
F	{100}	*0.000445	*0.000242	0.23596	0.126619	0.12265		0.181107
G	{500}	*0.003592	*0.001313	0.531813	0.62354	*0.015845	0.181107	

Newman-Keuls test; MN_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		50.18200	52.61700	52.67400	39.42400	22.82700	1.295000	.2075000
A	{0}		0.478841	0.741581	*0.006365	*0.000181	*0.000196	*0.000150
B	{0.01	0.478841		0.98676	*0.004042	*0.000196	*0.000150	*0.000158
C	{0.1}	0.741581	0.98676		*0.006990	*0.000151	*0.000158	*0.000174
D	{1}	*0.006365	*0.004042	*0.006990134716033		*0.000363	*0.000180	*0.000196
E	{10}	*0.000181	*0.000196	*0.000151	*0.000363767147064		*0.000186	*0.000196
F	{100}	*0.000196	*0.000150	*0.000158	*0.000180	*0.000186622142791		0.750053
G	{500}	*0.000150	*0.000158	*0.000174	*0.000196	*0.000196	0.750053	

Newman-Keuls test; AD_C_D4 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		53.85800	36.92800	47.83000	49.49700	40.21000	35.87800	0.000000
A	{0}		*0.000900	0.170964	0.187565	*0.003491	*0.000731	*0.000174
B	{0.01	*0.000900387763977		*0.010045	*0.006558	0.314733	0.743689	*0.000180
C	{0.1}	0.170964	*0.010045111179351		0.604693	*0.029747	*0.009458	*0.000150
D	{1}	0.187565	*0.006558	0.604693		*0.026773	*0.005357	*0.000158
E	{10}	*0.003491	0.314733	*0.029747	*0.026773691177368		0.379026	*0.000196
F	{100}	*0.000731	0.743689	*0.009458	*0.005357	0.379026		*0.000176
G	{500}	*0.000174	*0.000180	*0.000150	*0.000158	*0.000196	*0.000176668167114258	

Newman-Keuls test; AD_C_D10 (anova-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		50.18200	136.0700	139.2090	142.3980	132.9170	34.99600	0.000000
A	{0}		*0.000180	*0.000196	*0.000150	*0.000176	*0.004921	*0.000180
B	{0.01	*0.000180900096893		0.500221	0.369688	0.498344	*0.000196	*0.000150
C	{0.1}	*0.000196	0.500221		0.493536	0.373586	*0.000150	*0.000158
D	{1}	*0.000150	0.369688	0.493536		0.203541	*0.000158	*0.000174
E	{10}	*0.000176	0.498344	0.373586	0.203541		*0.000180	*0.000196
F	{100}	*0.004921	*0.000196	*0.000150	*0.000158	*0.000180900096893		*0.000177
G	{500}	*0.000180	*0.000150	*0.000158	*0.000174	*0.000196	*0.000177	

APPENDIX 29: ANOVA: *Chlorella vulgaris* UMACC 245: Superoxide dismutase (SOD) activity

* = Significant difference : p<0.05

ANOVA-SOD-CHL

Newman-Keuls test; BD_C_D4 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		53.85800	30.22200	33.86400	39.77200	41.51400	31.03300	0.000000
A	{0}		*0.000286	*0.000548	*0.004384	*0.004310	*0.000284	*0.000174
B	{0.01}	*0.000286		0.583744	0.080301	*0.049065	0.825651	*0.000176
C	{0.1}	*0.000548	0.583744		0.124219	0.12191	0.44624	*0.000196
D	{1}	*0.004384	0.080301	0.124219		0.637094	0.071799	*0.000150
E	{10}	*0.004310	*0.049065	0.12191	0.637094		0.050434	*0.000158
F	{100}	*0.000284	0.825651	0.44624	0.071799	0.050434		*0.000181
G	{500}	*0.000174	*0.000176	*0.000196	*0.000150	*0.000158	*0.000181	

Newman-Keuls test; BD_C_D10 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		50.18200	107.0090	137.2560	178.8470	184.0840	53.20600	0.000000
A	{0}		*0.000180	*0.000196	*0.000150	*0.000158	0.314869	*0.000176
B	{0.01}	*0.000180		*0.000176	*0.000180	*0.000196	*0.000176	*0.000196
C	{0.1}	*0.000196	*0.000176		*0.000176	*0.000180	*0.000180	*0.000150
D	{1}	*0.000150	*0.000180	*0.000176		0.09262	*0.000196	*0.000158
E	{10}	*0.000158	*0.000196	*0.000180	0.09262		*0.000150	*0.000174
F	{100}	0.314869	*0.000176	*0.000180	*0.000196	*0.000150		*0.000180
G	{500}	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174	*0.000180	

Newman-Keuls test; MC_C_D4 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		53.85800	34.61000	37.86500	44.85600	63.13500	25.14000	0.000000
A	{0}		*0.001028	*0.002487	*0.032884	*0.028648	*0.000170	*0.000158
B	{0.01}	*0.001028		0.406263	*0.043267	*0.000172	*0.025994	*0.000180
C	{0.1}	*0.002487	0.406263		0.08722	*0.000237	*0.012545	*0.000196
D	{1}	*0.032884	*0.043267	0.08722		*0.000893	*0.000858	*0.000150
E	{10}	*0.028648	*0.000172	*0.000237	*0.000893		*0.000158	*0.000174
F	{100}	*0.000170	*0.025994	*0.012545	*0.000858	*0.000158		*0.000183
G	{500}	*0.000158	*0.000180	*0.000196	*0.000150	*0.000174	*0.000183	

Newman-Keuls test; MC_C_D10 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		50.18200	109.3970	110.5690	202.9860	182.7230	105.9280	0.000000
A	{0}		*0.000180	*0.000196	*0.000158	*0.000150	*0.000176	*0.000176
B	{0.01}	*0.000180		0.811462	*0.000196	*0.000180	0.48353	*0.000196
C	{0.1}	*0.000196	0.811462		*0.000180	*0.000176	0.611001	*0.000150
D	{1}	*0.000158	*0.000196	*0.000180		*0.001020	*0.000150	*0.000174
E	{10}	*0.000150	*0.000180	*0.000176	*0.001020		*0.000196	*0.000158
F	{100}	*0.000176	0.48353	0.611001	*0.000150	*0.000196		*0.000180
G	{500}	*0.000176	*0.000196	*0.000150	*0.000174	*0.000158	*0.000180	

Newman-Keuls test; DD_C_D4 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		53.85800	33.28300	41.28700	45.69400	50.82300	27.33100	23.35500
A	{0}		*0.000150	*0.000213	*0.001220	0.108395	*0.000158	*0.000174
B	{0.01}	*0.000150		*0.000622	*0.000190	*0.000196	*0.004779	*0.000335
C	{0.1}	*0.000213	*0.000622		*0.026042	*0.000415	*0.000182	*0.000196
D	{1}	*0.001220	*0.000190	*0.026042		*0.011817	*0.000196	*0.000150
E	{10}	0.108395	*0.000196	*0.000415	*0.011817		*0.000150	*0.000158
F	{100}	*0.000158	*0.004779	*0.000182	*0.000196	*0.000150		*0.041372
G	{500}	*0.000174	*0.000335	*0.000196	*0.000150	*0.000158	*0.041372	

Newman-Keuls test; DD_C_D10 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		50.18200	45.98800	66.17000	53.22700	48.01700	44.29300	40.32600
A	{0}		0.346801	*0.000371	0.312589	0.468752	0.225014	*0.030266
B	{0.01}	0.346801		*0.000197	0.105097	0.496639	0.569119	0.162062
C	{0.1}	*0.000371	*0.000197		*0.000690	*0.000284	*0.000177	*0.000175
D	{1}	0.312589	0.105097	*0.000690		0.207884	0.053989	*0.006038
E	{10}	0.468752	0.496639	*0.000284	0.207884		0.428058	0.08005
F	{100}	0.225014	0.569119	*0.000177	0.053989	0.428058		0.19387
G	{500}	*0.030266	0.162062	*0.000175	*0.006038	0.08005	0.19387	

Newman-Keuls test; MA_C_D4 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		53.85800	41.75600	50.28000	55.23700	47.84100	48.38300	19.48000
A	{0}		*0.020768	0.305922	0.688388	0.3194	0.267575	*0.000158
B	{0.01}	*0.020768		0.097862	*0.013352	0.092315	0.156751	*0.000183
C	{0.1}	0.305922	0.097862		0.333168	0.753413	0.582111	*0.000151
D	{1}	0.688388	*0.013352	0.333168		0.236483	0.221718	*0.000174
E	{10}	0.3194	0.092315	0.753413	0.236483		0.874503	*0.000181
F	{100}	0.267575	0.156751	0.582111	0.221718	0.874503		*0.000196
G	{500}	*0.000158	*0.000183	*0.000151	*0.000174	*0.000181	*0.000196	

Newman-Keuls test; MA_C_D10 (anova-chl.sta)

Probabilities for Post Hoc Tests

MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		50.18200	76.17000	81.48100	71.92100	66.93100	37.21900	26.57700
A	{0}		*0.000196	*0.000150	*0.000183	*0.000205	*0.000579	*0.000181
B	{0.01}	*0.000196		0.081991	0.15603	*0.014837	*0.000150	*0.000158
C	{0.1}	*0.000150	0.081991		*0.011949	*0.000927	*0.000158	*0.000174
D	{1}	*0.000183	0.15603	*0.011949		0.100134	*0.000196	*0.000150
E	{10}	*0.000205	*0.014837	*0.000927	0.100134		*0.000180	*0.000196
F	{100}	*0.000579	*0.000150	*0.000158	*0.000196	*0.000180		*0.002283
G	{500}	*0.000181	*0.000158	*0.000174	*0.000150	*0.000196	*0.002283	

APPENDIX 30: ANOVA: *Tetraselmis tetrahele* UMACC 144: Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-TET

Newman-Keuls test; CD_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.614000	{0.01 mg/L} 1.559000	{0.1 mg/L} 1.562000	{1 mg/L} 1.540000	{10 mg/L} 1.348000	{100 mg/L} .9780000	{500 mg/L} 0.000000
A	{0}		0.301939	0.166825	0.208459	*0.000173	*0.000158	*0.000174
B	{0.01	0.301939		0.934229	0.602508	*0.000268	*0.000196	*0.000150
C	{0.1}	0.166825	0.934229		0.813315	*0.000348	*0.000150	*0.000158
D	{1}	0.208459	0.602508	0.813315		*0.000257	*0.000180	*0.000196
E	{10}	*0.000173	*0.000268	*0.000348	*0.000257194042205		*0.000176	*0.000180
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176668167114		*0.000176
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; CO_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.614000	{0.01 mg/L} 1.506000	{0.1 mg/L} 1.543000	{1 mg/L} 1.570000	{10 mg/L} 1.506000	{100 mg/L} 1.313000	{500 mg/L} 0.000000
A	{0}		0.058494	0.150882	0.237496	*0.039932	*0.000161	*0.000174
B	{0.01	0.058494		0.566241	0.31587	1	*0.000252	*0.000180
C	{0.1}	0.150882	0.566241		0.461505	0.316996	*0.000256	*0.000150
D	{1}	0.237496	0.31587	0.461505		0.20705	*0.000182	*0.000158
E	{10}	*0.039932	1	0.316996	0.20705		*0.000405	*0.000196
F	{100}	*0.000161	*0.000252	*0.000256	*0.000182	*0.000405073165893		*0.000176
G	{500}	*0.000174	*0.000180	*0.000150	*0.000158	*0.000196	*0.000176	

Newman-Keuls test; CR_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.614000	{0.01 mg/L} 1.528000	{0.1 mg/L} 1.570000	{1 mg/L} 1.537000	{10 mg/L} 1.411000	{100 mg/L} 1.258000	{500 mg/L} 0.000000
A	{0}		0.120229	0.237496	0.11355	*0.000571	*0.000158	*0.000174
B	{0.01	0.120229		0.484739	0.80447	*0.005598	*0.000183	*0.000196
C	{0.1}	0.237496	0.484739		0.370436	*0.002802	*0.000152	*0.000158
D	{1}	0.11355	0.80447	0.370436		*0.008781	*0.000200	*0.000150
E	{10}	*0.000571	*0.005598	*0.002802	*0.008781433105468		*0.000886	*0.000180
F	{100}	*0.000158	*0.000183	*0.000152	*0.000200	*0.000886440277099		*0.000176
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; CU_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.614000	{0.01 mg/L} 1.478000	{0.1 mg/L} 1.545000	{1 mg/L} 1.340000	{10 mg/L} .8740000	{100 mg/L} 0.000000	{500 mg/L} 0.000000
A	{0}		*0.002384	*0.049886	*0.000197	*0.000150	*0.000174	*0.000158
B	{0.01	*0.002384245395660		0.05598	*0.000883	*0.000180	*0.000150	*0.000196
C	{0.1}	*0.049886	0.05598		*0.000214	*0.000196	*0.000158	*0.000150
D	{1}	*0.000197	*0.000883	*0.000214040279388		*0.000176	*0.000196	*0.000180
E	{10}	*0.000150	*0.000180	*0.000196	0*.000176668167114		*0.000180	*0.000176
F	{100}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180900096893		1
G	{500}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176	1	

Newman-Keuls test; CD_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .7590000	{0.01 mg/L} .7900000	{0.1 mg/L} .8030000	{1 mg/L} .7960000	{10 mg/L} .6580000	{100 mg/L} .5360000	{500 mg/L} 0.000000
A	{0}		0.420164	0.649055	0.593911	*0.017177	*0.000260	*0.000196
B	{0.01	0.420164		0.935661	0.874658	*0.008729	*0.000226	*0.000150
C	{0.1}	0.649055	0.935661		0.853994	*0.012098	*0.000197	*0.000174
D	{1}	0.593911	0.874658	0.853994		*0.011401	*0.000195	*0.000158
E	{10}	*0.017177	*0.008729	*0.012098	*0.011401534080505		*0.005734	*0.000180
F	{100}	*0.000260	*0.000226	*0.000197	*0.000195	*0.005734682083129		*0.000176
G	{500}	*0.000196	*0.000150	*0.000174	*0.000158	*0.000180	*0.000176	

Newman-Keuls test; CO_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .7590000	{0.01 mg/L} .7730000	{0.1 mg/L} .7710000	{1 mg/L} .7540000	{10 mg/L} .6970000	{100 mg/L} .5820000	{500 mg/L} 0.000000
A	{0}		0.925813	0.752617	0.895433	0.253962	*0.001715	*0.000150
B	{0.01	0.925813		0.958129	0.95559	0.299178	*0.001841	*0.000174
C	{0.1}	0.752617	0.958129		0.892784	0.240278	*0.001470	*0.000158
D	{1}	0.895433	0.95559	0.892784		0.148995	*0.001230	*0.000196
E	{10}	0.253962	0.299178	0.240278	0.148995		*0.008279	*0.000180
F	{100}	*0.001715	*0.001841	*0.001470	*0.001230	*0.008279621601104		*0.000176
G	{500}	*0.000150	*0.000174	*0.000158	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; CR_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .7590000	{0.01 mg/L} .7400000	{0.1 mg/L} .7520000	{1 mg/L} .7070000	{10 mg/L} .5920000	{100 mg/L} .4910000	{500 mg/L} .2820000
A	{0}		0.886119	0.864962	0.58494	*0.007612	*0.000267	*0.000174
B	{0.01	0.886119		0.7708	0.427599	*0.006848	*0.000299	*0.000150
C	{0.1}	0.864962	0.7708		0.521224	*0.006964	*0.000254	*0.000158
D	{1}	0.58494	0.427599	0.521224		*0.013051	*0.000434	*0.000196
E	{10}	*0.007612	*0.006848	*0.006964	*0.013051390647888		*0.025533	*0.000183
F	{100}	*0.000267	*0.000299	*0.000254	*0.000434	*0.025533020496368		*0.000298
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000183	*0.000298	

Newman-Keuls test; CU_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .7590000	{0.01 mg/L} .7720000	{0.1 mg/L} .7810000	{1 mg/L} .7610000	{10 mg/L} .5160000	{100 mg/L} 0.000000	{500 mg/L} 0.000000
A	{0}		0.922969	0.914711	0.953979	*0.000178	*0.000196	*0.000180
B	{0.01	0.922969		0.795037	0.750995	*0.000203	*0.000158	*0.000150
C	{0.1}	0.914711	0.795037		0.828363	*0.000158	*0.000174	*0.000158
D	{1}	0.953979	0.750995	0.828363		*0.000187	*0.000150	*0.000196
E	{10}	*0.000178	*0.000203	*0.000158	*0.000187218189239		*0.000180	*0.000176
F	{100}	*0.000196	*0.000158	*0.000174	*0.000150	*0.000180		1
G	{500}	*0.000180	*0.000150	*0.000158	*0.000196	*0.000176	1	

APPENDIX 30: ANOVA: *Tetraselmis tetrahele* UMACC 144: Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-TET

Newman-Keuls test; FE_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.614000	1.486000	1.528000	1.471000	1.275000	1.117000	0.000000
A	{0}		*0.007891	*0.030265	*0.006352	*0.000151	*0.000158	*0.000174
B	{0.01}	*0.007891		0.258458	0.680416	*0.000268	*0.000196	*0.000150
C	{0.1}	*0.030265	0.258458		0.278534	*0.000213	*0.000150	*0.000158
D	{1}	*0.006352	0.680416	0.278534		*0.000241	*0.000180	*0.000196
E	{10}	*0.000151	*0.000268	*0.000213	*0.000241		*0.000712	*0.000180
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000712		*0.000176
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; MN_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.614000	1.489000	1.471000	1.475000	1.396000	1.117000	0.000000
A	{0}		*0.003640	*0.006352	*0.004397	*0.000348	*0.000158	*0.000174
B	{0.01}	*0.003640		0.8702	0.700548	0.085556	*0.000150	*0.000158
C	{0.1}	*0.006352	0.8702		0.912366	0.054049	*0.000180	*0.000196
D	{1}	*0.004397	0.700548	0.912366		0.103041	*0.000196	*0.000150
E	{10}	*0.000348	0.085556	0.054049	0.103041		*0.000177	*0.000180
F	{100}	*0.000158	*0.000150	*0.000180	*0.000196	*0.000177		*0.000176
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; ZN_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.614000	1.451000	1.506000	1.451000	1.239000	1.105000	0.000000
A	{0}		*0.002302	*0.009166	*0.001309	*0.000150	*0.000158	*0.000174
B	{0.01}	*0.002302		0.301939	1	*0.000203	*0.000180	*0.000196
C	{0.1}	*0.009166	0.301939		0.145282	*0.000204	*0.000150	*0.000158
D	{1}	*0.001309	1	0.145282		*0.000264	*0.000196	*0.000150
E	{10}	*0.000150	*0.000203	*0.000204	*0.000264		*0.002270	*0.000180
F	{100}	*0.000158	*0.000180	*0.000150	*0.000196	*0.002270		*0.000176
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; AD_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.614000	1.401000	1.443000	1.425000	1.375000	1.144000	0.000000
A	{0}		*0.000355	*0.000436	*0.000458	*0.000219	*0.000158	*0.000174
B	{0.01}	*0.000355		0.484739	0.511884	0.477951	*0.000187	*0.000196
C	{0.1}	*0.000436	0.484739		0.621575	0.268946	*0.000153	*0.000158
D	{1}	*0.000458	0.511884	0.621575		0.366219	*0.000199	*0.000150
E	{10}	*0.000219	0.477951	0.268946	0.366219		*0.000185	*0.000180
F	{100}	*0.000158	*0.000187	*0.000153	*0.000199	*0.000185		*0.000176
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; FE_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.7590000	.7890000	.8060000	.8260000	.8000000	.7270000	0.000000
A	{0}		0.434965	0.601581	0.413404	0.530334	0.405696	*0.000180
B	{0.01}	0.434965		0.892784	0.756521	0.772584	0.253962	*0.000196
C	{0.1}	0.601581	0.892784		0.600472	0.874658	0.266395	*0.000158
D	{1}	0.413404	0.756521	0.600472		0.769264	0.147788	*0.000174
E	{10}	0.530334	0.772584	0.874658	0.769264		0.250152	*0.000150
F	{100}	0.405696	0.253962	0.266395	0.147788	0.250152		*0.000176
G	{500}	*0.000180	*0.000196	*0.000158	*0.000174	*0.000150	*0.000176	

Newman-Keuls test; MN_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.7590000	.7880000	.7570000	.7450000	.6970000	.5190000	0.000000
A	{0}		0.450097	0.958129	0.925813	0.378586	*0.000260	*0.000158
B	{0.01}	0.450097		0.690759	0.664805	0.161764	*0.000194	*0.000174
C	{0.1}	0.958129	0.690759		0.752617	0.274876	*0.000265	*0.000150
D	{1}	0.925813	0.664805	0.752617		0.219251	*0.000249	*0.000196
E	{10}	0.378586	0.161764	0.274876	0.219251		*0.000449	*0.000180
F	{100}	*0.000260	*0.000194	*0.000265	*0.000249	*0.000449		*0.000176
G	{500}	*0.000158	*0.000174	*0.000150	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; ZN_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.7590000	.7570000	.7860000	.8080000	.7710000	.6700000	0.000000
A	{0}		0.958129	0.753933	0.570005	0.752617	0.076434	*0.000196
B	{0.01}	0.958129		0.863496	0.656881	0.925813	*0.035290	*0.000180
C	{0.1}	0.753933	0.863496		0.564962	0.693864	0.050712	*0.000158
D	{1}	0.570005	0.656881	0.564962		0.593911	*0.023411	*0.000174
E	{10}	0.752617	0.925813	0.693864	0.593911		0.071871	*0.000150
F	{100}	0.076434	*0.035290	0.050712	*0.023411	0.071871		*0.000176
G	{500}	*0.000196	*0.000180	*0.000158	*0.000174	*0.000150	*0.000176	

Newman-Keuls test; AD_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.7590000	.7320000	.7360000	.7470000	.7360000	.5780000	0.000000
A	{0}		0.947473	0.925204	0.752617	0.813707	*0.002906	*0.000174
B	{0.01}	0.947473		0.916272	0.977227	0.993767	*0.001163	*0.000180
C	{0.1}	0.925204	0.916272		0.953475	1	*0.002374	*0.000196
D	{1}	0.752617	0.977227	0.953475		0.772584	*0.003721	*0.000158
E	{10}	0.813707	0.993767	1	0.772584		*0.004204	*0.000150
F	{100}	*0.002906	*0.001163	*0.002374	*0.003721	*0.004204		*0.000176
G	{500}	*0.000174	*0.000180	*0.000196	*0.000158	*0.000150	*0.000176	

APPENDIX 30: ANOVA: *Tetraselmis tetrahele* UMACC 144: Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-TET

Newman-Keuls test; BD_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.614000	1.380000	1.402000	1.357000	1.297000	1.114000	0.000000
A	{0}		*0.000203	*0.000203	*0.000209	*0.000151	*0.000158	*0.000174
B	{0.01	*0.000203	9074897766	0.547168	0.52936	0.084598	*0.000204	*0.000150
C	{0.1}	*0.000203	0.547168		0.438273	*0.046621	*0.000155	*0.000158
D	{1}	*0.000209	0.52936	0.438273		0.114616	*0.000194	*0.000196
E	{10}	*0.000151	0.084598	*0.046621	0.114616		*0.000309	*0.000180
F	{100}	*0.000158	*0.000204	*0.000155	*0.000194	*0.000309586524963		*0.000176
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; BD_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.7590000	.7320000	.7430000	.7420000	.7330000	0.000000	0.000000
A	{0}		0.927929	0.645037	0.872339	0.868667	*0.000174	*0.000158
B	{0.01	0.927929		0.987817	0.953612	0.977052	*0.000180	*0.000176
C	{0.1}	0.645037	0.987817		0.977052	0.953612	*0.000158	*0.000150
D	{1}	0.872339	0.953612	0.977052		0.795037	*0.000150	*0.000196
E	{10}	0.868667	0.977052	0.953612	0.795037		*0.000196	*0.0001809
F	{100}	*0.000174	*0.000180	*0.000158	*0.000150	*0.000196		1
G	{500}	*0.000158	*0.000176	*0.000150	*0.000196	*0.000180	1	

Newman-Keuls test; MC_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.614000	1.364000	1.364000	1.312000	1.099000	.2750000	0.000000
A	{0}		*0.000190	*0.000179	*0.000197	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000190	2580261230	1	0.166825	*0.000184	*0.000196	*0.000150
C	{0.1}	*0.000179	1		0.339532	*0.000205	*0.000150	*0.000158
D	{1}	*0.000197	0.166825	0.339532		*0.000201	*0.000180	*0.000196
E	{10}	*0.000150	*0.000184	*0.000205	*0.000201582908630		*0.000176	*0.000180
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176668167114		*0.000177
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000177	

Newman-Keuls test; MC_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.7590000	.7850000	.7750000	.7730000	.5270000	.3220000	0.000000
A	{0}		0.896652	0.904346	0.713238	*0.000192	*0.000180	*0.000196
B	{0.01	0.896652		0.792712	0.944885	*0.000199	*0.000158	*0.000174
C	{0.1}	0.904346	0.792712		0.958129	*0.000237	*0.000150	*0.000158
D	{1}	0.713238	0.944885	0.958129		*0.000202	*0.000196	*0.000150
E	{10}	*0.000192	*0.000199	*0.000237	*0.000202715396881		*0.000241	*0.000180
F	{100}	*0.000180	*0.000158	*0.000150	*0.000196	*0.000241696834564		*0.000176
G	{500}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; DD_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.614000	.9730000	.9680000	.9680000	.9460000	.9090000	0.000000
A	{0}		*0.000176	*0.000196	*0.000180	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176	6681671142	0.989305	0.890583	0.872068	0.413564	*0.000158
C	{0.1}	*0.000196	0.989305		1	0.547168	0.256458	*0.000196
D	{1}	*0.000180	0.890583	1		0.813315	0.381853	*0.000150
E	{10}	*0.000150	0.872068	0.547168	0.813315		0.316996	*0.000180
F	{100}	*0.000158	0.413564	0.256458	0.381853	0.316996		*0.000176
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; DD_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.7590000	.5550000	.5590000	.5810000	.5360000	.5150000	.3260000
A	{0}		*0.001048	*0.000721	*0.000739	*0.000728	*0.000474	*0.000174
B	{0.01	*0.001048	3860969543	0.922605	0.7988	0.64533	0.594498	*0.0004749
C	{0.1}	*0.000721	0.922605		0.594538	0.838248	0.701275	*0.000518
D	{1}	*0.000739	0.7988	0.594538		0.686953	0.50111	*0.000350
E	{10}	*0.000728	0.64533	0.838248	0.686953		0.611248	*0.000516
F	{100}	*0.000474	0.594498	0.701275	0.50111	0.611248		*0.000503
G	{500}	*0.000174	*0.000474	*0.000518	*0.000350	*0.000516	*0.000503	

Newman-Keuls test; MA_T_D4 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.614000	1.086000	1.040000	.9970000	.9630000	.8820000	.8050000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176	6681671142	0.218318	0.062774	0.01829	*0.000554	*0.000168
C	{0.1}	*0.000180	0.218318		0.248217	0.113945	*0.002967	*0.000235
D	{1}	*0.000196	*0.062773	0.248217		0.356962	*0.015950	*0.000662
E	{10}	*0.000150	*0.018289	*0.113945	*0.356962084770203		*0.039640	*0.001685
F	{100}	*0.000158	*0.000554	*0.002967	*0.015950	*0.039640605449676		*0.048897
G	{500}	*0.000174	*0.000168	*0.000235	*0.000662	*0.001685	*0.048897	

Newman-Keuls test; MA_T_D10 (anova-tet-chl.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.7590000	.5010000	.5350000	.5530000	.5710000	.4600000	.3260000
A	{0}		*0.000269	*0.000542	*0.000587	*0.000519	*0.000182	*0.000174
B	{0.01	*0.000269	1745758056	0.414062	0.424655	0.343924	0.327237	*0.001984
C	{0.1}	*0.000542	0.414062		0.662684	0.654224	0.187708	*0.000873
D	{1}	*0.000587	0.424655	0.662684		0.662684	0.144465	*0.000628
E	{10}	*0.000519	0.343924	0.654224	0.662684		0.096003	*0.000461
F	{100}	*0.000182	0.327237	0.187708	0.144465	0.096003		*0.005218
G	{500}	*0.000174	*0.001984	*0.000873	*0.000628	*0.000461	*0.005218	

APPENDIX 31: ANOVA: *Tetraselmis tetrahele* UMACC 144: Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-TET

Newman-Keuls test; CD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	3.311300	4.194600	2.348500	1.724400	.8846000	.0729700
A	{0}		*0.000182	*0.000180	*0.000196	*0.000151	*0.000158	*0.000174
B	{0.01	*0.000182	2710037231	0.296989	0.257372	0.162523	*0.044023	*0.010318
C	{0.1}	*0.000180	0.296989		0.094685	*0.039901	*0.008756	*0.002045
D	{1}	*0.000196	0.257372	0.094685		0.456801	0.206938	0.061735
E	{10}	*0.000151	0.162523	*0.039901	0.456801		0.320512	0.142578
F	{100}	*0.000158	*0.044023	*0.008756	0.206938	0.320512		0.336461
G	{500}	*0.000174	*0.010318	*0.002045	0.061736	0.142578	0.336461	

Newman-Keuls test; CO_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	4.552000	4.181800	3.651700	2.926300	1.450300	0.000000
A	{0}		*0.000187	*0.000194	*0.000204	*0.000153	*0.000158	*0.000174
B	{0.01	*0.000187	5758171081	0.659006	0.531496	0.241242	*0.014756	*0.000931
C	{0.1}	*0.000194	0.659006		0.528968	0.307676	*0.022899	*0.001402
D	{1}	*0.000204	0.531496	0.528968		0.391903	*0.044453	*0.002859
E	{10}	*0.000153	0.241242	0.307676	0.391903		0.093849	*0.008284
F	{100}	*0.000158	*0.014756	*0.022899	*0.044453	0.093849		*0.099137
G	{500}	*0.000174	*0.000931	*0.001402	*0.002859	*0.008284	*0.099137	

Newman-Keuls test; CR_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	6.536700	6.569300	5.769200	4.795600	1.592000	0.000000
A	{0}		*0.003259	*0.001410	*0.001107	*0.000307	*0.000158	*0.000174
B	{0.01	*0.003259	7184181213	0.968289	0.355034	0.111518	*0.000299	*0.000155
C	{0.1}	*0.001410	0.968289		0.59044	0.168047	*0.000314	*0.000163
D	{1}	*0.001107	0.355034	0.59044		0.245024	*0.000512	*0.000210
E	{10}	*0.000307	0.111518	0.168047	0.245024		*0.001475	*0.000260
F	{100}	*0.000158	*0.000299	*0.000314	*0.000512	*0.001475	4533767700	0.067256
G	{500}	*0.000174	*0.000155	*0.000163	*0.000210	*0.000260	0.067256	

Newman-Keuls test; CU_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	9.038500	13.31970	7.500000	4.840400	.7575800	0.000000
A	{0}		0.271206	*0.000262	*0.009729	*0.000203	*0.000150	*0.000158
B	{0.01	0.271206		*0.000207	*0.035063	*0.000214	*0.000196	*0.000150
C	{0.1}	*0.000262	*0.000207	3049545288	*0.000196	*0.000150	*0.000158	*0.000174
D	{1}	*0.009729	*0.035063	*0.000196	6953277587	*0.001369	*0.000180	*0.000196
E	{10}	*0.000203	*0.000214	*0.000150	*0.001369	0590858459	*0.000192	*0.000185
F	{100}	*0.000150	*0.000196	*0.000158	*0.000180	*0.000192	7018165588	0.269582
G	{500}	*0.000158	*0.000150	*0.000174	*0.000196	*0.000185	0.269582	

Newman-Keuls test; CD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	35.57690	34.37500	31.15380	24.09090	19.58330	0.000000
A	{0}		*0.000338	*0.000484	*0.007458	0.152919	*0.001538	*0.000196
B	{0.01	*0.000338	196754555	*0.442418	0.028955	*0.000170	0.*000158	*0.000174
C	{0.1}	*0.000484	*0.442418	277263641	0.052536	*0.000229	*0.000151	*0.000158
D	{1}	*0.007458	0.028955	0.052536		*0.001157	*0.000202	*0.000150
E	{10}	0.152919	*0.000170	*0.000229	*0.001157999038696	2	*0.010380	*0.000180
F	{100}	*0.001538	*0.000158	*0.000151	*0.000202	*0.010380268096923	8	*0.000176
G	{500}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; CO_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	24.54270	23.03370	24.46430	16.42240	4.932430	0.000000
A	{0}		0.148037	0.062632	0.279507	*0.000154	*0.000158	*0.000174
B	{0.01	0.148037		0.443968	0.949154	*0.000231	*0.000150	*0.000158
C	{0.1}	0.062632	0.443968		0.255167	*0.000242	*0.000180	*0.00019
D	{1}	0.279507	0.949154	0.255167		*0.000199	*0.000196	*0.000150
E	{10}	*0.000154	*0.000231	*0.000242	*0.000199496746063	2	*0.000176	*0.000180
F	{100}	*0.000158	*0.000150	*0.000180	*0.000196	*0.000176727771759	0	*0.001236
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000180	*0.001236	

Newman-Keuls test; CR_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	41.16380	34.63110	30.75400	28.73380	15.07630	0.000000
A	{0}		*0.000150	*0.000325	*0.016153	0.106113	*0.000176	*0.000180
B	{0.01	*0.000150	9785652	*0.000427	*0.000183	*0.000196	*0.000158	*0.000174
C	{0.1}	*0.000325	*0.000427603721618	6	*0.012833	*0.001946	*0.000150	*0.000158
D	{1}	*0.016153	*0.000183	*0.012833297252655		0.158899	*0.000196	*0.000150
E	{10}	0.106113	*0.000196	*0.001946	0.158899		*0.000180	*0.000196
F	{100}	*0.000176	*0.000158	*0.000150	*0.000196	*0.000180900096893	3	*0.000176
G	{500}	*0.000180	*0.000174	*0.000158	*0.000150	*0.000196	*0.000176	

Newman-Keuls test; CU_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	30.33090	22.12840	18.32390	2.307690	0.000000	0.000000
A	{0}		*0.002108	*0.001204	*0.000182	*0.000196	*0.000158	*0.000150
B	{0.01	*0.002108	0374717712	*0.000182	*0.000196	*0.000150	*0.000174	*0.000158
C	{0.1}	*0.001204	*0.000182449817657	4	*0.002687	*0.000180	*0.000150	*0.000196
D	{1}	*0.000182	*0.000196	*0.002687275409698	4	*0.000176	*0.000196	*0.000180
E	{10}	*0.000196	*0.000150	*0.000180	*0.000176668167114	2	0.101545	*0.043190
F	{100}	*0.000158	*0.000174	*0.000150	*0.000196	0.101545		1
G	{500}	*0.000150	*0.000158	*0.000196	*0.000180	*0.043190	1	

APPENDIX 31: ANOVA: *Tetraselmis tetrahele* UMACC 144: Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-TET

Newman-Keuls test; FE_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	8.582100	7.979700	7.094600	4.518100	.4961800	0.000000
A	{0}		0.147117	0.089133	*0.019228	*0.000221	*0.000158	*0.000174
B	{0.01	0.147117		0.457964	0.179511	*0.000905	*0.000150	*0.000158
C	{0.1}	0.089133	0.457964		0.280898	*0.001807	*0.000196	*0.000151
D	{1}	*0.019228	0.179511	0.280898		*0.005779	*0.000181	*0.000196
E	{10}	*0.000221	*0.000905	*0.001807	*0.0057792663574		*0.000319	*0.000305
F	{100}	*0.000158	*0.000150	*0.000196	*0.000181	*0.000319	4212913513	0.539667
G	{500}	*0.000174	*0.000158	*0.000151	*0.000196	*0.000305	0.539667	

Newman-Keuls test; MN_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	10.50000	9.750000	8.406300	6.073200	2.357100	0.000000
A	{0}		0.312725	0.9497	0.135465	*0.000562	*0.000150	*0.000158
B	{0.01	0.312725		0.522768	*0.034805	*0.000238	*0.000158	*0.000174
C	{0.1}	0.9497	0.522768		0.066367	*0.000390	*0.000196	*0.000150
D	{1}	0.135465	*0.034805	0.066367		*0.003993	*0.000181	*0.000196
E	{10}	*0.000562	*0.000238	*0.000390	*0.0039934515953064		*0.000239	*0.000181
F	{100}	*0.000150	*0.000158	*0.000196	*0.000181	*0.000239	7894859	*0.003726
G	{500}	*0.000158	*0.000174	*0.000150	*0.000196	*0.000181	*0.003726	

Newman-Keuls test; ZN_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	3.462800	3.750000	3.125000	2.755900	1.828100	.3488000
A	{0}		*0.000189	*0.000181	*0.000204	*0.000158	*0.000159	*0.000174
B	{0.01	*0.000189423561096		0.753386	0.7119	0.715638	0.303104	*0.025892
C	{0.1}	*0.000181	0.753386		0.768808	0.689778	0.255638	*0.019556
D	{1}	*0.000204	0.7119	0.768808		0.686708	0.344718	*0.035127
E	{10}	*0.000158	0.715638	0.689778	0.686708		0.318041	*0.044057
F	{100}	*0.000159	0.303104	0.255638	0.344718	0.318041		0.12107
G	{500}	*0.000174	*0.025892	*0.019556	*0.035127	*0.044057	0.12107	

Newman-Keuls test; AD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	4.675600	3.062900	1.936600	1.098900	0.000000	0.000000
A	{0}		*0.000193	*0.000181	*0.000196	*0.000150	*0.000174	*0.000158
B	{0.01	*0.000193357467651		0.072054	*0.013609	*0.003619	*0.000807	*0.000610
C	{0.1}	*0.000181	0.072054		0.19559	0.078467	*0.017193	*0.011434
D	{1}	*0.000196	*0.013609	0.19559		0.329223	0.136446	0.08323
E	{10}	*0.000150	*0.003619	0.078467	0.329223		0.40449	0.206032
F	{100}	*0.000174	*0.000807	*0.017193	0.136446	0.40449		1
G	{500}	*0.000158	*0.000610	*0.011434	0.08323	0.206032	1	

Newman-Keuls test; FE_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	13.57950	17.55320	20.29700	20.65220	1.925680	0.000000
A	{0}		*0.000150	*0.000196	*0.000236	*0.000210	*0.000158	*0.000174
B	{0.01	*0.000150978565216		*0.001377	*0.000194	*0.000210	*0.000176	*0.000180
C	{0.1}	*0.000196	*0.001377224922180		*0.014758	*0.018500	*0.000180	*0.000196
D	{1}	*0.000236	*0.000194	*0.014758586883544		0.723951	*0.000196	*0.000150
E	{10}	*0.000210	*0.000210	*0.018500	0.723951		*0.000150	*0.000158
F	{100}	*0.000158	*0.000176	*0.000180	*0.000196	*0.000150978565216		0.071031
G	{500}	*0.000174	*0.000180	*0.000196	*0.000150	*0.000158	0.071031	

Newman-Keuls test; MN_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	24.42310	38.61110	18.83330	11.37930	4.000000	0.000000
A	{0}		0.172141	*0.000176	*0.000360	*0.000196	*0.000150	*0.000158
B	{0.01	0.172141		*0.000180	*0.001234	*0.000180	*0.000196	*0.000150
C	{0.1}	*0.000176	*0.000180900096893		*0.000196	*0.000150	*0.000158	*0.000174
D	{1}	*0.000360	*0.001234	*0.000196039676666		*0.000246	*0.000180	*0.000196
E	{10}	*0.000196	*0.000180	*0.000150	*0.000246524810791		*0.000254	*0.000181
F	{100}	*0.000150	*0.000196	*0.000158	*0.000180	*0.000254571437835		*0.011151
G	{500}	*0.000158	*0.000150	*0.000174	*0.000196	*0.000181	*0.011151	

Newman-Keuls test; ZN_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	26.06380	28.16270	30.42760	31.07140	19.33960	10.37500
A	{0}		0.791866	0.164212	*0.012671	*0.008174	*0.000283	*0.000196
B	{0.01	0.791866		0.226429	*0.013400	*0.007477	*0.000232	*0.000180
C	{0.1}	0.164212	0.226429		0.081919	0.07339	*0.000207	*0.000150
D	{1}	*0.012671	*0.013400	0.081919		0.602528	*0.000151	*0.000158
E	{10}	*0.008174	*0.007477	0.07339	0.602528		*0.000158	*0.000174
F	{100}	*0.000283	*0.000232	*0.000207	*0.000151	*0.000158309936523		*0.0001779
G	{500}	*0.000196	*0.000180	*0.000150	*0.000158	*0.000174	*0.000177	

Newman-Keuls test; AD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	29.86110	32.69230	24.84380	24.61540	0.000000	0.000000
A	{0}		*0.013666	*0.000567	0.229465	0.34705	*0.000150	*0.000196
B	{0.01	*0.013666510581970		*0.037255	*0.003134	*0.003963	*0.000158	*0.000150
C	{0.1}	*0.000567	*0.037255942821502		*0.000264	*0.000237	*0.000174	*0.000158
D	{1}	0.229465	*0.003134	*0.000264227390289		0.85539	*0.000196	*0.000180
E	{10}	0.34705	*0.003963	*0.000237	0.85539		*0.000180	*0.000176
F	{100}	*0.000150	*0.000158	*0.000174	*0.000196	*0.000180		1
G	{500}	*0.000196	*0.000150	*0.000158	*0.000180	*0.000176	1	

APPENDIX 31: ANOVA: *Tetraselmis tetrahele* UMACC 144: Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-TET

Newman-Keuls test; BD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	4.391900	2.997400	2.747300	2.631600	0.000000	0.000000
A	{0}		*0.000182	*0.000181	*0.000196	*0.000151	*0.000174	*0.000158
B	{0.01}	*0.000182	4498176574	0.106157	0.139823	0.176504	*0.001091	*0.000824
C	{0.1}	*0.000181	0.106157		0.761374	0.893868	*0.016681	*0.011089
D	{1}	*0.000196	0.139823	0.761374		0.888186	*0.019861	*0.011288
E	{10}	*0.000151	0.176504	0.893868	0.888186		*0.014857	*0.005841
F	{100}	*0.000174	*0.001091	*0.016681	*0.019861	*0.014857		1
G	{500}	*0.000158	*0.000824	*0.011089	*0.011288	*0.005841	1	

Newman-Keuls test; BD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	22.53090	38.72280	16.92480	14.69510	0.000000	0.000000
A	{0}		*0.002085	*0.000176	*0.000180	*0.000196	*0.000158	*0.000150
B	{0.01}	*0.002085	0896835327	*0.000180	*0.000237	*0.000183	*0.000150	*0.000196
C	{0.1}	*0.000176	*0.000180	9000968933	*0.000196	*0.000150	*0.000174	*0.000158
D	{1}	*0.000180	*0.000237	*0.000196	0396766662	*0.045246	*0.000196	*0.000180
E	{10}	*0.000196	*0.000183	*0.000150	*0.045246	9583762605	*0.000180	*0.000176
F	{100}	*0.000158	*0.000150	*0.000174	*0.000196	*0.000180		1
G	{500}	*0.000150	*0.000196	*0.000158	*0.000180	*0.000176	1	

Newman-Keuls test; MC_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	2.636100	3.645800	2.011500	.7576000	0.000000	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000174	*0.000158
B	{0.01}	*0.000180	9597015380	0.189386	0.407876	0.054965	*0.020487	*0.013659
C	{0.1}	*0.000176	0.189386		0.099994	*0.007170	*0.002320	*0.001687
D	{1}	*0.000196	0.407876	0.099994		0.108777	*0.066651	*0.039225
E	{10}	*0.000150	0.054965	*0.007170	0.108777		0.567808	0.318181
F	{100}	*0.000174	*0.020487	*0.002320	*0.066651	0.567808		1
G	{500}	*0.000158	*0.013659	*0.001687	*0.039225	0.318181	1	

Newman-Keuls test; MC_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	24.05910	29.77940	21.46740	13.31080	0.000000	0.000000
A	{0}		*0.039353	*0.005313	*0.000902	*0.000196	*0.000158	*0.000150
B	{0.01}	*0.039353	3191852569	*0.000344	*0.024187	*0.000180	*0.000150	*0.000196
C	{0.1}	*0.005313	*0.000344	0380096435	*0.000198	*0.000150	*0.000174	*0.000158
D	{1}	*0.000902	*0.024187	*0.000198	3642578125	*0.000177	*0.000196	*0.000180
E	{10}	*0.000196	*0.000180	*0.000150	*0.000177	0257949825	*0.000180	*0.000176
F	{100}	*0.000158	*0.000150	*0.000174	*0.000196	*0.000180		1
G	{500}	*0.000150	*0.000196	*0.000158	*0.000180	*0.000176	1	

Newman-Keuls test; DD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	1.755300	1.579900	1.204800	1.124500	.7116000	.0281590
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01}	*0.000176	6681671142	0.807867	0.721976	0.809201	0.593091	0.206998
C	{0.1}	*0.000180	0.807867		0.604306	0.798836	0.620502	0.237608
D	{1}	*0.000196	0.721976	0.604306		0.911339	0.768992	0.377636
E	{10}	*0.000150	0.809201	0.798836	0.911339		0.568781	0.298877
F	{100}	*0.000158	0.593091	0.620502	0.768992	0.568781		0.350422
G	{500}	*0.000174	0.206998	0.237609	0.377637	0.298877	0.350422	

Newman-Keuls test; DD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	4.284500	4.102200	4.028600	3.952400	3.299300	.3916700
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01}	*0.000176	6681671142	0.766219	0.905632	0.944363	0.498437	*0.000302
C	{0.1}	*0.000180	0.766219		0.904388	0.966501	0.556698	*0.000324
D	{1}	*0.000196	0.905632	0.904388		0.901029	0.464983	*0.000336
E	{10}	*0.000150	0.944363	0.966501	0.901029		0.295618	*0.000268
F	{100}	*0.000158	0.498437	0.556698	0.464983	0.295618		*0.000415
G	{500}	*0.000174	*0.000302	*0.000324	*0.000336	*0.000268	*0.000415	

Newman-Keuls test; MA_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.793400	1.877700	1.857200	2.944200	2.099600	1.680700	.2649000
A	{0}		*0.000196	*0.000150	*0.000176	*0.000180	*0.000158	*0.000174
B	{0.01}	*0.000196	0396766662	0.978055	0.336881	0.7652	0.960662	0.167104
C	{0.1}	*0.000150	0.978055		0.467393	0.941095	0.812136	0.108486
D	{1}	*0.000176	0.336881	0.467393		0.265655	0.445609	*0.024262
E	{10}	*0.000180	0.7652	0.941095	0.265655		0.937934	0.141782
F	{100}	*0.000158	0.960662	0.812136	0.445609	0.937934		0.072377
G	{500}	*0.000174	0.167105	0.108486	*0.024262	0.141783	0.072377	

Newman-Keuls test; MA_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		26.38890	2.453300	4.222400	4.207900	3.936600	2.893000	.3551100
A	{0}		*0.000158	*0.000176	*0.000180	*0.000196	*0.000150	*0.000174
B	{0.01}	*0.000158	1907272338	0.077507	0.055706	0.07343	0.487285	*0.004416
C	{0.1}	*0.000176	0.077507		0.981672	0.889109	0.183106	*0.000363
D	{1}	*0.000180	0.055706	0.981672		0.666517	0.118675	*0.000301
E	{10}	*0.000196	0.07343	0.889109	0.666517		0.112511	*0.000407
F	{100}	*0.000150	0.487285	0.183106	0.118675	0.112511		*0.002923
G	{500}	*0.000174	*0.004416	*0.000363	*0.000301	*0.000407	*0.002923	

APPENDIX 31: ANOVA: *Tetraselmis tetrahele* UMACC 144: Biochemical composition (Protein)

ANOVA-PROTEIN-TET

Newman-Keuls test; CD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	35.22200	35.99300	32.59300	24.14100	.3719200	.0488490
A	{0}		*0.016858	*0.010926	*0.005779	*0.000204	*0.000158	*0.000174045562744141
B	{0.01}	*0.016858		0.802719	0.399804	*0.006897	*0.000196	*0.000150978565216064
C	{0.1}	*0.010926	0.802719		0.516006	*0.007588	*0.000150	*0.000158190727233887
D	{1}	*0.005779	0.399804	0.516006		*0.014530	*0.000180	*0.00019603967666626
E	{10}	*0.000204	*0.006897	*0.007588	*0.014530		*0.000177	*0.000182271003723145
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000177		0.91662
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000182	0.91662	

Newman-Keuls test; CD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	45.35600	62.34600	61.28200	44.68000	21.97500	0.000000
A	{0}		*0.000176	0.114354	0.361276	*0.000180	*0.000196	*0.000150978565216064
B	{0.01}	*0.000176		*0.000196	*0.000180	0.454294	*0.000180	*0.00019603967666626
C	{0.1}	0.114354	*0.000196		0.245736	*0.000150	*0.000158	*0.000174045562744141
D	{1}	0.361276	*0.000180	0.245736		*0.000196	*0.000150	*0.000158190727233887
E	{10}	*0.000180	0.454294	*0.000150	*0.000196		*0.000176	*0.000180900096893311
F	{100}	*0.000196	*0.000180	*0.000158	*0.000150	*0.000176		*0.000176668167114258
G	{500}	*0.000150	*0.000196	*0.000174	*0.000158	*0.000180	*0.000176	

Newman-Keuls test; CO_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	31.04300	32.32300	30.25400	24.09300	19.09800	0.000000
A	{0}		*0.001520	*0.001284	*0.001727	*0.000216	*0.000162	*0.000174045562744141
B	{0.01}	*0.001520		0.684846	0.802128	0.096951	*0.008289	*0.00015103816986084
C	{0.1}	*0.001284	0.684846		0.784297	0.077355	*0.005809	*0.000158250331878662
D	{1}	*0.001727	0.802128	0.784297		0.065942	*0.007559	*0.0001960992813
E	{10}	*0.000216	0.096951	0.077355	0.065942		0.128129	*0.0001828074455
F	{100}	*0.000162	*0.008289	*0.005809	*0.007559	0.128129		*0.000193119049072266
G	{500}	*0.000174	*0.000151	*0.000158	*0.000196	*0.000182	*0.000193119049072266	

Newman-Keuls test; CO_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	54.43500	58.58100	65.71400	54.25300	13.93400	0.000000
A	{0}		*0.000205	0.061661	*0.000218	*0.000231	*0.000150	*0.000158190727233887
B	{0.01}	*0.000205		*0.000644	*0.000196	0.84632	*0.000180	*0.00019603967666626
C	{0.1}	0.061661	*0.000644		*0.000182	*0.001064	*0.000196	*0.000150978565216064
D	{1}	*0.000218	*0.000196	*0.000182		*0.000150	*0.000158	*0.000174045562744141
E	{10}	*0.000231	0.84632	*0.001064	*0.000150		*0.000176	*0.000180900096893311
F	{100}	*0.000150	*0.000180	*0.000196	*0.000158	*0.000176		*0.000176668167114258
G	{500}	*0.000158	*0.000196	*0.000150	*0.000174	*0.000180	*0.000176	

Newman-Keuls test; CR_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	50.28900	40.11900	42.22200	34.38200	25.75100	6.670000
A	{0}		0.172163	0.433702	0.489238	*0.060805	*0.001390	*0.000158309936523437
B	{0.01}	0.172163		0.071452	0.115577	*0.006299	*0.000287	*0.000174045562744141
C	{0.1}	0.433702	0.071452		0.584167	0.148706	*0.005022	*0.000196456909179687
D	{1}	0.489238	0.115577	0.584167		0.128136	*0.003179	*0.000151216983795166
E	{10}	*0.060805	*0.006299	0.148706	0.128136		0.037466	*0.000185310840606689
F	{100}	*0.001390	*0.000287	*0.005022	*0.003179	*0.037466		*0.000323116779327393
G	{500}	*0.000158	*0.000174	*0.000196	*0.000151	*0.000185	*0.000323116779327393	

Newman-Keuls test; CR_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	42.42700	41.76100	33.35700	7.629000	.3765900	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176		0.395891	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	0.395891		*0.000176	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.000176		*0.000176	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176		0.628106
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	0.628106	

Newman-Keuls test; CU_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	44.21700	49.25300	40.98800	11.56820	.8271600	0.000000
A	{0}		0.533363	*0.001108	*0.006251	*0.000196	*0.000150	*0.000158190727233887
B	{0.01}	0.533363		*0.000914	*0.008405	*0.000180	*0.000196	*0.000150978565216064
C	{0.1}	*0.001108	*0.000914752483367		*0.000199	*0.000150	*0.000158	*0.000174045562744141
D	{1}	*0.006251	*0.008405	*0.000199854373931		*0.000176	*0.000180	*0.00019603967666626
E	{10}	*0.000196	*0.000180	*0.000150	*0.000176		*0.000176	*0.000180900096893311
F	{100}	*0.000150	*0.000196	*0.000158	*0.000180	*0.000176		0.44424
G	{500}	*0.000158	*0.000150	*0.000174	*0.000196	*0.000180	0.44424	

Newman-Keuls test; CU_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	59.32500	55.78600	34.31000	15.89700	.4349200	0.000000
A	{0}		0.119314	*0.000193	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	0.119314		*0.000291	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000193	*0.000291466712951		*0.000176	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.000176668167114		*0.000176	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176		0.532667
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	0.532667	

APPENDIX 31: ANOVA: *Tetraselmis tetrahele* UMACC 144: Biochemical composition (Protein)

ANOVA-PROTEIN-TET

Newman-Keuls test; FE_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	40.29900	44.50500	37.13700	32.75300	.4116500	0.000000
A	{0}		0.37798	0.910131	0.138455	*0.018897	*0.000158	*0.000174045562744141
B	{0.01	0.37798		0.227067	0.358362	0.09424	*0.000196	*0.000150978565216064
C	{0.1}	0.910131	0.227067		0.103473	*0.015624	*0.000150	*0.000158190727233887
D	{1}	0.138455	0.358362	0.103473		0.209022	*0.000180	*0.000196039676666626
E	{10}	*0.018897	0.09424	*0.015624	0.209022		*0.000176	*0.000180959701538086
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176	0.903444	
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	0.903444	

Newman-Keuls test; FE_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	49.19200	45.56300	35.15200	23.44600	2.611300	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.00017666816		*0.000193	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	*0.000193119049072		*0.000176	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.000176668167114		*0.000176	*0.000180	*0.000196039676666626
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176668167114		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176668167114		*0.000693619251251221
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000693619251251221	

Newman-Keuls test; MN_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	50.37000	50.37000	54.50600	64.41700	15.64020	0.000000
A	{0}		*0.000866	*0.002085	*0.000203	*0.000150	*0.000176	*0.000180900096893311
B	{0.01	*0.000866711139678		1	*0.015182	*0.000196	*0.000180	*0.000196039676666626
C	{0.1}	*0.002085	1		*0.005970	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000203	*0.015182	*0.005970418453		*0.000177	*0.000150	*0.000158190727233887
E	{10}	*0.000150	*0.000196	*0.000180	*0.000177204608917		*0.000158	*0.000174045562744141
F	{100}	*0.000176	*0.000180	*0.000196	*0.000150	*0.000158		*0.000176668167114258
G	{500}	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174	*0.000176668167114258	

Newman-Keuls test; MN_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	33.67500	51.24800	17.53100	4.764000	.6604900	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180900096893		*0.000176	*0.000176	*0.000180	*0.000196	*0.000150978565216064
C	{0.1}	*0.000176	*0.000176668167114		*0.000180	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000196	*0.000176	*0.000180900096893		*0.000176	*0.000180	*0.000196039676666626
E	{10}	*0.000150	*0.000180	*0.000196	*0.000176668167114		*0.000198	*0.000190377235412598
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000198628281036		0.347798
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000190	0.347798	

Newman-Keuls test; ZN_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	36.83700	41.93700	40.08700	42.92800	23.88900	6.296000
A	{0}		0.253401	0.715971	0.58793	0.608806	*0.000846	*0.000174224376678467
B	{0.01	0.253401		0.386021	0.399991	0.395813	*0.003986	*0.000181734561920166
C	{0.1}	0.715971	0.386021		0.628875	0.795143	*0.001506	*0.000151216983795166
D	{1}	0.58793	0.399991	0.628875		0.733291	*0.002006	*0.000196456909179687
E	{10}	0.608806	0.395813	0.795143	0.733291		*0.001419	*0.000158309936523437
F	{100}	*0.000846	*0.003986	*0.001506	*0.002006	*0.001419544219970		*0.000490784645080566
G	{500}	*0.000174	*0.000181	*0.000151	*0.000196	*0.000158	*0.000490784645080566	

Newman-Keuls test; ZN_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	21.86000	32.55700	40.40900	42.75100	20.48430	0.000000
A	{0}		*0.000150	*0.000196	*0.000180	*0.000176	*0.000158	*0.000174045562744141
B	{0.01	*0.000150978565216		*0.000176	*0.000180	*0.000196	0.062044	*0.000180900096893311
C	{0.1}	*0.000196	*0.000176668167114		*0.000176	*0.000180	*0.000180	*0.000196039676666626
D	{1}	*0.000180	*0.000180	*0.000176668167114		*0.004028	*0.000196	*0.000150978565216064
E	{10}	*0.000176	*0.000196	*0.000180	*0.004028379917144		*0.000150	*0.000158190727233887
F	{100}	*0.000158	0.062044	*0.000180	*0.000196	*0.000150978565216		*0.000176668167114258
G	{500}	*0.000174	*0.000180	*0.000196	*0.000150	*0.000158	*0.000176668167114258	

Newman-Keuls test; AD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	42.01300	36.44800	38.86300	30.36200	10.18400	0.000000
A	{0}		0.379523	0.077482	0.174673	*0.003362	*0.000158	*0.000174045562744141
B	{0.01	0.379523		0.219719	0.336926	*0.011832	*0.000151	*0.000158190727233887
C	{0.1}	0.077482	0.219719		0.458548	0.075362	*0.000181	*0.000196039676666626
D	{1}	0.174673	0.336926	0.458548		*0.044275	*0.000196	*0.000150978565216064
E	{10}	*0.003362	*0.011832	0.075362	*0.044275462627410		*0.000187	*0.000180959701538086
F	{100}	*0.000158	*0.000151	*0.000181	*0.000196	*0.000187933444976		*0.006367623805999976
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000180	*0.006367623805999976	

Newman-Keuls test; AD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	42.20200	49.11700	36.11100	28.66100	0.000000	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000180900096893		*0.000176	*0.000176	*0.000180	*0.000150	*0.000196039676666626
C	{0.1}	*0.000176	*0.000176727771759		*0.000180	*0.000196	*0.000158	*0.000150978565216064
D	{1}	*0.000196	*0.000176	*0.000180900096893		*0.000176	*0.000196	*0.000180900096893311
E	{10}	*0.000150	*0.000180	*0.000196	*0.000176668167114		*0.000180	*0.000176668167114258
F	{100}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180900096893		1
G	{500}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176	1	

APPENDIX 31: ANOVA: *Tetraselmis tetrahele* UMACC 144: Biochemical composition (Protein)

ANOVA-PROTEIN-TET

Newman-Keuls test; BD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	36.88700	27.70200	29.99600	21.10300	.7427700	0.000000
A	{0}		*0.017520	*0.000415	*0.000656	*0.000156	*0.000158	*0.000174045562744141
B	{0.01	*0.017520		*0.020337	*0.035903	*0.000717	*0.000150	*0.000158190727233887
C	{0.1}	*0.000415	*0.020337		0.452408	*0.043219	*0.000180	*0.000196218490600586
D	{1}	*0.000656	*0.035903	0.452408		*0.024527	*0.000196	*0.00015103816986084
E	{10}	*0.000156	*0.000717	*0.043219	*0.024527		*0.000180	*0.000188648700714111
F	{100}	*0.000158	*0.000150	*0.000180	*0.000196	*0.000180721829585		0.806078
G	{500}	*0.000174	*0.000158	*0.000196	*0.000151	*0.000188	0.806078	

Newman-Keuls test; BD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	42.06200	24.73400	11.65500	6.215000	0.000000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000176668167114		*0.000176	*0.000180	*0.000196	*0.000158	*0.000150978565216064
C	{0.1}	*0.000180	*0.000176668167114		*0.000176	*0.000180	*0.000150	*0.00019603967666626
D	{1}	*0.000196	*0.000180	*0.000176668167114		*0.000176	*0.000196	*0.000180900096893311
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176727771759		*0.000180	*0.000176668167114258
F	{100}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180900096893		1
G	{500}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	1	

Newman-Keuls test; MC_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	38.04500	46.42000	32.05600	22.20000	.4681200	0.000000
A	{0}		0.060765	0.654954	*0.005048	*0.000229	*0.000150	*0.000158190727233887
B	{0.01	0.060765		0.062454	0.095932	*0.001021	*0.000196	*0.000150978565216064
C	{0.1}	0.654954	0.062454		*0.003851	*0.000181	*0.000158	*0.000174045562744141
D	{1}	*0.005048	0.095932	*0.003851234912872		*0.010938	*0.000180	*0.000196158885955811
E	{10}	*0.000229	*0.001021	*0.000181	*0.010938227176666		*0.000185	*0.00020146369934082
F	{100}	*0.000150	*0.000196	*0.000158	*0.000180	*0.000185668468475		0.891114
G	{500}	*0.000158	*0.000150	*0.000174	*0.000196	*0.000201	0.891114	

Newman-Keuls test; MC_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	25.07400	25.06400	23.36600	15.86600	0.000000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000176668167114		0.986482	*0.025911	*0.000196	*0.000158	*0.000150978565216064
C	{0.1}	*0.000180	0.986482		*0.010676	*0.000180	*0.000150	*0.00019603967666626
D	{1}	*0.000196	*0.025911	*0.010676145553588		*0.000176	*0.000196	*0.000180900096893311
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176668167114		*0.000180	*0.000176668167114258
F	{100}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180900096893		1
G	{500}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	1	

Newman-Keuls test; DD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	16.28600	14.44800	11.67600	7.381000	1.630000	.2028200
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114		0.112671	*0.002323	*0.000198	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	0.112671		*0.023101	*0.000206	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.002323	*0.023101687431335		*0.001575	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000198	*0.000206	*0.001575350761413		*0.000272	*0.000201940536499023
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000272572040557		0.209842
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000201	0.209842	

Newman-Keuls test; DD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	19.34300	18.07900	18.22800	17.70500	5.792800	.2518500
A	{0}		*0.000176	*0.000196	*0.000180	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114		0.64239	0.435098	0.648128	*0.000151	*0.000158190727233887
C	{0.1}	*0.000196	0.64239		0.916109	0.791504	*0.000181	*0.00019603967666626
D	{1}	*0.000180	0.435098	0.916109		0.925126	*0.000196	*0.000150978565216064
E	{10}	*0.000150	0.648128	0.791504	0.925126		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000151	*0.000181	*0.000196	*0.000176846981048		*0.00147342681884766
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000180	*0.00147342681884766	

Newman-Keuls test; MA_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		44.88800	17.10400	17.71400	16.84500	15.82800	7.284000	.2452800
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180900096		0.612115	0.828938	0.538276	*0.000197	*0.000150978565216064
C	{0.1}	*0.000176	0.612115		0.744956	0.407783	*0.000151	*0.000158190727233887
D	{1}	*0.000196	0.828938	0.744956		0.401787	*0.000181	*0.00019603967666626
E	{10}	*0.000150	0.538276	0.407783	0.401787		*0.000178	*0.0001809000968
F	{100}	*0.000158	*0.000197	*0.000151	*0.000181	*0.000178337097167		*0.000201046466827393
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000201046466827393	

Newman-Keuls test; MA_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		60.45300	17.29900	19.16700	19.30500	22.33900	11.83600	2.174500
A	{0}		*0.000150	*0.000196	*0.000180	*0.000176	*0.000158	*0.000174045562744141
B	{0.01	*0.000150978565216		0.278267	0.466015	*0.038874	*0.005412	*0.000180959701538086
C	{0.1}	*0.000196	0.278267		0.934856	0.170889	*0.001685	*0.000196099281311035
D	{1}	*0.000180	0.466015	0.934856		0.088331	*0.002561	*0.000150978565216064
E	{10}	*0.000176	*0.038874	0.170889	0.088331		*0.000279	*0.000158190727233887
F	{100}	*0.000158	*0.005412	*0.001685	*0.002561	*0.000279128551483		*0.000209569931030273
G	{500}	*0.000174	*0.000180	*0.000196	*0.000150	*0.000158	*0.000209569931030273	

APPENDIX 31: ANOVA: *Tetraselmis tetrahele* UMACC 144: Biochemical composition (Lipid)

ANOVA-LIPID-TET

Newman-Keuls test; CD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	4.376821	4.489933	3.968485	2.826282	.4651748	.0603423
A	{0}		*0.042982	*0.027462	*0.014977	*0.000419	*0.000158	*0.000174045562744141
B	{0.01}	*0.042982		0.816628	0.407864	*0.015405	*0.000198	*0.000151753425598145
C	{0.1}	*0.027462	0.816628		0.535489	*0.017271	*0.000153	*0.000158727169036865
D	{1}	*0.014977	0.407864	0.535489		*0.031734	*0.000185	*0.000198185443878174
E	{10}	*0.000419	*0.015405	*0.017271	*0.031734		*0.000373	*0.0002937912940979
F	{100}	*0.000158	*0.000198	*0.000153	*0.000185	*0.000373		0.411798
G	{500}	*0.000174	*0.000151	*0.000158	*0.000198	*0.000293	0.411798	

Newman-Keuls test; CD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	3.969230	2.233300	1.918460	.8654550	.0716670	0.000000
A	{0}		*0.000179	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000179		0.053803	0.06345	*0.010043	*0.002605	*0.00308698415756226
C	{0.1}	*0.000180	0.053803		0.708289	0.254681	0.083459	0.102721
D	{1}	*0.000196	0.06345	0.708289		0.222221	0.098683	0.138668
E	{10}	*0.000150	*0.010043	0.254681	0.222221		0.351912	0.558989
F	{100}	*0.000158	*0.002605	0.083459	0.098683	0.351912		0.932035
G	{500}	*0.000174	*0.003086	0.102721	0.138668	0.558989	0.932035	

Newman-Keuls test; CO_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	3.849200	4.483640	4.113480	3.614103	.7569000	0.000000
A	{0}		*0.006386	*0.020692	*0.010806	*0.003739	*0.000158	*0.000174045562744141
B	{0.01}	*0.006386		0.368504	0.569708	0.612637	*0.000194	*0.000197112560272217
C	{0.1}	*0.020692	0.368504		0.428496	0.265598	*0.000154	*0.000158309936523437
D	{1}	*0.010806	0.569708	0.428496		0.529419	*0.000205	*0.000151634216308594
E	{10}	*0.003739	0.612637	0.265598	0.529419		*0.000189	*0.000182271003723145
F	{100}	*0.000158	*0.000194	*0.000154	*0.000205	*0.000189		0.117654
G	{500}	*0.000174	*0.000197	*0.000158	*0.000151	*0.000182	0.117654	

Newman-Keuls test; CO_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	4.634150	4.899000	5.228600	2.896550	1.567600	0.000000
A	{0}		*0.000255	*0.000241	*0.000217	*0.000152	*0.000158	*0.000174045562744141
B	{0.01}	*0.000255		0.74754	0.746038	*0.049174	*0.005271	*0.000440895557403564
C	{0.1}	*0.000241	0.74754		0.689014	0.064028	*0.005092	*0.000362813472747803
D	{1}	*0.000217	0.746038	0.689014		0.051397	*0.003649	*0.000300705432891846
E	{10}	*0.000152	*0.049174	0.064028	0.051397		0.121698	0.007868
F	{100}	*0.000158	*0.005271	*0.005092	*0.003649	0.121698		0.072371
G	{500}	*0.000174	*0.000440	*0.000362	*0.000300	0.007868	0.072371	

Newman-Keuls test; CR_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	5.036700	3.080300	2.892300	1.238990	.1509000	0.000000
A	{0}		0.264144	*0.000954	*0.000956	*0.000155	*0.000158	*0.000174224376678467
B	{0.01}	0.264144		*0.003025	*0.003993	*0.000217	*0.000151	*0.000158727169036865
C	{0.1}	*0.000954	*0.003025		0.73439	*0.011553	*0.000649	*0.000586748123168945
D	{1}	*0.000956	*0.003993	0.73439		*0.008889	*0.000629	*0.000709772109985352
E	{10}	*0.000155	*0.000217	*0.011553	*0.008889		0.064893	0.091776
F	{100}	*0.000158	*0.000151	*0.000649	*0.000629	0.064893		0.785215
G	{500}	*0.000174	*0.000158	*0.000586	*0.000709	0.091776	0.785215	

Newman-Keuls test; CR_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	7.172410	4.590000	4.127000	3.494000	1.397000	0.000000
A	{0}		*0.019372	*0.000516	*0.000480	*0.000292	*0.000162	*0.000174462795257568
B	{0.01}	*0.019372		*0.022929	*0.023679	*0.012661	*0.000553	*0.000201940536499023
C	{0.1}	*0.000516	*0.022929		0.653749	0.538214	*0.031224	*0.0036165714263916
D	{1}	*0.000480	*0.023679	0.653749		0.540968	*0.042682	*0.00552713871002197
E	{10}	*0.000292	*0.012661	0.538214	0.540968		0.056844	*0.0101306438446045
F	{100}	*0.000162	*0.000553	*0.031224	*0.042682	0.056844		0.188293
G	{500}	*0.000174	*0.000201	*0.003616	*0.005527	*0.010130	0.188293	

Newman-Keuls test; CU_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	4.738460	4.295100	3.250000	2.914890	.9300000	0.000000
A	{0}		0.322547	0.314215	0.077114	0.057729	*0.001544	*0.000467896461486816
B	{0.01}	0.322547		0.632524	0.261597	0.230265	*0.006758	*0.00154471397399902
C	{0.1}	0.314215	0.632524		0.26844	0.310848	*0.011125	*0.00256937742233276
D	{1}	0.077114	0.261597	0.26844		0.717312	0.055797	*0.0141230821609497
E	{10}	0.057729	0.230265	0.310848	0.717312		*0.046133	*0.0161961913108826
F	{100}	*0.001544	*0.006758	*0.011125	0.055797	*0.046133	0.816242218	*0.322504460811615
G	{500}	*0.000467	*0.001544	*0.002569	*0.014123	*0.016196	*0.322504460811615	

Newman-Keuls test; CU_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	2.161760	3.554100	1.675000	1.353850	.2441600	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000180		0.092118	0.537473	0.559332	0.105055	0.086589
C	{0.1}	*0.000176	0.092118		0.069119	0.054664	*0.005636	*0.00438809394836426
D	{1}	*0.000196	0.537473	0.069119		0.682965	0.187303	0.177847
E	{10}	*0.000150	0.559332	0.054664	0.682965		0.171515	0.219146
F	{100}	*0.000158	0.105055	*0.005636	0.187303	0.171515		0.755894
G	{500}	*0.000174	0.086589	*0.004388	0.177847	0.219146	0.755894	

APPENDIX 31: ANOVA: *Tetraselmis tetrahele* UMACC 144: Biochemical composition (Lipid)

ANOVA-LIPID-TET

Newman-Keuls test; FE_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	2.440300	1.484600	1.439200	.4536140	.1984700	0.000000
A	{0}		*0.000181	*0.000181	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000181		0.065996	0.128013	*0.004949	*0.002841	*0.0019189715385437
C	{0.1}	*0.000181	0.065996		0.925947	0.115089	0.074757	0.051616
D	{1}	*0.000196	0.128013	0.925947		0.058887	0.052695	*0.0418398976325989
E	{10}	*0.000150	*0.004949	0.115089	0.058887		0.602762	0.620834
F	{100}	*0.000158	*0.002841	0.074757	0.052695	0.602762		0.68504
G	{500}	*0.000174	*0.001918	0.051616	*0.041839	0.620834	0.68504	

Newman-Keuls test; FE_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	2.238640	3.117000	1.168300	.1543480	.0336940	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180		0.194584	0.11915	*0.015628	*0.019222	*0.0260227918624878
C	{0.1}	*0.000176	0.194584		*0.023346	*0.002205	*0.002371	*0.00298351049423218
D	{1}	*0.000196	0.11915	*0.023346364498138		0.138147	0.218648	0.308328
E	{10}	*0.000150	*0.015628	*0.002205	0.138147		0.854314	0.969035
F	{100}	*0.000158	*0.019222	*0.002371	0.218648	0.854314		0.959164
G	{500}	*0.000174	*0.026022	*0.002983	0.308328	0.969035	0.959164	

Newman-Keuls test; MN_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	1.450000	1.880000	2.270800	.3658500	.0520000	0.000000
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196		0.154219	0.030868	*0.002111	*0.000781	*0.00100433826446533
C	{0.1}	*0.000180	0.154219		0.192541	*0.000456	*0.000261	*0.000235021114349365
D	{1}	*0.000176	*0.030868	0.192541		*0.000235	*0.000159	*0.000166773796081543
E	{10}	*0.000150	*0.002111	*0.000456	*0.000235	0.290086	0.427774	
F	{100}	*0.000158	*0.000781	*0.000261	*0.000159	0.290086		0.858141
G	{500}	*0.000174	*0.001004	*0.000235	*0.000166	0.427774	0.858141	

Newman-Keuls test; MN_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	5.246150	5.388900	1.253300	.7965500	.3595200	0.000000
A	{0}		*0.000437	*0.000298	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000437		0.870714	*0.000530	*0.000536	*0.000471	*0.000355124473571777
C	{0.1}	*0.000298	0.870714		*0.000898	*0.000700	*0.000474	*0.000368931461458 93
D	{1}	*0.000196	*0.000530	*0.000898301601409		0.603961	0.565751	0.487429
E	{10}	*0.000150	*0.000536	*0.000700	0.603961		0.619538	0.633605
F	{100}	*0.000158	*0.000471	*0.000474	0.565751	0.619538		0.682519
G	{500}	*0.000174	*0.000355	*0.000368	0.487429	0.633605	0.682519	

Newman-Keuls test; ZN_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	1.601350	1.769200	1.661800	.3771650	.1268800	0.000000
A	{0}		*0.000196	*0.000176	*0.000181	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196		0.932825	0.899838	*0.021172	*0.019049	*0.0200146436691284
C	{0.1}	*0.000176	0.932825		0.823062	*0.045811	*0.025395	*0.0210708379745483
D	{1}	*0.000181	0.899838	0.823062		*0.040851	*0.026080	*0.0235268473625183
E	{10}	*0.000150	*0.021172	*0.045811	*0.040851	0.603643	0.708754	
F	{100}	*0.000158	*0.019049	*0.025395	*0.026080	0.603643		0.791706
G	{500}	*0.000174	*0.020014	*0.021070	*0.023526	0.708754	0.791706	

Newman-Keuls test; ZN_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	5.189190	4.313300	3.940500	2.957140	.3212600	0.000000
A	{0}		*0.000208	*0.000191	*0.000205	*0.000152	*0.000158	*0.000174045562744141
B	{0.01	*0.000208		0.289821	0.290979	0.060317	*0.000348	*0.000292122364044189
C	{0.1}	*0.000191	0.289821		0.646868	0.238392	*0.001109	*0.000851094722747803
D	{1}	*0.000205	0.290979	0.646868		0.237098	*0.001367	*0.0012266039848
E	{10}	*0.000152	0.060317	0.238392	0.237098		*0.005290	*0.00622844696044922
F	{100}	*0.000158	*0.000348	*0.001109	*0.001367	*0.005290985107421	8	0.692722
G	{500}	*0.000174	*0.000292	*0.000851	*0.001226	*0.006228	0.692722	

Newman-Keuls test; AD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	1.732820	1.887420	1.752113	.6250000	0.000000	0.000000
A	{0}		*0.000197	*0.000176	*0.000181	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000197		0.940719	0.967471	*0.031414	*0.010489	*0.00590896606445312
C	{0.1}	*0.000176	0.940719		0.774484	0.069343	*0.011690	*0.00849109888076782
D	{1}	*0.000181	0.967471	0.774484		0.069948	*0.014598	*0.00970149040222168
E	{10}	*0.000150	*0.031414	0.069343	0.069948		0.392399	0.198525
F	{100}	*0.000174	*0.010489	*0.011690	*0.014598	0.392399		1
G	{500}	*0.000158	*0.005908	*0.008491	*0.009701	0.198525	1	

Newman-Keuls test; AD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	3.648150	3.661540	3.725000	3.307690	0.000000	0.000000
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000196		0.976524	0.98377	0.456713	*0.000197	*0.000181734561920166
C	{0.1}	*0.000180	0.976524		0.888659	0.711623	*0.000154	*0.000197887420654297
D	{1}	*0.000176	0.98377	0.888659		0.785084	*0.000161	*0.000153660774230957
E	{10}	*0.000150	0.456713	0.711623	0.785084		*0.000184	*0.000177919864654541
F	{100}	*0.000174	*0.000197	*0.000154	*0.000161	*0.000184893608093	2	1
G	{500}	*0.000158	*0.000181	*0.000197	*0.000153	*0.000177	9	1

APPENDIX 31: ANOVA: *Tetraselmis tetrahele* UMACC 144: Biochemical composition (Lipid)

ANOVA-LIPID-TET

Newman-Keuls test; BD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	4.783780	4.367400	3.648400	1.533800	0.000000	0.000000
A	{0}		*0.036086	*0.011108	*0.000738	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.036086916923522		0.293378	*0.025472	*0.000197	*0.000158	*0.000150978565216064
C	{0.1}	*0.011108	0.293378		0.080381	*0.000184	*0.000150	*0.00019603967666626
D	{1}	*0.000738	*0.025472	0.080381		*0.000235	*0.000196	*0.000180959701538086
E	{10}	*0.000150	*0.000197	*0.000184	*0.000235319137573		*0.003492	*0.0014030933380127
F	{100}	*0.000174	*0.000158	*0.000150	*0.000196	*0.003492355346		1
G	{500}	*0.000158	*0.000150	*0.000196	*0.000180	*0.001403	1	

Newman-Keuls test; BD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	4.382720	4.282600	3.360470	1.853660	0.000000	0.000000
A	{0}		*0.000177	*0.000181	*0.000196	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000177025794982		0.885833	0.323188	*0.011424	*0.000321	*0.0002657175064
C	{0.1}	*0.000181	0.885833		0.199121	*0.008510	*0.000299	*0.000282227993011475
D	{1}	*0.000196	0.323188	0.199121		*0.044971	*0.001301	*0.000765085220336914
E	{10}	*0.000150	*0.011424	*0.008510	*0.044971883296966		*0.042170	*0.0170744657516479
F	{100}	*0.000174	*0.000321	*0.000299	*0.001301	*0.042170405387878		1
G	{500}	*0.000158	*0.000265	*0.000282	*0.000765	*0.017074	1	

Newman-Keuls test; MC_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	1.972790	1.800000	1.212600	.3704550	0.000000	0.000000
A	{0}		*0.000176	*0.000181	*0.000196	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000176966190338		0.713069	0.257726	*0.017143	*0.007970	*0.00577634572982788
C	{0.1}	*0.000181	0.713069		0.222702	*0.019937	*0.011552	*0.0076630711555481
D	{1}	*0.000196	0.257726	0.222702		0.088766	0.081727	*0.0485192537307739
E	{10}	*0.000150	*0.017143	*0.019937	0.088766		0.70621	0.434457
F	{100}	*0.000174	*0.007970	*0.011552	0.081727	0.70621		1
G	{500}	*0.000158	*0.005776	*0.007663	*0.048519	0.434457	1	

Newman-Keuls test; MC_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	2.204300	2.294100	1.250000	.3770270	0.000000	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000174	*0.0001581907272
B	{0.01	*0.000180900096893		0.867592	0.092564	*0.010167	*0.007124	*0.00471585988998413
C	{0.1}	*0.000176	0.867592		0.154842	*0.013012	*0.007211	*0.00522541999816895
D	{1}	*0.000196	0.092564	0.154842		0.120873	0.130144	0.079156
E	{10}	*0.000150	*0.010167	*0.013012	0.120873		0.759723	0.48738
F	{100}	*0.000174	*0.007124	*0.007211	0.130144	0.759723		1
G	{500}	*0.000158	*0.004715	*0.005225	0.079156	0.48738	1	

Newman-Keuls test; DD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	2.965960	1.542800	1.530000	1.166010	.2573000	.0182000
A	{0}		*0.000177	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000177383422851		*0.001348	*0.003130	0*.000952	*0.000167	*0.000161945819854736
C	{0.1}	*0.000180	*0.001348316669464		0.97162	0.546625	*0.012392	*0.00530076026916504
D	{1}	*0.000196	*0.003130	0.97162		0.318464	*0.007495	*0.00375080108642578
E	{10}	*0.000150	*0.000952	0.546625	0.318464		*0.021820	*0.0147833824157715
F	{100}	*0.000158	*0.000167	*0.012392	*0.007495	*0.021820843219	7	0.507939
G	{500}	*0.000174	*0.000161	*0.005300	*0.003750	*0.014783	0.507939	

Newman-Keuls test; DD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	1.236750	1.715200	1.720500	1.078080	.2268000	0.000000
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196039676666		0.330027	0.576581	0.742898	0.119403	0.085505
C	{0.1}	*0.000180	0.330027		0.991355	0.39539	*0.032533	*0.019880473613739
D	{1}	*0.000176	0.576581	0.991355		0.545408	*0.046963	*0.0265456438064575
E	{10}	*0.000150	0.742898	0.39539	0.545408		0.094222	0.093047
F	{100}	*0.000158	0.119403	*0.032533	*0.046963	0.094222		0.63982
G	{500}	*0.000174	0.085505	*0.019880	*0.026545	0.093047	0.63982	

Newman-Keuls test; MA_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.668376	3.008580	2.293000	2.247900	1.937500	.7857000	.0484770
A	{0}		*0.000177	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000177741050720		0.063526	0.116906	*0.040833	*0.000298	*0.000162243843078613
C	{0.1}	*0.000180	0.063526		0.900819	0.587963	*0.004113	*0.000283718109130859
D	{1}	*0.000196	0.116906	0.900819		0.396765	*0.002922	*0.000293374061584473
E	{10}	*0.000150	*0.040833	0.587963	0.396765		*0.006015	*0.000447928905487061
F	{100}	*0.000158	*0.000298	*0.004113	*0.002922	*0.006015241146087	6	0.056811
G	{500}	*0.000174	*0.000162	*0.000283	*0.000293	*0.000447	0.056811	

Newman-Keuls test; MA_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.843056	.9519000	1.532000	2.089100	.6304300	.2567900	0.000000
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196039676666		0.230782	0.067227	0.498919	0.320034	0.21491
C	{0.1}	*0.000180	0.230782		0.248859	0.162268	0.065956	*0.03519606590271
D	{1}	*0.000176	0.067227	0.248859		*0.031879	*0.010592	*0.00529134273529053
E	{10}	*0.000150	0.498919	0.162268	*0.031879425048828	1	0.433238	0.386445
F	{100}	*0.000158	0.320034	0.065956	*0.010592	0.433238		0.587964
G	{500}	*0.000174	0.21491	*0.035196	*0.005291	0.386445	0.587964	

APPENDIX 32: ANOVA: *Tetraselmis tetrahele* UMACC 144: DNA Damage (RAPD-Appearance of new band)

* = Significant difference : p<0.05

ANOVA-APPEARANCE-TET

Newman-Keuls test; CD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	44.17000	43.08000	38.36000	33.71000	23.25000	21.79000
A	{0}		*0.000439	*0.000418	*0.000773	*0.001521	*0.013199	*0.007776
B	{0.01	*0.000439		0.87857	0.691164	0.466313	0.0629	0.057985
C	{0.1}	*0.000418	0.87857		0.511174	0.398114	0.057207	0.057213
D	{1}	*0.000773	0.691164	0.511174		0.517361	0.113759	0.129735
E	{10}	*0.001521	0.466313	0.398114	0.517361		0.157342	0.238606
F	{100}	*0.013199	0.0629	0.057207	0.113759	0.157342		0.837861
G	{500}	*0.007776	0.057985	0.057214	0.129736	0.238606	0.837861	

Newman-Keuls test; CD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	29.82000	22.68000	22.93000	21.28000	17.67000	16.00000
A	{0}		*0.000194	*0.000484	*0.000572	*0.000595	*0.001383	*0.001196
B	{0.01	*0.000194		0.194704	0.098566	0.17273	*0.049554	*0.030660
C	{0.1}	*0.000484	0.194704		0.949783	0.724556	0.424997	0.352104
D	{1}	*0.000572	0.098566	0.949783		0.906412	0.547675	0.421401
E	{10}	*0.000595	0.17273	0.724556	0.906412		0.369545	0.389091
F	{100}	*0.001383	*0.049554	0.424997	0.547675	0.369545		0.67452
G	{500}	*0.001196	*0.030660	0.352104	0.421401	0.389092	0.67452	

Newman-Keuls test; CR_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	50.40000	31.43000	31.43000	30.56000	28.28000	8.040000
A	{0}		*0.000283	*0.006253	*0.008629	*0.005148	*0.005127	0.296178
B	{0.01	*0.000283		0.055586	*0.022772	0.075617	0.063238	*0.000726
C	{0.1}	*0.006253	0.055586		1	0.908284	0.905861	*0.031480
D	{1}	*0.008629	*0.022772	1		0.992498	0.973259	*0.046426
E	{10}	*0.005148	0.075617	0.908284	0.992498		0.76289	*0.022608
F	{100}	*0.005127	0.063238	0.905861	0.973259	0.76289		*0.016340
G	{500}	0.296179	*0.000726	*0.031480	*0.046426	*0.022608	*0.016340	

Newman-Keuls test; CR_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	38.75000	28.75000	25.83000	23.21000	16.68000	13.63000
A	{0}		*0.000219	*0.001205	*0.002171	*0.003453	*0.019370	*0.023231
B	{0.01	*0.000219		0.08246	0.072115	*0.049937	*0.007697	*0.003785
C	{0.1}	*0.001205	0.08246		0.593498	0.566983	0.155512	0.083467
D	{1}	*0.002171	0.072115	0.593498		0.63165	0.235353	0.149405
E	{10}	*0.003453	*0.049937	0.566983	0.63165		0.241991	0.207913
F	{100}	*0.019370	*0.007697	0.155512	0.235353	0.241991		0.57734
G	{500}	*0.023231	*0.003785	0.083468	0.149406	0.207914	0.57734	

Newman-Keuls test; CU_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	57.09000	69.76000	57.07000	44.24000	35.66000	26.21000
A	{0}		*0.002781	*0.000666	*0.002023	*0.009807	*0.022361	*0.042052
B	{0.01	*0.002781		0.297534	0.998781	0.531052	0.300609	0.116263
C	{0.1}	*0.000666	0.297534		0.539053	0.176761	0.072069	*0.022513
D	{1}	*0.002023	0.998781	0.539053		0.291716	0.196462	0.081556
E	{10}	*0.009807	0.531052	0.176761	0.291716		0.475872	0.303148
F	{100}	*0.022361	0.300609	0.072069	0.196462	0.475872		0.433201
G	{500}	*0.042052	0.116263	*0.022513	0.081557	0.303148	0.433201	

Newman-Keuls test; CU_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	41.23000	25.13000	23.68000	22.81000	19.72000	12.32000
A	{0}		*0.000363	*0.014582	*0.016228	*0.013948	*0.019992	0.072955
B	{0.01	*0.000363		*0.023946	*0.038192	0.050665	*0.030541	*0.004932
C	{0.1}	*0.014582	*0.023946		0.822835	0.929541	0.829045	0.307782
D	{1}	*0.016228	*0.038192	0.822835		0.893131	0.80992	0.318898
E	{10}	*0.013948	0.050665	0.929541	0.893131		0.634268	0.257807
F	{100}	*0.019992	*0.030541	0.829045	0.80992	0.634268		0.26356
G	{500}	0.072955	*0.004932	0.307783	0.318898	0.257807	0.26356	

Newman-Keuls test; AD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	42.26000	27.98000	26.79000	23.22000	20.63000	0.000000
A	{0}		*0.000174	*0.000190	*0.000197	*0.000343	*0.000433	1
B	{0.01	*0.000174		*0.002508	*0.003551	*0.001247	*0.000638	*0.000158
C	{0.1}	*0.000190	*0.002508		0.762204	0.453192	0.269243	*0.000180
D	{1}	*0.000197	*0.003551	0.762204		0.370218	0.278898	*0.000219
E	{10}	*0.000343	*0.001247	0.453192	0.370218		0.512708	*0.000253
F	{100}	*0.000433	*0.000638	0.269243	0.278898	0.512708		*0.000262
G	{500}	1	*0.000158	*0.000180	*0.000219	*0.000253	*0.000262	

Newman-Keuls test; AD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	21.02000	32.08000	45.62000	28.96000	23.61000	0.000000
A	{0}		*0.004427	*0.000534	*0.000178	*0.000917	*0.003258	1
B	{0.01	*0.004427		0.217083	*0.003529	0.333625	0.638767	*0.001755
C	{0.1}	*0.000534	0.217083		*0.025145	0.572412	0.29056	*0.000416
D	{1}	*0.000178	*0.003529	*0.025145		*0.020656	*0.005607	*0.000161
E	{10}	*0.000917	0.333625	0.572412	*0.020656		0.338374	*0.000674
F	{100}	*0.003258	0.638767	0.29056	*0.005607	0.338374		*0.001845
G	{500}	1	*0.001755	*0.000416	*0.000161	*0.000674	*0.001845	

APPENDIX 32: ANOVA: *Tetraselmis tetrahele* UMACC 144: DNA Damage (RAPD-Appearance of new band)

* = Significant difference : p<0.05

ANOVA-APPEARANCE-TET

Newman-Keuls test; BD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	39.53000	53.61000	42.34000	37.03000	36.67000	0.000000
A	{0}		*0.001120	*0.000234	*0.000849	*0.001318	*0.000831	1
B	{0.01	*0.001120	3289031982	0.185489	0.715434	0.74557	0.924445	*0.000803
C	{0.1}	*0.000234	0.185489		0.157811	0.172199	0.220243	*0.000202
D	{1}	*0.000849	0.715434	0.157811		0.765566	0.874794	*0.000640
E	{10}	*0.001318	0.74557	0.172199	0.765566		0.962757	*0.000774
F	{100}	*0.000831	0.924445	0.220243	0.874794	0.962757		*0.000406
G	{500}	1	*0.000803	*0.000202	*0.000640	*0.000774	*0.000406	

Newman-Keuls test; MC_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	21.50000	28.89000	26.11000	17.33000	15.89000	0.000000
A	{0}		*0.006093	*0.000929	*0.001692	*0.018837	*0.018465	1
B	{0.01	*0.006093	5616493225	0.337416	0.376949	0.42297	0.523345	*0.004034
C	{0.1}	*0.000929	0.337416		0.59079	0.147923	0.129413	*0.000724
D	{1}	*0.001692	0.376949	0.59079		0.226093	0.225968	*0.001242
E	{10}	*0.018837	0.42297	0.147923	0.226093		0.779831	*0.010699
F	{100}	*0.018465	0.523345	0.129413	0.225968	0.779831		*0.007295
G	{500}	1	*0.004034	*0.000724	*0.001242	*0.010699	*0.007295	

Newman-Keuls test; DD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	41.61000	34.32000	29.49000	26.71000	25.32000	22.05000
A	{0}		*0.000876	*0.003455	*0.008386	*0.011398	*0.009270	*0.008754
B	{0.01	*0.000876	3670921325	0.329988	0.247711	0.212585	0.21621	0.134933
C	{0.1}	*0.003455	0.329988		0.514616	0.55683	0.609473	0.465125
D	{1}	*0.008386	0.247711	0.514616		0.706166	0.834226	0.735094
E	{10}	*0.011398	0.212585	0.55683	0.706166		0.85024	0.798049
F	{100}	*0.009270	0.21621	0.609473	0.834226	0.85024		0.657744
G	{500}	*0.008754	0.134934	0.465125	0.735094	0.798049	0.657744	

Newman-Keuls test; MA_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	42.04000	55.19000	50.60000	40.83000	37.85000	33.56000
A	{0}		*0.013204	*0.002757	*0.004362	*0.010781	*0.010205	*0.008567
B	{0.01	*0.013204	6937942505	0.472063	0.447579	0.913703	0.922995	0.86476
C	{0.1}	*0.002757	0.472063		0.681596	0.571165	0.530329	0.40156
D	{1}	*0.004362	0.447579	0.681596		0.653894	0.657875	0.546156
E	{10}	*0.010781	0.913703	0.571165	0.653894		0.789617	0.7878
F	{100}	*0.010205	0.922995	0.530329	0.657875	0.789617		0.701267
G	{500}	*0.008567	0.86476	0.40156	0.546156	0.787801	0.701267	

Newman-Keuls test; BD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	29.06000	38.96000	32.88000	32.53000	28.54000	0.000000
A	{0}		*0.001974	*0.000469	*0.001432	*0.001153	*0.001299	1
B	{0.01	*0.001974	1654396057	0.416231	0.815712	0.586746	0.934819	*0.001131
C	{0.1}	*0.000469	0.416231		0.346149	0.570203	0.480544	*0.000374
D	{1}	*0.001432	0.815712	0.346149		0.956127	0.89694	*0.001057
E	{10}	*0.001153	0.586746	0.570203	0.956127		0.800981	*0.000824
F	{100}	*0.001299	0.934819	0.480544	0.89694	0.800981		*0.000578
G	{500}	1	*0.001131	*0.000374	*0.001057	*0.000824	*0.000578	

Newman-Keuls test; MC_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	32.50000	24.06000	21.86000	20.86000	14.21000	0.000000
A	{0}		*0.005074	*0.035495	*0.046826	*0.041506	0.137008	1
B	{0.01	*0.005074	3818283081	0.24369	0.30549	0.370118	0.116196	*0.003873
C	{0.1}	*0.035495	0.24369		0.755806	0.890159	0.507741	*0.026162
D	{1}	*0.046826	0.30549	0.755806		0.88749	0.527684	*0.031763
E	{10}	*0.041506	0.370118	0.890159	0.88749		0.353882	*0.024004
F	{100}	0.137008	0.116196	0.507741	0.527684	0.353882		0.059744
G	{500}	1	*0.003873	*0.026162	*0.031763	*0.024004	0.059744	

Newman-Keuls test; DD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	16.82000	18.64000	23.95000	22.81000	13.58000	13.35000
A	{0}		*0.022458	*0.017117	*0.004515	*0.005155	*0.043357	*0.019194
B	{0.01	*0.022458	1360816956	0.723445	0.510969	0.478767	0.530729	0.773879
C	{0.1}	*0.017117	0.723445		0.556796	0.421928	0.586355	0.724032
D	{1}	*0.004515	0.510969	0.556796		0.824388	0.290262	0.339061
E	{10}	*0.005155	0.478767	0.421928	0.824388		0.30003	0.372109
F	{100}	*0.043357	0.530729	0.586355	0.290262	0.30003		0.964353
G	{500}	*0.019194	0.773879	0.724032	0.339061	0.372109	0.964353	

Newman-Keuls test; MA_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	36.87000	47.41000	50.76000	53.57000	49.06000	31.59000
A	{0}		*0.003964	*0.000999	*0.001086	*0.000897	*0.001076	*0.004570
B	{0.01	*0.003964	1857147216	0.277423	0.469034	0.415837	0.414413	0.580304
C	{0.1}	*0.000999	0.277423		0.931752	0.910101	0.862197	0.240984
D	{1}	*0.001086	0.469034	0.931752		0.767693	0.858062	0.291183
E	{10}	*0.000897	0.415837	0.910101	0.767693		0.880144	0.235581
F	{100}	*0.001076	0.414413	0.862197	0.858062	0.880144		0.282602
G	{500}	*0.004570	0.580305	0.240985	0.291183	0.235582	0.282602	

APPENDIX 32: ANOVA: *Tetraselmis tetrahele* UMACC 144: DNA Damage (RAPD-Disappearance of band)

* = Significant difference : p<0.05

ANOVA-DISAPPEARANCE-TET

Newman-Keuls test; CD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	24.17000	26.70000	29.32000	40.55000	43.35000	37.93000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000158	*0.000174	*0.000150978565216064
B	{0.01	*0.000176668167114		0.116963	*0.011318	*0.000150	*0.000158	*0.000196456909179687
C	{0.1}	*0.000180	0.116963		0.105572	*0.000196	*0.000150	*0.000185072422027588
D	{1}	*0.000196	*0.011318	0.105572		*0.000185	*0.000196	*0.000220894813537598
E	{10}	*0.000158	*0.000150	*0.000196	*0.000185072422027		0.085694	0.105572
F	{100}	*0.000174	*0.000158	*0.000150	*0.000196	0.085694		*0.00804728269577026
G	{500}	*0.000150	*0.000196	*0.000185	*0.000220	0.105572	*0.00804728269577026	

Newman-Keuls test; CD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	23.32000	29.78000	40.43000	51.65000	54.18000	66.92000
A	{0}		*0.031014	*0.021588	*0.004815	*0.000992	*0.000891	*0.00026375055313110
B	{0.01	*0.031014025211		0.517147	0.218521	*0.049278	*0.044938	*0.00554633140563965
C	{0.1}	*0.021588	0.517147		0.291709	0.097045	0.101548	*0.0136277079582214
D	{1}	*0.004815	0.218521	0.291709		0.267701	0.360258	0.069468
E	{10}	*0.000992	*0.049278	0.097045	0.267701		0.798504	0.289902
F	{100}	*0.000891	*0.044938	0.101548	0.360258	0.798504		0.211052
G	{500}	*0.000263	*0.005546	*0.013627	0.069468	0.289902	0.211052	

Newman-Keuls test; CR_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	59.82000	63.39000	66.27000	66.67000	68.75000	66.97000
A	{0}		*0.000232	*0.000270	*0.000297	*0.000312	*0.000393	*0.00037693977355957
B	{0.01	*0.000232040882110		0.744384	0.821827	0.917865	0.956309	0.960544
C	{0.1}	*0.000270	0.744384		0.792397	0.950052	0.986127	0.986706
D	{1}	*0.000297	0.821827	0.792397		0.970922	0.995502	0.997757
E	{10}	*0.000312	0.917865	0.950052	0.970922		0.97961	0.978202
F	{100}	*0.000393	0.956309	0.986127	0.995502	0.97961		0.870716
G	{500}	*0.000376	0.960544	0.986706	0.997757	0.978202	0.870716	

Newman-Keuls test; CR_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	49.20000	52.40000	53.83000	54.05000	58.51000	61.61000
A	{0}		*0.001995	*0.003144	*0.004554	*0.006668	*0.004902	*0.00418835878372192
B	{0.01	*0.001995980739593		0.807006	0.93132	0.980986	0.947182	0.921309
C	{0.1}	*0.003144	0.807006		0.913052	0.991034	0.963273	0.949102
D	{1}	*0.004554	0.93132	0.913052		0.986691	0.929881	0.928652
E	{10}	*0.006668	0.980986	0.991034	0.986691		0.73372	0.828453
F	{100}	*0.004902	0.947182	0.963273	0.929881	0.73372		0.812911
G	{500}	*0.004188	0.921309	0.949102	0.928652	0.828453	0.812911	

Newman-Keuls test; CU_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	34.11000	46.11000	45.43000	46.44000	57.28000	86.88000
A	{0}		*0.004048	*0.001957	*0.001257	*0.002746	*0.000653	*0.00017625093460083
B	{0.01	*0.004048407077789		0.464569	0.271336	0.608669	0.188205	*0.00128382444381714
C	{0.1}	*0.001957	0.464569		0.94621	0.97397	0.512097	*0.00515085458755493
D	{1}	*0.001257	0.271336	0.94621		0.994342	0.637365	*0.00683754682540894
E	{10}	*0.002746	0.608669	0.97397	0.994342		0.291305	*0.00307309627532959
F	{100}	*0.000653	0.188205	0.512097	0.637365	0.291305		*0.00980204343795776
G	{500}	*0.000176	*0.001283	*0.005150	*0.006837	*0.003073	*0.00980204343795776	

Newman-Keuls test; CU_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	64.97000	66.25000	67.14000	69.01000	83.25000	85.21000
A	{0}		*0.000178	*0.000185	*0.000205	*0.000169	*0.000158	*0.00017470121383667
B	{0.01	*0.000178694725036		0.88952	0.968873	0.969189	0.305293	0.280365
C	{0.1}	*0.000185	0.88952		0.923065	0.950156	0.279589	0.274076
D	{1}	*0.000205	0.968873	0.923065		0.839166	0.211245	0.234499
E	{10}	*0.000169	0.969189	0.950156	0.839166		0.137575	0.207992
F	{100}	*0.000158	0.305293	0.279589	0.211245	0.137575		0.831551
G	{500}	*0.000174	0.280365	0.274076	0.234499	0.207992	0.831551	

Newman-Keuls test; AD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	59.53000	68.88000	70.58000	72.03000	76.19000	100.0000
A	{0}		*0.000183	*0.000183	*0.000199	*0.000156	*0.000161	*0.000174105167388916
B	{0.01	*0.000183045864105		0.314619	0.454267	0.522628	0.381287	*0.00526565313339233
C	{0.1}	*0.000183	0.314619		0.852391	0.934571	0.846156	*0.0260651707649231
D	{1}	*0.000199	0.454267	0.852391		0.873901	0.808583	*0.0249246954917908
E	{10}	*0.000156	0.522628	0.934571	0.873901		0.649796	*0.0193889141082764
F	{100}	*0.000161	0.381287	0.846156	0.808583	0.649796		*0.0189324617385864
G	{500}	*0.000174	*0.005265	*0.026065	*0.024924	*0.019388	*0.0189324617385864	

Newman-Keuls test; AD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	77.59000	74.52000	74.52000	77.54000	79.17000	100.0000
A	{0}		*0.000152	*0.000176	*0.000181	*0.000196	*0.000159	*0.000174105167388916
B	{0.01	*0.000152289867401		0.985493	0.937296	0.995716	0.862214	0.061063
C	{0.1}	*0.000176	0.985493		1	0.93925	0.983813	0.105766
D	{1}	*0.000181	0.937296	1		0.740366	0.952758	0.080036
E	{10}	*0.000196	0.995716	0.93925	0.740366		0.981908	0.100797
F	{100}	*0.000159	0.862214	0.983813	0.952758	0.981908		*0.0352303981781006
G	{500}	*0.000174	0.061063	0.105766	0.080036	0.100797	*0.0352303981781006	

APPENDIX 32: ANOVA: *Tetraselmis tetrahele* UMACC 144: DNA Damage (RAPD-Disappearance of band)

* = Significant difference : p<0.05

ANOVA-DISAPPEARANCE-TET

Newman-Keuls test; BD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	75.03000	76.57000	77.42000	78.61000	82.77000	100.0000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000151	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176		0.847415	0.950456	0.967385	0.857282	0.059775
C	{0.1}	*0.000180	0.847415		0.915429	0.963645	0.85795	0.063434
D	{1}	*0.000196	0.950456	0.915429		0.881789	0.777941	0.052921
E	{10}	*0.000151	0.967385	0.963645	0.881789		0.604601	*0.0410385727882385
F	{100}	*0.000158	0.857282	0.85795	0.777941	0.604601		*0.0456660389900208
G	{500}	*0.000174	0.059775	0.063434	0.052921	*0.041038	*0.0456660389900208	

Newman-Keuls test; BD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	76.11000	78.10000	80.16000	81.27000	82.86000	100.0000
A	{0}		*0.000179	*0.000185	*0.000203	*0.000169	*0.000170	*0.00017470121383667
B	{0.01}	*0.000179		0.854983	0.924302	0.961642	0.967283	0.281453
C	{0.1}	*0.000185	0.854983		0.849939	0.952862	0.969449	0.293453
D	{1}	*0.000203	0.924302	0.849939		0.918821	0.965554	0.289195
E	{10}	*0.000169	0.961642	0.952862	0.918821		0.883903	0.220901
F	{100}	*0.000170	0.967283	0.969449	0.965554	0.883903		0.130984
G	{500}	*0.000174	0.281453	0.293453	0.289195	0.220901	0.130984	

Newman-Keuls test; MC_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	73.82000	79.79000	80.94000	82.54000	80.52000	100.0000
A	{0}		*0.000176	*0.000180	*0.000150	*0.000158	*0.000196	*0.000174045562744141
B	{0.01}	*0.000176		0.348487	0.662017	0.627382	0.536125	*0.00844436883926392
C	{0.1}	*0.000180	0.348487		0.981039	0.969199	0.907355	*0.0368280410766602
D	{1}	*0.000150	0.662017	0.981039		0.798731	0.946636	*0.0202595591545105
E	{10}	*0.000158	0.627382	0.969199	0.798731		0.942645	*0.013317883014679
F	{100}	*0.000196	0.536125	0.907355	0.946636	0.942645		*0.0309924483299255
G	{500}	*0.000174	*0.008444	*0.036828	*0.020259	*0.013317	*0.0309924483299255	

Newman-Keuls test; MC_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	63.14000	71.36000	77.19000	84.24000	85.70000	100.0000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176		0.145347	0.29248	0.072187	0.073921	*0.00090719135284424
C	{0.1}	*0.000180	0.145347		0.29248	0.072187	0.073921	*0.00090719135284424
D	{1}	*0.000196	*0.048347	0.29248		0.207117	0.279324	*0.00386673212051392
E	{10}	*0.000150	*0.007001	0.072187	0.207117		0.788204	*0.0264561772346497
F	{100}	*0.000158	*0.006360	0.073921	0.279324	0.788204		*0.0179697871208191
G	{500}	*0.000174	*0.000226	*0.000907	*0.003866	*0.026456	*0.0179697871208191	

Newman-Keuls test; DD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	53.63000	57.80000	59.29000	74.61000	72.30000	73.87000
A	{0}		*0.000208	*0.000219	*0.000254	*0.000182	*0.000157	*0.000165283679962158
B	{0.01}	*0.000208		0.656581	0.813472	0.261725	0.222154	0.233346
C	{0.1}	*0.000219	0.656581		0.873435	0.394735	0.286181	0.335696
D	{1}	*0.000254	0.813472	0.873435		0.374633	0.178199	0.282596
E	{10}	*0.000182	0.261725	0.394735	0.374633		0.965839	0.936964
F	{100}	*0.000157	0.222154	0.286181	0.178199	0.965839		0.866701
G	{500}	*0.000165	0.233346	0.335696	0.282596	0.936964	0.866701	

Newman-Keuls test; DD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	52.13000	50.46000	59.04000	68.26000	70.19000	70.19000
A	{0}		*0.001394	*0.000760	*0.000925	*0.000420	*0.000441	*0.000553250312805176
B	{0.01}	*0.001394		0.886678	0.557481	0.365915	0.424984	0.537389
C	{0.1}	*0.000760	0.886678		0.740696	0.436999	0.45565	0.543577
D	{1}	*0.000925	0.557481	0.740696		0.436045	0.606924	0.768295
E	{10}	*0.000420	0.365915	0.436999	0.436045		0.869174	0.984676
F	{100}	*0.000441	0.424984	0.45565	0.606924	0.869174		1
G	{500}	*0.000553	0.537389	0.543577	0.768295	0.984676	1	

Newman-Keuls test; MA_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	52.38000	55.40000	54.55000	57.67000	61.67000	69.65000
A	{0}		*0.000526	*0.001297	*0.000853	*0.001353	*0.001042	*0.000507712364196777
B	{0.01}	*0.000526		0.9614	0.85021	0.964638	0.918754	0.651142
C	{0.1}	*0.001297	0.9614		0.94105	0.843404	0.845054	0.598864
D	{1}	*0.000853	0.85021	0.94105		0.958854	0.920157	0.672689
E	{10}	*0.001353	0.964638	0.843404	0.958854		0.728085	0.551468
F	{100}	*0.001042	0.918754	0.845054	0.920157	0.728085		0.490726
G	{500}	*0.000507	0.651142	0.598864	0.672689	0.551468	0.490726	

Newman-Keuls test; MA_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	46.34000	45.66000	48.54000	49.20000	53.97000	78.48000
A	{0}		*0.001595	*0.000758	*0.001949	*0.002595	*0.001637	*0.000200033187866211
B	{0.01}	*0.001595		0.948874	0.835592	0.959362	0.881968	0.052386
C	{0.1}	*0.000758	0.948874		0.958805	0.985908	0.926599	0.062416
D	{1}	*0.001949	0.835592	0.958805		0.950376	0.861919	0.052616
E	{10}	*0.002595	0.959362	0.985908	0.950376		0.653591	*0.0345895290374756
F	{100}	*0.001637	0.881968	0.926599	0.861919	0.653591		*0.0336272120475769
G	{500}	*0.000200	0.052386	0.062416	0.052616	*0.034589	*0.0336272120475769	

APPENDIX 32: ANOVA: *Tetraselmis tetrahele* UMACC 245: DNA Damage (RAPD-Similarity of band)

* = Significant difference : p<0.05

ANOVA-SIMILIAR-TET

Newman-Keuls test; CD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	75.83000	68.83000	68.57000	59.45000	56.65000	56.65000
A	{0}		*0.011814	*0.005953	*0.009967	*0.002060	*0.002104	*0.00161182880401611
B	{0.01	*0.011814		0.415341	0.666702	0.247015	0.256288	0.201619
C	{0.1}	*0.005953	0.415341		0.975687	0.515025	0.601634	0.484923
D	{1}	*0.009967	0.666702	0.975687		0.292518	0.502511	0.35309
E	{10}	*0.002060	0.247015	0.515025	0.292518		0.940047	0.742067
F	{100}	*0.002104	0.256288	0.601634	0.502511	0.940047		1
G	{500}	*0.001611	0.201619	0.484923	0.35309	0.742067	1	

Newman-Keuls test; CD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	76.68000	70.22000	59.57000	48.35000	45.82000	33.08000
A	{0}		0.050589	*0.040445	*0.011167	*0.002561	*0.002364	*0.000531554222106934
B	{0.01	0.050589		0.562938	0.290497	0.086997	0.083156	*0.0134560465812683
C	{0.1}	*0.040445	0.562938		0.345178	0.147194	0.160651	*0.0293700695037842
D	{1}	*0.011167	0.290497	0.345178		0.320816	0.438676	0.116586
E	{10}	*0.002561	0.086997	0.147194	0.320816		0.819906	0.366971
F	{100}	*0.002364	0.083156	0.160651	0.438676	0.819906		0.262048
G	{500}	*0.000531	*0.013456	*0.029370	0.116586	0.366971	0.262048	

Newman-Keuls test; CR_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	45.92000	46.03000	45.46000	44.90000	31.25000	25.85000
A	{0}		*0.000216	*0.000188	*0.000262	*0.000255	*0.000165	*0.000176727771759033
B	{0.01	*0.000216		0.989998	0.957852	0.992217	0.349996	0.185357
C	{0.1}	*0.000188	0.989998		0.997619	0.999187	0.446324	0.232119
D	{1}	*0.000262	0.957852	0.997619		0.948648	0.25203	0.145211
E	{10}	*0.000255	0.992217	0.999187	0.948648		0.1318	0.099798
F	{100}	*0.000165	0.349996	0.446324	0.25203	0.1318		0.536815
G	{500}	*0.000176	0.185357	0.232119	0.145211	0.099798	0.536815	

Newman-Keuls test; CR_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	40.46000	45.40000	50.40000	54.47000	51.12000	38.39000
A	{0}		*0.000637	*0.000974	*0.001465	*0.000702	*0.000952	*0.00062251091003418
B	{0.01	*0.000637		0.637401	0.607036	0.656846	0.729584	0.842967
C	{0.1}	*0.000974	0.637401		0.633343	0.812683	0.844137	0.77655
D	{1}	*0.001465	0.607036	0.633343		0.917324	0.945086	0.653448
E	{10}	*0.000702	0.656846	0.812683	0.917324		0.748738	0.629715
F	{100}	*0.000952	0.729584	0.844137	0.945086	0.748738		0.72832
G	{500}	*0.000622	0.842967	0.77655	0.653448	0.629715	0.72832	

Newman-Keuls test; CU_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	65.89000	62.50000	62.75000	53.56000	42.72000	13.13000
A	{0}		*0.006271	*0.015252	*0.009027	*0.004797	*0.001140	*0.000181317329406738
B	{0.01	*0.006271		0.945302	0.771151	0.657333	0.239175	*0.00230669975280762
C	{0.1}	*0.015252	0.945302		0.981603	0.412567	0.184231	*0.00195872783660889
D	{1}	*0.009027	0.771151	0.981603		0.668168	0.274807	*0.00279587507247925
E	{10}	*0.004797	0.657333	0.412567	0.668168		0.323134	*0.00510108470916748
F	{100}	*0.001140	0.239175	0.184231	0.274807	0.323134		*0.0144362449645996
G	{500}	*0.000181	*0.002306	*0.001958	*0.002795	*0.005101	*0.014436	2449645996

Newman-Keuls test; CU_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	24.14000	35.13000	34.45000	30.94000	16.75000	14.79000
A	{0}		*0.000151	*0.000176	*0.000181	*0.000196	*0.000158	*0.000174051673888916
B	{0.01	*0.000151	0.038169860	0.491834	0.387809	0.385345	0.346605	0.454461
C	{0.1}	*0.000176	0.491834		0.929945	0.847028	0.165994	0.141143
D	{1}	*0.000181	0.387809	0.929945		0.650811	0.137411	0.125657
E	{10}	*0.000196	0.385345	0.847028	0.650811		0.183817	0.191714
F	{100}	*0.000158	0.346605	0.165994	0.137411	0.183817		0.79999
G	{500}	*0.000174	0.454461	0.141143	0.125657	0.191714	0.79999	

Newman-Keuls test; AD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	40.48000	36.90000	34.33000	30.95000	28.57000	0.000000
A	{0}		*0.000177	*0.000182	*0.000197	*0.000152	*0.000159	*0.000174045562744141
B	{0.01	*0.000177	8006553649	0.65942	0.724652	0.637374	0.579674	*0.00192219018936157
C	{0.1}	*0.000182	0.65942		0.751325	0.739413	0.725059	*0.00304538011550903
D	{1}	*0.000197	0.724652	0.751325		0.677223	0.753304	*0.00360506772994995
E	{10}	*0.000152	0.637374	0.739413	0.677223		0.769118	*0.00444036722183228
F	{100}	*0.000159	0.579674	0.725059	0.753304	0.769118		*0.00307768583297729
G	{500}	*0.000174	*0.001922	*0.003045	*0.003605	*0.004440	*0.003077	68583297729

Newman-Keuls test; AD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	22.41000	33.98000	25.48000	22.47000	20.84000	0.000000
A	{0}		*0.000150	*0.000176	*0.000180	*0.000196	*0.000158	*0.000174045562744141
B	{0.01	*0.000150	978565216	0.251447	0.863851	0.992169	0.79497	*0.00548213720321655
C	{0.1}	*0.000176	0.251447		0.173417	0.163513	0.229077	*0.00070954877471924
D	{1}	*0.000180	0.863851	0.173417		0.619425	0.860848	*0.0056261420249939
E	{10}	*0.000196	0.992169	0.163513	0.619425		0.959334	*0.00955265760421753
F	{100}	*0.000158	0.79497	0.229077	0.860848	0.959334		*0.00356435775756836
G	{500}	*0.000174	*0.005482	*0.000709	*0.005626	*0.009552	*0.003564	35775756836

APPENDIX 32: ANOVA: *Tetraselmis tetrahele* UMACC 245: DNA Damage (RAPD-Similarity of band)

* = Significant difference : p<0.05

ANOVA-SIMILIAR-TET

Newman-Keuls test; BD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	45.79000	44.01000	39.25000	31.22000	17.23000	0.000000
A	{0}		*0.000203	*0.000240	*0.000235	*0.000171	*0.000159	*0.000174105167388916
B	{0.01}	*0.000203	0.848053	0.848053	0.757357	0.410489	*0.048455	*0.002155721187591 55
C	{0.1}	*0.000240	0.848053		0.609723	0.365775	*0.047204	*0.002186596393585 21
D	{1}	*0.000235	0.757357	0.609723		0.393253	0.072286	*0.003685712814331 05
E	{10}	*0.000171	0.410489	0.365775	0.393253		0.147144	*0.0108139514923096
F	{100}	*0.000159	*0.048455	*0.047204	0.072286	0.147144		0.079647
G	{500}	*0.000174	*0.002155	*0.002186	*0.003685	*0.010813	0.079647	

Newman-Keuls test; BD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	33.44000	30.67000	26.22000	21.08000	21.25000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000158	*0.000150	*0.000174045562744141
B	{0.01}	*0.000176	0.686842	0.686842	0.545268	0.391978	0.308473	*0.002363681793212 89
C	{0.1}	*0.000180	0.686842		0.519129	0.504883	0.36733	*0.003527104854583 74
D	{1}	*0.000196	0.545268	0.519129		0.730332	0.472341	*0.007851898670196 53
E	{10}	*0.000158	0.391978	0.504883	0.730332		0.980297	*0.007474064826965 33
F	{100}	*0.000150	0.308473	0.36733	0.472341	0.980297		*0.018020570278167 7
G	{500}	*0.000174	*0.002363	*0.003527	*0.007851	*0.007474	*0.018020570278167 7	

Newman-Keuls test; MC_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	34.91000	40.42000	30.50000	28.83000	29.83000	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000158	*0.000150	*0.000174045562744141
B	{0.01}	*0.000180	9000968933	0.383711	0.483523	0.755964	0.691622	*0.000567674636840 82
C	{0.1}	*0.000176	0.383711		0.270266	0.364931	0.346111	*0.000274837017059326
D	{1}	*0.000196	0.483523	0.270266		0.96005	0.91456	*0.001172065734861 28
E	{10}	*0.000158	0.755964	0.364931	0.96005		0.872752	*0.000486850738525391
F	{100}	*0.000150	0.691622	0.346111	0.91456	0.872752		*0.000815153121948242
G	{500}	*0.000174	*0.000567	*0.000274	*0.001172	*0.000486	*0.000815153121948242	

Newman-Keuls test; MC_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	42.13000	32.73000	26.07000	18.02000	16.35000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176	727771759	0.158628	0.057231	*0.009069	*0.008361	*0.000260233879089355
C	{0.1}	*0.000180	0.158628		0.309219	0.08419	0.087558	*0.001210033893585 21
D	{1}	*0.000196	0.057231	0.309219		0.222925	0.30304	*0.005095720291137 7
E	{10}	*0.000150	*0.009069	0.08419	0.222925		0.795251	*0.032087922096252 4
F	{100}	*0.000158	*0.008361	0.087558	0.30304	0.795251		*0.021492362022399 9
G	{500}	*0.000174	*0.000260	*0.001210	*0.005095	*0.032087	*0.021492362022399 9	

Newman-Keuls test; DD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	46.37000	42.20000	40.72000	29.01000	31.90000	26.13000
A	{0}		*0.000208	*0.000218	*0.000253	*0.000170	*0.000173	*0.000183582305908203
B	{0.01}	*0.000208	1394195556	0.655873	0.813324	0.363022	0.420046	0.292127
C	{0.1}	*0.000218	0.655873		0.874001	0.496375	0.515109	0.434721
D	{1}	*0.000253	0.813324	0.874001		0.429328	0.351816	0.413196
E	{10}	*0.000170	0.363022	0.496375	0.429328		0.757027	0.757839
F	{100}	*0.000173	0.420046	0.515109	0.351816	0.757027		0.806257
G	{500}	*0.000183	0.292127	0.434721	0.413196	0.757839	0.806257	

Newman-Keuls test; DD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	45.11000	49.54000	40.96000	31.74000	29.81000	29.81000
A	{0}		*0.000193	*0.000189	*0.000206	*0.000152	*0.000176	*0.000159859657287598
B	{0.01}	*0.000193	7400164 7	0.588875	0.612422	0.250813	0.355885	0.267553
C	{0.1}	*0.000189	0.588875		0.546245	0.164743	0.199875	0.155172
D	{1}	*0.000206	0.612422	0.546245		0.268861	0.523773	0.371101
E	{10}	*0.000152	0.250813	0.164743	0.268861		0.968625	0.813114
F	{100}	*0.000176	0.355885	0.199875	0.523773	0.968625		1
G	{500}	*0.000159	0.267553	0.155172	0.371101	0.813114	1	

Newman-Keuls test; MA_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	47.62000	49.96000	45.45000	42.33000	42.92000	30.35000
A	{0}		*0.000710	*0.000465	*0.000881	*0.001051	*0.000862	*0.000330209732055664
B	{0.01}	*0.000710	1297378540	0.827807	0.840122	0.95747	0.897242	0.499891
C	{0.1}	*0.000465	0.827807		0.904951	0.9476	0.907726	0.46344
D	{1}	*0.000881	0.840122	0.904951		0.953204	0.814098	0.501838
E	{10}	*0.001051	0.95747	0.9476	0.953204		0.956298	0.275386
F	{100}	*0.000862	0.897242	0.907726	0.814098	0.956298		0.477375
G	{500}	*0.000330	0.499891	0.46344	0.501838	0.275386	0.477375	

Newman-Keuls test; MA_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	48.04000	50.68000	51.46000	50.68000	46.03000	21.52000
A	{0}		*0.001374	*0.001446	*0.000449	*0.000843	*0.001357	*0.000192999839782715
B	{0.01}	*0.001374	4831085205	0.799159	0.986418	0.963747	0.846345	0.051112
C	{0.1}	*0.001446	0.799159		0.996838	1	0.892159	0.053909
D	{1}	*0.000449	0.986418	0.996838		0.940073	0.982269	0.090731
E	{10}	*0.000843	0.963747	1	0.940073		0.96718	0.078262
F	{100}	*0.001357	0.846345	0.892159	0.982269	0.96718		*0.030468642711639 4
G	{500}	*0.000192	0.051112	0.053909	0.090731	0.078262	*0.030468642711639 4	

APPENDIX 32: ANOVA: *Tetraselmis tetrahele* UMACC 245: DNA Damage (RAPD-Intensity of band)

* = Significant difference : p<0.05

ANOVA-INTENSITY-TET

Newman-Keuls test; CD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	94.81000	129.2000	156.6900	170.7700	165.2900	166.0900
A	{0}		0.783748	0.13774	*0.021875	*0.018836	*0.015909	*0.0219802260398865
B	{0.01	0.783748		0.188515	*0.022490	*0.014638	*0.014156	*0.0179108381271362
C	{0.1}	0.13774	0.188515		0.160447	0.220826	0.162551	0.237983
D	{1}	*0.021875	*0.022490	0.160447		0.871226	0.650026	0.869234
E	{10}	*0.018836	*0.014638	0.220826	0.871226		0.953245	0.804516
F	{100}	*0.015909	*0.014156	0.162551	0.650026	0.953245		0.966305
G	{500}	*0.021980	*0.017910	0.237983	0.869234	0.804516	0.966305	

Newman-Keuls test; CD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	97.29000	95.77000	110.2800	102.8100	100.8300	89.52000
A	{0}		0.875756	0.966626	0.928994	0.985157	0.961873	0.925223
B	{0.01	0.875756		0.930138	0.936843	0.987724	0.976502	0.892183
C	{0.1}	0.966626	0.930138		0.951644	0.993189	0.990501	0.718792
D	{1}	0.928994	0.936843	0.951644		0.667245	0.845298	0.874778
E	{10}	0.985157	0.987724	0.993189	0.667245		0.909061	0.96629
F	{100}	0.961873	0.976502	0.990501	0.845298	0.909061		0.960799
G	{500}	0.925223	0.892183	0.718792	0.874778	0.96629	0.960799	

Newman-Keuls test; CR_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	145.0900	148.2000	154.0400	164.5500	119.5000	43.60000
A	{0}		*0.025699	*0.030311	*0.022040	*0.008429	0.219508	*0.02244492292404175
B	{0.01	*0.025699913501739		0.840586	0.827647	0.587774	0.113818	*0.000233888626098633
C	{0.1}	*0.030311	0.840586		0.706104	0.542542	0.177539	*0.000201106071472168
D	{1}	*0.022040	0.827647	0.706104		0.499817	0.150805	*0.000189363956451416
E	{10}	*0.008429	0.587774	0.542542	0.499817		0.065061	*0.00018543004989624
F	{100}	0.219508	0.113818	0.177539	0.150805	0.065061		*0.000670909881591797
G	{500}	*0.002444	*0.000233	*0.000201	*0.000189	*0.000185	*0.000670909881591797	

Newman-Keuls test; CR_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	109.9300	111.7900	124.3900	121.7100	109.8400	73.04000
A	{0}		0.832621	0.899528	0.712142	0.713038	0.57435	0.137438
B	{0.01	0.832621		0.915062	0.832117	0.773865	0.995974	0.183433
C	{0.1}	0.899528	0.915062		0.746384	0.571279	0.99293	0.212997
D	{1}	0.712142	0.832117	0.746384		0.877844	0.909971	0.102664
E	{10}	0.713038	0.773865	0.571279	0.877844		0.897748	0.107192
F	{100}	0.57435	0.995974	0.99293	0.909971	0.897748		0.115236
G	{500}	0.137438	0.183433	0.212997	0.102664	0.107192	0.115236	

Newman-Keuls test; CU_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	111.2300	126.3400	131.1000	149.5900	143.9700	130.8600
A	{0}		0.482071	0.241763	0.314824	0.074319	0.110297	0.23956
B	{0.01	0.482071		0.347646	0.590601	0.198848	0.27075	0.438028
C	{0.1}	0.241763	0.347646		0.949892	0.581321	0.6755	0.775604
D	{1}	0.314824	0.590601	0.949892		0.478393	0.421745	0.988002
E	{10}	0.074319	0.198848	0.581321	0.478393		0.723203	0.633915
F	{100}	0.110297	0.27075	0.6755	0.421745	0.723203		0.683241
G	{500}	0.23956	0.438028	0.775604	0.988002	0.633915	0.683241	

Newman-Keuls test; CU_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	80.41000	89.02000	110.1900	93.70000	78.07000	66.70000
A	{0}		0.295938	0.569696	0.354503	0.563277	0.288872	0.065366
B	{0.01	0.295938		0.432	0.087967	0.445348	0.829185	0.424177
C	{0.1}	0.569696	0.432		0.237845	0.666828	0.571375	0.20128
D	{1}	0.354503	0.087967	0.237845		0.298864	0.079426	*0.0148702263832092
E	{10}	0.563277	0.445348	0.666828	0.298864		0.480357	0.137446
F	{100}	0.288872	0.829185	0.571375	0.079426	0.480357		0.30334
G	{500}	0.065366	0.424177	0.20128	*0.014870	0.137446	0.30334	

Newman-Keuls test; AD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	114.3600	189.3900	278.6700	175.0300	167.9200	0.000000
A	{0}		0.183899	*0.000152	*0.000158	*0.000207	*0.000201	*0.000176727771759033
B	{0.01	0.183899		*0.000207	*0.000150	*0.000270	*0.000289	*0.000180900096893311
C	{0.1}	*0.000152	*0.000207		*0.000176	0.183899	0.12785	*0.000158190727233887
D	{1}	*0.000158	*0.000150	*0.000176		*0.000180	*0.000196	*0.000174045562744141
E	{10}	*0.000207	*0.000270	0.183899	*0.000180		0.500207	*0.000150978565216064
F	{100}	*0.000201	*0.000289	0.12785	*0.000196	0.500207		*0.000196039676666626
G	{500}	*0.000176	*0.000180	*0.000158	*0.000174	*0.000150	*0.000196039676666626	

Newman-Keuls test; AD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	90.86000	141.1600	146.5700	139.3900	82.45000	0.000000
A	{0}		0.38907	*0.003610	*0.002476	*0.001989	0.23708	*0.000196099281311035
B	{0.01	0.38907		*0.001342	*0.000849	*0.001026	0.427102	*0.000181078910827637
C	{0.1}	*0.003610	*0.001342594623565		0.607024	0.865859	*0.000558	*0.000158190727233887
D	{1}	*0.002476	*0.000849	0.607024		0.768296	*0.000378	*0.000174045562744141
E	{10}	*0.001989	*0.001026	0.865859	0.768296		*0.000547	*0.000150978565216064
F	{100}	0.23708	0.427102	*0.000558	*0.000378	*0.000547289848327		*0.000176966190338135
G	{500}	*0.000196	*0.000181	*0.000158	*0.000174	*0.000150	*0.000176966190338135	

APPENDIX 32: ANOVA: *Tetraselmis tetrahele* UMACC 245: DNA Damage (RAPD-Intensity of band)

* = Significant difference : p<0.05

ANOVA-INTENSITY-TET

Newman-Keuls test; BD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	125.7600	131.2400	155.1600	213.9100	181.3500	0.000000
A	{0}		*0.031004	*0.028936	*0.000922	*0.000158	*0.000170	*0.000176727771759033
B	{0.01}	*0.031004428863525	0.617758	*0.039954	*0.000154	*0.000870	*0.000180900096893311
C	{0.1}	*0.028936	0.617758		*0.042875	*0.000201	*0.001118	*0.00019603967666626
D	{1}	*0.000922	*0.039954	*0.042875707149505	*0.000381	*0.028723	*0.0001509785652	
E	{10}	*0.000158	*0.000154	*0.000201	*0.000381410121917	*0.009100	*0.000174045562744141	
F	{100}	*0.000170	*0.000870	*0.001118	*0.028723	*0.009100914001464	*0.000158190727233887	
G	{500}	*0.000176	*0.000180	*0.000196	*0.000150	*0.000174	*0.000158190727233887	

Newman-Keuls test; BD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	108.5700	115.0800	135.8000	121.4000	100.8200	0.000000
A	{0}		0.723292	0.539423	0.053582	0.343217	0.941949	*0.000176787376403809
B	{0.01	0.723292		0.565012	0.109731	0.493986	0.494429	*0.000196099281311035
C	{0.1}	0.539423	0.565012		0.182121	0.576289	0.422788	*0.000150978565216064
D	{1}	0.053582	0.109731	0.182121		0.213339	*0.045580	*0.000174045562744141
E	{10}	0.343217	0.493986	0.576289	0.213339		0.286649	*0.000158190727233887
F	{100}	0.941949	0.494429	0.422788	*0.045580	0.286649		*0.000180959701538086
G	{500}	*0.000176	*0.000196	*0.000150	*0.000174	*0.000158	*0.000180959701538086	

Newman-Keuls test; MC_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	108.0900	154.0000	162.2600	178.1200	157.6800	0.000000
A	{0}		0.601638	*0.008266	*0.007958	*0.001722	*0.009263	*0.000183582305908203
B	{0.01}	0.601638		*0.009124	*0.014312	*0.003141	*0.014439	*0.000188231468200684
C	{0.1}	*0.008266	*0.009124398231506		0.850452	0.413549	0.811621	*0.000196099281311035
D	{1}	*0.007958	*0.014312	0.850452		0.312748	0.766858	*0.000158190727233887
E	{10}	*0.001722	*0.003141	0.413549	0.312748		0.392462	*0.000174045562744141
F	{100}	*0.009263	*0.014439	0.811621	0.766858	0.392462		*0.000150978565216064
G	{500}	*0.000183	*0.000188	*0.000196	*0.000158	*0.000174	*0.000150978565216064	

Newman-Keuls test; MC_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	104.4200	116.5700	128.8800	112.3700	87.69000	0.000000
A	{0}		0.71634	0.524969	0.165779	0.566113	0.319167	*0.000181436538696289
B	{0.01	0.71634		0.577114	0.216062	0.515626	0.365509	*0.000196754932403564
C	{0.1}	0.524969	0.577114		0.319167	0.729846	0.165779	*0.000158309936523437
D	{1}	0.165779	0.216062	0.319167		0.374602	*0.036385	*0.000174105167388916
E	{10}	0.566113	0.515626	0.729846	0.374602		0.209958	*0.000151336193084717
F	{100}	0.319167	0.365509	0.165779	*0.036385	0.209958		*0.000178158283233643
G	{500}	*0.000181	*0.000196	*0.000158	*0.000174	*0.000151	*0.000178158283233643	

Newman-Keuls test; DD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	99.73000	113.0300	110.7400	136.2500	115.4100	112.0400
A	{0}		0.980205	0.621838	0.329738	*0.039682	0.608611	0.51094
B	{0.01}	0.980205		0.723436	0.567747	*0.048330	0.684468	0.661744
C	{0.1}	0.621838	0.723436		0.974883	0.10901	0.826241	0.927249
D	{1}	0.329738	0.567747	0.974883		0.172525	0.970698	0.904551
E	{10}	*0.039682	*0.048330	0.10901	0.172525		0.070349	0.150942
F	{100}	0.608611	0.684468	0.826241	0.970698	0.070349		0.946437
G	{500}	0.51094	0.661744	0.927249	0.904551	0.150942	0.946437	

Newman-Keuls test; DD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	98.03000	98.03000	98.66000	102.7800	98.85000	95.01000
A	{0}		0.999939	0.999313	0.996051	0.861554	0.942505	0.999474
B	{0.01	0.999939		1	0.999178	0.999586	0.99995	0.849737
C	{0.1}	0.999313	1		0.968576	0.997973	0.998571	0.979761
D	{1}	0.996051	0.999178	0.968576		0.993357	0.990605	0.99537
E	{10}	0.861554	0.999586	0.997973	0.993357		0.965951	0.998514
F	{100}	0.942505	0.99995	0.998571	0.990605	0.965951		0.999135
G	{500}	0.999474	0.849737	0.979761	0.99537	0.998514	0.999135	

Newman-Keuls test; MA_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	117.7600	118.0200	118.4400	127.2900	136.0500	124.2500
A	{0}		0.120955	0.248596	0.352706	0.177984	0.055874	0.216345
B	{0.01}	0.120955		0.981157	0.997892	0.897314	0.552467	0.929309
C	{0.1}	0.248596	0.981157		0.969534	0.823985	0.4774	0.833204
D	{1}	0.352706	0.997892	0.969534		0.695385	0.390511	0.597557
E	{10}	0.177984	0.897314	0.823985	0.695385		0.429021	0.781633
F	{100}	0.055874	0.552467	0.4774	0.390511	0.429021		0.531142
G	{500}	0.216345	0.929309	0.833204	0.597557	0.781633	0.531142	

Newman-Keuls test; MA_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	93.95000	92.88000	90.86000	85.31000	82.40000	77.40000
A	{0}		0.598519	0.804089	0.846826	0.690638	0.630353	0.448143
B	{0.01	0.598519		0.925526	0.959303	0.86681	0.838035	0.684661
C	{0.1}	0.804089	0.925526		0.859892	0.781953	0.787716	0.649826
D	{1}	0.846826	0.959303	0.859892		0.62882	0.736507	0.637409
E	{10}	0.690638	0.86681	0.781953	0.62882		0.799355	0.764834
F	{100}	0.630353	0.838035	0.787716	0.736507	0.799355		0.662969
G	{500}	0.448143	0.684661	0.649826	0.637409	0.764834	0.662969	

APPENDIX 32: ANOVA: *Tetraselmis tetrahele* UMACC 245: DNA Damage (RAPD-Genomic Template Stability)

* = Significant difference : p<0.05

ANOVA-GTS-TET

Newman-Keuls test; CD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	53.75000	41.73000	39.11000	38.29000	37.57000	36.51000
A	{0}		*0.000385	*0.000236	*0.000255	*0.000241	*0.000270	*0.000297963619232178
B	{0.01}	*0.000385	824470520	0.223178	0.297689	0.389501	0.455776	0.479903
C	{0.1}	*0.000236	0.223178		0.785265	0.929685	0.970307	0.979707
D	{1}	*0.000255	0.297689	0.785265		0.932024	0.985497	0.992406
E	{10}	*0.000241	0.389501	0.929685	0.932024		0.940302	0.980657
F	{100}	*0.000270	0.455776	0.970307	0.985497	0.940302		0.912193
G	{500}	*0.000297	0.479903	0.979707	0.992406	0.980657	0.912193	

Newman-Keuls test; CD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	61.77000	53.21000	31.70000	25.21000	23.27000	18.04000
A	{0}		*0.000391	*0.000258	*0.000196	*0.000151	*0.000158	*0.000174224376678467
B	{0.01}	*0.000391	542911529	0.291989	*0.004850	*0.001917	*0.001854	*0.000865757465362549
C	{0.1}	*0.000258	0.291989		*0.015724	*0.008014	*0.008899	*0.00392228364944458
D	{1}	*0.000196	*0.004850	*0.015724539756774		0.4204	0.542178	0.337355
E	{10}	*0.000151	*0.001917	*0.008014	0.4204		0.807688	0.638649
F	{100}	*0.000158	*0.001854	*0.008899	0.542178	0.807688		0.514404
G	{500}	*0.000174	*0.000865	*0.003922	0.337355	0.638649	0.514404	

Newman-Keuls test; CR_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	43.81000	43.81000	30.36000	27.70000	15.48000	14.76000
A	{0}		*0.000183	*0.000177	*0.000196	*0.000151	*0.000158	*0.000174045562744141
B	{0.01}	*0.000183	2842826843	1	0.087525	0.105752	*0.008258	*0.0103773474693298
C	{0.1}	*0.000177	1		0.193697	0.170775	*0.012444	*0.0142464637756348
D	{1}	*0.000196	0.087525	0.193697		0.721808	0.140829	0.190949
E	{10}	*0.000151	0.105752	0.170775	0.721808		0.117291	0.216039
F	{100}	*0.000158	*0.008258	*0.012444	0.140829	0.117291		0.923141
G	{500}	*0.000174	*0.010377	*0.014246	0.190949	0.216039	0.923141	

Newman-Keuls test; CR_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	45.64000	45.46000	42.28000	39.96000	38.08000	20.98000
A	{0}		*0.000324	*0.000592	*0.000652	*0.000639	*0.000663	*0.000209689140319824
B	{0.01}	*0.000324	845314025	0.986932	0.947417	0.950223	0.951673	0.255089
C	{0.1}	*0.000592	0.986932		0.770871	0.865949	0.899447	0.205956
D	{1}	*0.000652	0.947417	0.770871		0.831635	0.919175	0.237839
E	{10}	*0.000639	0.950223	0.865949	0.831635		0.863205	0.214293
F	{100}	*0.000663	0.951673	0.899447	0.919175	0.863205		0.132571
G	{500}	*0.000209	0.255089	0.205956	0.237839	0.214293	0.132571	

Newman-Keuls test; CU_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	55.83000	55.63000	46.00000	25.63000	13.13000	6.560000
A	{0}		*0.000559	*0.001205	*0.000498	*0.000159	*0.000159	*0.000174462795257568
B	{0.01}	*0.000559	4491958615	0.983784	0.574523	*0.032175	*0.004308	*0.00180017948150635
C	{0.1}	*0.001205	0.983784		0.332906	*0.019225	*0.002970	*0.00136381387710571
D	{1}	*0.000498	0.574523	0.332906		0.052271	*0.010850	*0.00531172752380371
E	{10}	*0.000159	*0.032175	*0.019225	0.052271		0.213963	0.152165
F	{100}	*0.000159	*0.004308	*0.002970	*0.010850	0.213963		0.505022
G	{500}	*0.000174	*0.001800	*0.001363	*0.005311	0.152165	0.505022	

Newman-Keuls test; CU_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	24.05000	27.94000	32.72000	25.02000	16.23000	13.40000
A	{0}		*0.000150	*0.000180	*0.000176	*0.000196	*0.000158	*0.000174045562744141
B	{0.01}	*0.000150	9785652160	0.669781	0.260343	0.832298	0.103815	0.078511
C	{0.1}	*0.000180	0.669781		0.305497	0.526423	0.085958	*0.0402531027793884
D	{1}	*0.000176	0.260343	0.305497		0.234814	*0.018073	*0.00777989625930786
E	{10}	*0.000196	0.832298	0.526423	0.234814		0.159945	0.089038
F	{100}	*0.000158	0.103815	0.085958	*0.018073	0.159945		0.539065
G	{500}	*0.000174	0.078511	*0.040253	*0.007779	0.089038	0.539065	

Newman-Keuls test; AD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	20.84000	21.43000	13.89000	7.740000	3.570000	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000180	9000968933	0.828668	*0.021159	*0.000780	*0.000255	*0.000159144401550293
C	{0.1}	*0.000176	0.828668		*0.034342	*0.000948	*0.000222	*0.000165879726409912
D	{1}	*0.000196	*0.021159	*0.034342586994171		*0.037477	*0.004744	*0.00085151195526123
E	{10}	*0.000150	*0.000780	*0.000948	*0.037477254867553		0.141336	*0.0298186540603638
F	{100}	*0.000158	*0.000255	*0.000222	*0.004744	0.141336		0.203258
G	{500}	*0.000174	*0.000159	*0.000165	*0.000851	*0.029818	0.203258	

Newman-Keuls test; AD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	5.560000	7.410000	8.330000	6.940000	2.780000	0.000000
A	{0}		*0.000150	*0.000180	*0.000176	*0.000196	*0.000158	*0.000174045562744141
B	{0.01}	*0.000150	9785652160	0.161662	*0.048898	0.167983	*0.011125	*0.000278890132904053
C	{0.1}	*0.000180	0.161662		0.348798	0.628148	*0.001372	*0.000158786773681641
D	{1}	*0.000176	*0.048898	0.348798		0.336626	*0.000466	*0.000159800052642822
E	{10}	*0.000196	0.167983	0.628148	0.336626		*0.001817	*0.000207424163818359
F	{100}	*0.000158	*0.011125	*0.001372	*0.000466	*0.001817584037780		*0.0111252069473267
G	{500}	*0.000174	*0.000278	*0.000158	*0.000159	*0.000207	*0.0111252069473267	

APPENDIX 32: ANOVA: *Tetraselmis tetrahele* UMACC 245: DNA Damage (RAPD-Genomic Template Stability)

* = Significant difference : p<0.05

ANOVA-GTS-TET

Newman-Keuls test; BD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	28.57000	28.57000	28.57000	28.57000	16.67000	0.000000
A	{0}		*0.000150	*0.000196	*0.000180	*0.000176	*0.000158	*0.000174045562744141
B	{0.01	*0.000150	9785652160	1	1	1	0.088342	*0.00177061557769775
C	{0.1}	*0.000196	1		1	1	0.195314	*0.00312221050262451
D	{1}	*0.000180	1	1		1	0.299755	*0.00470346212387085
E	{10}	*0.000176	1	1	1		0.394515	*0.00648856163024902
F	{100}	*0.000158	0.088342	0.195314	0.299755	0.394515		*0.0225073099136353
G	{500}	*0.000174	*0.001770	*0.003122	*0.004703	*0.006488	*0.0225073099136353	

Newman-Keuls test; BD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	39.59000	20.84000	15.28000	14.58000	13.20000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	*0.001893	*0.000675	*0.000916	*0.000841	*0.00016409158706665
C	{0.1}	*0.000180	*0.001893	1031227111	0.272186	0.424968	0.425037	*0.00578862428665161
D	{1}	*0.000196	*0.000675	0.272186		0.887715	0.904815	*0.0324156880378723
E	{10}	*0.000150	*0.000916	0.424968	0.887715		0.780853	*0.0244919061660767
F	{100}	*0.000158	*0.000841	0.425037	0.904815	0.780853		*0.0169317722320557
G	{500}	*0.000174	*0.000164	*0.005788	*0.032415	*0.024491	*0.0169317722320557	

Newman-Keuls test; MC_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	22.55000	37.50000	26.25000	21.63000	19.76000	0.000000
A	{0}		*0.000196	*0.000176	*0.000180	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196	0396766662	*0.009683	0.403313	0.833463	0.795424	*0.00078332424163818 4
C	{0.1}	*0.000176	*0.009683	3109855651	*0.020271	*0.011418	*0.007648	*0.00015997886657748
D	{1}	*0.000180	0.403313	*0.020271	9569206238	0.543444	0.456647	*0.00034850835800170 9
E	{10}	*0.000150	0.833463	*0.011418	0.543444		0.669808	*0.00063848495483398 4
F	{100}	*0.000158	0.795424	*0.007648	0.456647	0.669808		*0.00055748224258422 9
G	{500}	*0.000174	*0.000783	*0.000159	*0.000348	*0.000638	*0.00055748224258422 9	

Newman-Keuls test; MC_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	49.82000	28.75000	17.22000	14.91000	11.57000	0.000000
A	{0}		*0.000177	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000177	2642135620	*0.005936	*0.000646	*0.000656	*0.000437	*0.00017249584197998
C	{0.1}	*0.000180	*0.005936	0860817782	0.096986	0.118333	0.07935	*0.00439518690109253
D	{1}	*0.000196	*0.000646	0.096986		0.726887	0.666037	0.078486
E	{10}	*0.000150	*0.000656	0.118333	0.726887		0.614386	0.088776
F	{100}	*0.000158	*0.000437	0.07935	0.666037	0.614386		0.095943
G	{500}	*0.000174	*0.000172	*0.004395	0.078486	0.088776	0.095943	

Newman-Keuls test; DD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	30.36000	25.95000	18.45000	14.28000	10.32000	5.610000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	0.266911	*0.019288	*0.004340	*0.001086	*0.00029915571212768 6
C	{0.1}	*0.000180	0.266911		0.069451	*0.021748	*0.005405	*0.00096380710601806 6
D	{1}	*0.000196	*0.019288	0.069451		0.292678	0.119108	*0.0212603807449341
E	{10}	*0.000150	*0.004340	*0.021748	0.292678		0.316721	0.093157
F	{100}	*0.000158	*0.001086	*0.005405	0.119108	0.316721		0.237157
G	{500}	*0.000174	*0.000299	*0.000963	*0.021260	*0.093156	0.237157	

Newman-Keuls test; DD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	36.90000	42.52000	29.05000	28.57000	23.51000	14.62000
A	{0}		*0.000181	*0.000177	*0.000196	*0.000151	*0.000158	*0.000174105167388916
B	{0.01	*0.000181	7345619201	0.477937	0.325679	0.540721	0.341985	0.074814
C	{0.1}	*0.000177	0.477937		0.222764	0.309233	0.154472	*0.0269766449928284
D	{1}	*0.000196	0.325679	0.222764		0.951307	0.756593	0.282922
E	{10}	*0.000151	0.540721	0.309233	0.951307		0.522154	0.202201
F	{100}	*0.000158	0.341985	0.154472	0.756593	0.522154		0.268015
G	{500}	*0.000174	0.074814	*0.026976	0.282922	0.202201	0.268015	

Newman-Keuls test; MA_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	27.33000	26.39000	25.83000	25.83000	21.47000	20.83000
A	{0}		*0.000176	*0.000180	*0.000150	*0.000196	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	0.879311	0.994531	0.967094	0.866272	0.885146
C	{0.1}	*0.000180	0.879311		0.99541	0.927942	0.848691	0.886286
D	{1}	*0.000150	0.994531	0.99541		1	0.484753	0.695246
E	{10}	*0.000196	0.967094	0.927942	1		0.757205	0.842574
F	{100}	*0.000158	0.866272	0.848691	0.484753	0.757205		0.917686
G	{500}	*0.000174	0.885146	0.886286	0.695246	0.842574	0.917686	

Newman-Keuls test; MA_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	18.75000	19.87000	18.30000	14.73000	10.51000	6.250000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180	90096893	0.773425	0.907857	0.556636	0.182271	*0.0372980833053589
C	{0.1}	*0.000176	0.773425		0.911461	0.549787	0.157736	*0.0295262932777405
D	{1}	*0.000196	0.907857	0.911461		0.365245	0.138591	*0.0313571691513062
E	{10}	*0.000150	0.556636	0.549787	0.365245		0.287232	0.101706
F	{100}	*0.000158	0.182271	0.157736	0.138591	0.287232		0.282875
G	{500}	*0.000174	*0.037298	*0.029526	*0.031357	0.101706	0.282875	

APPENDIX 33: ANOVA: *Tetraselmis tetrahele* UMACC 144: DNA Damage (AP-Site)

* = Significant difference : p<0.05

ANOVA-AP-SITE-TET

Newman-Keuls test; CR_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		13.12600	25.16900	20.27800	13.42700	9.675000	4.131000	2.824000
A	{0}		*0.0002196	*0.0029165	0.864917	0.066808	*0.0005291	*0.000367
B	{0.01}	*0.0002196431159973		*0.0138394	*0.0001963	*0.0001518	*0.0001581	*0.000174
C	{0.1}	*0.0029165	*0.0138394832611084		*0.0016011	*0.0003108	*0.0001515	*0.000158
D	{1}	0.864917	*0.0001963	*0.0016011595726013		0.113219	*0.0006840	*0.000351
E	{10}	0.066808	*0.0001518	*0.0003108	0.113219		*0.0066503	*0.004015
F	{100}	*0.0005291	*0.0001581	*0.0001515	*0.0006840	*0.0066503286361694		0.464049
G	{500}	*0.0003671	*0.0001740	*0.0001583	*0.0003510	*0.0040151	0.464049	

Newman-Keuls test; CR_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		10.42100	18.50300	19.43900	22.57300	18.20100	7.832000	2.690000
A	{0}		*0.0001971	*0.0002036	*0.0001510	*0.0001856	*0.0487686	*0.000209
B	{0.01}	*0.0001971721649169		0.448119	*0.0114796	0.804909	*0.0001965	*0.00015
C	{0.1}	*0.0002036	0.448119		*0.0205581	0.569423	*0.0001511	*0.000158
D	{1}	*0.0001510	*0.0114796	*0.0205581188201904		*0.0125697	*0.0001581	*0.000174
E	{10}	*0.0001856	0.804909	0.569423	*0.0125697255134583		*0.0001812	*0.000196
F	{100}	*0.0487686	*0.0001965	*0.0001511	*0.0001581	*0.0001812577247619		*0.000891
G	{500}	*0.0002098	*0.0001509	*0.0001581	*0.0001740	*0.0001960	*0.000891	

Newman-Keuls test; CU_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		13.12600	31.71900	36.45900	41.09900	49.82600	102.8760	0.000000
A	{0}		*0.0002648	*0.0001984	*0.0001987	*0.0001509	*0.0001581	*0.002225
B	{0.01}	*0.0002648830413818		*0.1950039	*0.0434489	*0.0008440	*0.0001509	*0.000180
C	{0.1}	*0.0001984	*0.195003986358643		0.204025	*0.0049267	*0.0001960	*0.000196
D	{1}	*0.0001987	*0.0434489	0.204025		0.025286	*0.0001809	*0.000150
E	{10}	*0.0001509	*0.0008440	*0.0049267	0.025286		*0.0001766	*0.000158
F	{100}	*0.0001581	*0.0001509	*0.0001960	*0.0001809	*0.0001766681671142		*0.000174
G	{500}	*0.0022259	*0.0001809	*0.0001960	*0.0001509	*0.0001581	*0.000174	

Newman-Keuls test; CU_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		10.42100	26.00700	84.48200	93.20900	85.16900	17.44700	0.000000
A	{0}		*0.0001814	*0.0001960	*0.0001581	*0.0001509	*0.0023369	*0.000234
B	{0.01}	*0.0001814961433410		*0.0001766	*0.0001960	*0.0001809	*0.0005919	*0.000196
C	{0.1}	*0.0001960	*0.0001766681671142		*0.0011529	0.719935	*0.0001809	*0.000150
D	{1}	*0.0001581	*0.0001960	*0.0011529922485351		*0.0008986	*0.0001509	*0.000174
E	{10}	*0.0001509	*0.0001809	0.719935	*0.0008986592292785		*0.0001960	*0.000158
F	{100}	*0.0023369	*0.0005919	*0.0001809	*0.0001509	*0.0001960396766662		*0.000180
G	{500}	*0.0002346	*0.0001960	*0.0001509	*0.0001740	*0.0001581	*0.000180	

APPENDIX 34: ANOVA: *Tetraselmis tetrahele* UMACC 144: Superoxide dismutase (SOD) Activity

* = Significant difference : p<0.05

ANOVA-SOD-TET

Newman-Keuls test; CD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		45.76300	65.45300	68.38500	66.02400	68.20700	92.57800	97.91000
A	{0}		*0.000183	*0.000170	*0.000195	*0.000203	*0.000158	*0.000174
B	{0.01	*0.000183		0.762227	0.851312	0.636101	*0.000151	*0.000158
C	{0.1}	*0.000170	0.762227		0.71501	0.953437	*0.000176	*0.000180
D	{1}	*0.000195	0.851312	0.71501		0.477254	*0.000196	*0.000150
E	{10}	*0.000203	0.636101	0.953437	0.477254		*0.000181	*0.000196
F	{100}	*0.000158	*0.000151	*0.000176	*0.000196	*0.000181	7345619	*0.096161
G	{500}	*0.000174	*0.000158	*0.000180	*0.000150	*0.000196	*0.096161	

Newman-Keuls test; CD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		36.19200	40.67500	33.90700	34.89500	47.28400	68.72900	57.02400
A	{0}		*0.001289	0.131672	0.259018	*0.000180	*0.000150	*0.000196
B	{0.01	*0.001289		*0.000304	*0.000490	*0.000200	*0.000196	*0.000180
C	{0.1}	0.131672	*0.000304		0.385354	*0.000150	*0.000174	*0.000158
D	{1}	0.259018	*0.000490	0.385354		*0.000196	*0.000158	*0.000150
E	{10}	*0.000180	*0.000200	*0.000150	*0.000196	0396766662	*0.000180	*0.000176
F	{100}	*0.000150	*0.000196	*0.000174	*0.000158	*0.000180	0968933	*0.000176
G	{500}	*0.000196	*0.000180	*0.000158	*0.000150	*0.000176	*0.000176	

Newman-Keuls test; CR_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		45.76300	34.72100	52.78500	40.40400	38.44600	24.04700	4.330500
A	{0}		*0.000255	*0.001202	*0.007455	*0.002187	*0.000150	*0.000158
B	{0.01	*0.000255		*0.000150	*0.013131	*0.047003	*0.000191	*0.000180
C	{0.1}	*0.001202	*0.000150978565216		*0.000186	*0.000197	*0.000158	*0.000174
D	{1}	*0.007455	*0.013131	*0.000186860561370		0.271315	*0.000196	*0.000150
E	{10}	*0.002187	*0.047003	*0.000197	0.271315		*0.000181	*0.000196
F	{100}	*0.000150	*0.000191	*0.000158	*0.000196	*0.000181	3765340	*0.000176
G	{500}	*0.000158	*0.000180	*0.000174	*0.000150	*0.000196	*0.000176	

Newman-Keuls test; CR_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		36.19200	43.31500	45.68100	45.49500	19.31500	2.962000	2.434000
A	{0}		*0.000181	*0.000196	*0.000181	*0.000176	*0.000180	*0.000196
B	{0.01	*0.000181		0.096541	0.056881	*0.000180	*0.000196	*0.000150
C	{0.1}	*0.000196	0.096541		0.862043	*0.000150	*0.000158	*0.000174
D	{1}	*0.000181	0.056881	0.862043		*0.000196	*0.000150	*0.000158
E	{10}	*0.000176	*0.000180	*0.000150	*0.000196	0396766662	*0.000176	*0.000180
F	{100}	*0.000180	*0.000196	*0.000158	*0.000150	*0.000176	1671142	0.622983
G	{500}	*0.000196	*0.000150	*0.000174	*0.000158	*0.000180	0.622983	

Newman-Keuls test; CU_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		45.76300	51.04800	50.59700	49.74200	66.36400	84.33600	59.20000
A	{0}		0.286453	0.237906	0.182357	*0.000190	*0.000174	*0.002559
B	{0.01	0.286453		0.875989	0.890548	*0.000410	*0.000196	*0.012375
C	{0.1}	0.237906	0.875989		0.767522	*0.000533	*0.000150	*0.022852
D	{1}	0.182357	0.890548	0.767522		*0.000458	*0.000158	*0.022544
E	{10}	*0.000190	*0.000410	*0.000533	*0.000458538532257		*0.000188	*0.024309
F	{100}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000188	7083053588	*0.000181
G	{500}	*0.002559	*0.012375	*0.022852	*0.022544	*0.024309	*0.000181	

Newman-Keuls test; CU_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		36.19200	31.58200	36.73100	39.59300	15.25400	4.602000	0.000000
A	{0}		*0.000301	0.555715	*0.005200	*0.000180	*0.000196	*0.000150
B	{0.01	*0.000301		*0.000296	*0.000196	*0.000176	*0.000180	*0.000196
C	{0.1}	0.555715	*0.000296592712402		*0.006495	*0.000196	*0.000150	*0.000158
D	{1}	*0.005200	*0.000196	*0.006495058536529		*0.000150	*0.000158	*0.000174
E	{10}	*0.000180	*0.000176	*0.000196	*0.0001509785652		*0.000176	*0.000180
F	{100}	*0.000196	*0.000180	*0.000150	*0.000158	*0.000176	6681671142	*0.000303
G	{500}	*0.000150	*0.000196	*0.000158	*0.000174	*0.000180	*0.000303	

Newman-Keuls test; AD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		45.76300	38.59600	38.61500	43.93200	47.36400	51.01300	0.000000
A	{0}		0.194483	0.122797	0.59673	0.643244	0.297666	*0.000150
B	{0.01	0.194483		0.9957	0.286993	0.12528	*0.024540	*0.000176
C	{0.1}	0.122797	0.9957		0.138238	0.088775	*0.018170	*0.000180
D	{1}	0.59673	0.286993	0.138238		0.579828	0.202427	*0.000196
E	{10}	0.643244	0.12528	0.088775	0.579828		0.298779	*0.000158
F	{100}	0.297666	*0.024540	*0.018170	0.202427	0.298779		*0.000174
G	{500}	*0.000150	*0.000176	*0.000180	*0.000196	*0.000158	*0.000174	

Newman-Keuls test; AD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		36.19200	116.0740	121.0840	173.8860	147.0860	94.93200	0.000000
A	{0}		*0.000180	*0.000196	*0.000158	*0.000150	*0.000176	*0.000176
B	{0.01	*0.000180		*0.003995	*0.000196	*0.000180	*0.000176	*0.000196
C	{0.1}	*0.000196	*0.003995656967163		*0.000180	*0.000176	*0.000180	*0.000150
D	{1}	*0.000158	*0.000196	*0.000180900096893		*0.000176	*0.000150	*0.000174
E	{10}	*0.000150	*0.000180	*0.000176	*0.000176681671142		*0.000196	*0.000158
F	{100}	*0.000176	*0.000176	*0.000180	*0.000150	*0.000196	6766662	*0.000180
G	{500}	*0.000176	*0.000196	*0.000150	*0.000174	*0.000158	*0.000180	

APPENDIX 34: ANOVA: *Tetraselmis tetrahele* UMACC 144: Superoxide dismutase (SOD) Activity

* = Significant difference : p<0.05

ANOVA-SOD-TET

Newman-Keuls test; BD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		45.76300	52.04100	54.87400	56.63500	53.40900	58.07300	0.000000
A	{0}		*0.004607	*0.001307	*0.000460	*0.002915	*0.000267	*0.000176
B	{0.01}	*0.004607		0.308869	0.107853	0.473271	*0.039173	*0.000180
C	{0.1}	*0.001307	0.308869		0.35882	0.44309	0.231133	*0.000150
D	{1}	*0.000460	0.107853	0.35882		0.226047	0.451361	*0.000158
E	{10}	*0.002915	0.473271	0.44309	0.226047		0.101049	*0.000196
F	{100}	*0.000267	*0.039173	0.231133	0.451361	0.101049		*0.000174
G	{500}	*0.000176	*0.000180	*0.000150	*0.000158	*0.000196	*0.000174	

Newman-Keuls test; MC_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		45.76300	64.31800	52.56800	49.26000	44.01500	33.00000	0.000000
A	{0}		*0.000196	*0.000191	*0.003205	0.095886	*0.000180	*0.000196
B	{0.01}	*0.000196		*0.000176	*0.000180	*0.000150	*0.000158	*0.000174
C	{0.1}	*0.000191	*0.000176		*0.004640	*0.000196	*0.000150	*0.000158
D	{1}	*0.003205	*0.000180	*0.004640		*0.000429	*0.000196	*0.000150
E	{10}	0.095886	*0.000150	*0.000196	*0.000429		*0.000176	*0.000180
F	{100}	*0.000180	*0.000158	*0.000150	*0.000196	*0.000176		*0.000176
G	{500}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; DD_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		45.76300	36.06500	38.70300	47.94500	46.29600	30.91500	31.41500
A	{0}		*0.018047	*0.037522	0.761403	0.864793	*0.002163	*0.001934
B	{0.01}	*0.018047		0.404901	*0.012488	*0.022722	0.248125	0.152298
C	{0.1}	*0.037522	0.404901		*0.041418	0.065273	0.09715	0.078062
D	{1}	0.761403	*0.012488	*0.041418		0.59979	*0.001214	*0.001193
E	{10}	0.864793	0.022723	0.065273	0.59979		*0.002213	*0.002124
F	{100}	*0.002163	0.248125	0.09715	*0.001214	*0.002213		0.87309
G	{500}	*0.001934	0.152298	0.078063	*0.001193	*0.002124	0.87309	

Newman-Keuls test; MA_T_D4 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		45.76300	32.95900	42.91300	48.42700	31.75500	28.25900	21.44900
A	{0}		*0.000181	0.076898	0.095934	*0.000196	*0.000150	*0.000158
B	{0.01}	*0.000181		*0.000182	*0.000196	0.433279	*0.018322	*0.000201
C	{0.1}	0.076898	*0.000182		*0.006456	*0.000184	*0.000196	*0.000150
D	{1}	0.095934	*0.000196	*0.006456		*0.000150	*0.000158	*0.000174
E	{10}	*0.000196	0.433279	*0.000184	*0.000150		*0.034515	*0.000192
F	{100}	*0.000150	*0.018322	*0.000196	*0.000158	*0.034515		*0.000588
G	{500}	*0.000158	*0.000201	*0.000150	*0.000174	*0.000192	*0.000588	

Newman-Keuls test; BD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		36.19200	175.6140	163.0400	157.1500	135.1320	128.2630	0.000000
A	{0}		*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	*0.000176
B	{0.01}	*0.000158		*0.000176	*0.000180	*0.000196	*0.000150	*0.000174
C	{0.1}	*0.000150	*0.000176		*0.001502	*0.000180	*0.000196	*0.000158
D	{1}	*0.000196	*0.000180	*0.001502		*0.000176	*0.000180	*0.000150
E	{10}	*0.000180	*0.000196	*0.000180	*0.000176		*0.000527	*0.000196
F	{100}	*0.000176	*0.000150	*0.000196	*0.000180	*0.000527		*0.000180
G	{500}	*0.000176	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	

Newman-Keuls test; MC_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		36.19200	73.39000	122.4580	130.7740	147.6890	79.80800	0.000000
A	{0}		*0.000176	*0.000196	*0.000150	*0.000158	*0.000180	*0.000176
B	{0.01}	*0.000176		*0.000180	*0.000196	*0.000150	*0.000176	*0.000180
C	{0.1}	*0.000196	*0.000180		*0.000176	*0.000180	*0.000176	*0.000150
D	{1}	*0.000150	*0.000196	*0.000176		*0.000176	*0.000180	*0.000158
E	{10}	*0.000158	*0.000150	*0.000180	*0.000176		*0.000196	*0.000174
F	{100}	*0.000180	*0.000176	*0.000176	*0.000180	*0.000196		*0.000196
G	{500}	*0.000176	*0.000180	*0.000150	*0.000158	*0.000174	*0.000196	

Newman-Keuls test; DD_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		36.19200	31.98200	46.58800	38.62200	36.93500	32.84000	24.23000
A	{0}		*0.040260	*0.000229	0.286897	0.636927	*0.047155	*0.000200
B	{0.01}	*0.040260		*0.000158	*0.005500	*0.028148	0.586194	*0.000338
C	{0.1}	*0.000229	*0.000158		*0.000299	*0.000222	*0.000151	*0.000174
D	{1}	0.286897	*0.005500	*0.000299		0.291716	*0.010235	*0.000158
E	{10}	0.636927	*0.028148	*0.000222	0.291716		*0.046293	*0.000154
F	{100}	*0.047155	0.586194	*0.000151	*0.010235	*0.046293		*0.000341
G	{500}	*0.000200	*0.000338	*0.000174	*0.000158	*0.000154	*0.000341	

Newman-Keuls test; MA_T_D10 (anova-tet.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		36.19200	29.04400	27.73700	35.95800	14.82300	5.084000	1.198400
A	{0}		*0.001433	*0.000693	0.884621	*0.000150	*0.000158	*0.000174
B	{0.01}	*0.001433		0.422634	*0.000782	*0.000181	*0.000196	*0.000150
C	{0.1}	*0.000693	0.422634		*0.000519	*0.000176	*0.000180	*0.000196
D	{1}	0.884621	*0.000782	*0.000519		*0.000196	*0.000150	*0.000158
E	{10}	*0.000150	*0.000181	*0.000176	*0.000196		*0.000194	*0.000181
F	{100}	*0.000158	*0.000196	*0.000180	*0.000150	*0.000194		*0.027823
G	{500}	*0.000174	*0.000150	*0.000196	*0.000158	*0.000181	*0.027823	

APPENDIX 35: ANOVA: *Boergesenia forbesii* : Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.126000	{0.01 mg/L} 1.014000	{0.1 mg/L} 1.006000	{1 mg/L} 1.001000	{10 mg/L} .9720000	{100 mg/L} .9310000	{500 mg/L} .8690000
A	{0}		*0.009176	*0.015281	*0.020731	*0.007214	*0.001431	*0.000253
B	{0.01}	*0.009176		0.831933	0.934527	0.674477	0.219901	*0.015545
C	{0.1}	*0.015281	0.831933		0.894485	0.637358	0.224334	*0.016934
D	{1}	*0.020731	0.934527	0.894485		0.446063	0.17728	*0.014517
E	{10}	*0.007214	0.674477	0.637358	0.446063		0.286276	*0.036606
F	{100}	*0.001431	0.219901	0.224334	0.17728	0.286276		*0.115867
G	{500}	*0.000253	*0.015545	*0.016934	*0.014517	*0.036606	*0.115867	

Newman-Keuls test; CO_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.126000	{0.01 mg/L} .9480000	{0.1 mg/L} .9370000	{1 mg/L} .9430000	{10 mg/L} .9420000	{100 mg/L} .9000000	{500 mg/L} .3800000
A	{0}		*0.000429	*0.001373	*0.000729	*0.001185	*0.000441	*0.000174
B	{0.01}	*0.000429		0.990533	0.894569	0.985708	0.697182	*0.000158
C	{0.1}	*0.001373	0.990533		0.985708	0.894569	0.334454	*0.000180
D	{1}	*0.000729	0.894569	0.985708		0.978933	0.659186	*0.000150
E	{10}	*0.001185	0.985708	0.894569	0.978933		0.50937	*0.000196
F	{100}	*0.000441	0.697182	0.334454	0.659186	0.50937		*0.000176
G	{500}	*0.000174	*0.000158	*0.000180	*0.000150	*0.000196	*0.000176	

Newman-Keuls test; CR_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.126000	{0.01 mg/L} 1.016000	{0.1 mg/L} 1.060000	{1 mg/L} 1.033000	{10 mg/L} .9980000	{100 mg/L} .9530000	{500 mg/L} .4860000
A	{0}		*0.020844	0.062303	*0.032079	*0.011146	*0.001340	*0.000174
B	{0.01}	*0.020844		0.391856	0.609942	0.589283	0.165765	*0.000196
C	{0.1}	0.062303	0.391856		0.421114	0.270418	*0.036762	*0.000158
D	{1}	*0.032079	0.609942	0.421114		0.544455	0.111511	*0.000150
E	{10}	*0.011146	0.589283	0.270418	0.544455		0.188783	*0.000180
F	{100}	*0.001340	0.165765	*0.036762	0.111511	0.188783		*0.000176
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.126000	{0.01 mg/L} .9560000	{0.1 mg/L} .9300000	{1 mg/L} .9080000	{10 mg/L} .8180000	{100 mg/L} .6950000	{500 mg/L} .3030000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01}	*0.000176		0.221562	0.079619	*0.000227	*0.000150	*0.000158
C	{0.1}	*0.000180	0.221562		0.297297	*0.000367	*0.000196	*0.000150
D	{1}	*0.000196	0.079619	0.297297		*0.000715	*0.000180	*0.000196
E	{10}	*0.000150	*0.000227	*0.000367	*0.000715		*0.000198	*0.000180
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000198		*0.000176
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .4630000	{0.01 mg/L} .4280000	{0.1 mg/L} .4260000	{1 mg/L} .4390000	{10 mg/L} .4250000	{100 mg/L} .3450000	{500 mg/L} .1400000
A	{0}		0.58731	0.718327	0.503125	0.809477	*0.041795	*0.000174
B	{0.01}	0.58731		0.955222	0.757437	0.996029	0.127532	*0.000154
C	{0.1}	0.718327	0.955222		0.92687	0.977668	0.085799	*0.000198
D	{1}	0.503125	0.757437	0.92687		0.977382	0.105782	*0.000160
E	{10}	0.809477	0.996029	0.977668	0.977382		*0.038056	*0.000181
F	{100}	*0.041795	0.127532	0.085799	0.105782	*0.038056		*0.000207
G	{500}	*0.000174	*0.000154	*0.000198	*0.000160	*0.000181	*0.000207	

Newman-Keuls test; CO_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .4630000	{0.01 mg/L} .4230000	{0.1 mg/L} .4310000	{1 mg/L} .4130000	{10 mg/L} .3470000	{100 mg/L} .1810000	{500 mg/L} 0.000000
A	{0}		0.305936	0.240187	0.265233	*0.004314	*0.000158	*0.000174
B	{0.01}	0.305936		0.763693	0.707296	*0.028747	*0.000196	*0.000150
C	{0.1}	0.240187	0.763693		0.773061	*0.027989	*0.000151	*0.000158
D	{1}	0.265233	0.707296	0.773061		*0.024147	*0.000181	*0.000196
E	{10}	*0.004314	*0.028747	*0.027989	*0.024147987365722		*0.000188	*0.000180
F	{100}	*0.000158	*0.000196	*0.000151	*0.000181	*0.000188052654266		*0.000180
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000180	

Newman-Keuls test; CR_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .4630000	{0.01 mg/L} .4180000	{0.1 mg/L} .4180000	{1 mg/L} .4190000	{10 mg/L} .4180000	{100 mg/L} .3570000	{500 mg/L} .1890000
A	{0}		0.363043	0.273476	0.085037	0.176433	*0.005750	*0.000174
B	{0.01}	0.363043		1	0.999974	1	*0.022364	*0.000180
C	{0.1}	0.273476	1		0.999101	1	0.054637	*0.000196
D	{1}	0.085037	0.999974	0.999101		0.967103	0.121331	*0.000158
E	{10}	0.176433	1	1	0.967103		0.091548	*0.000151
F	{100}	*0.005750	*0.022364	0.054637	0.121331	0.091548		*0.000179
G	{500}	*0.000174	*0.000180	*0.000196	*0.000158	*0.000151	*0.000179	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .4630000	{0.01 mg/L} .4310000	{0.1 mg/L} .4380000	{1 mg/L} .4340000	{10 mg/L} .4000000	{100 mg/L} .3290000	{500 mg/L} .1650000
A	{0}		0.879154	0.572172	0.783715	0.603399	0.068792	*0.000262
B	{0.01}	0.879154		0.985732	0.945723	0.485039	0.07987	*0.000301
C	{0.1}	0.572172	0.985732		0.927659	0.815415	0.14099	*0.000349
D	{1}	0.783715	0.945723	0.927659		0.716912	0.116674	*0.000308
E	{10}	0.603399	0.485039	0.815415	0.716912		0.122727	*0.000394
F	{100}	0.068792	0.07987	0.14099	0.116674	0.122727		*0.002124
G	{500}	*0.000262	*0.000301	*0.000349	*0.000308	*0.000394	*0.002124	

APPENDIX 35: ANOVA: *Boergesenia forbesii* : Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-BOE

Newman-Keuls test; FE_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.126000	1.046000	1.036000	1.037000	.9740000	.7970000	.4800000
A	{0}		0.073079	0.176409	0.114175	*0.017626	*0.000166	*0.000174
B	{0.01	0.073079		0.9683	0.830601	0.338711	*0.000378	0*.000158
C	{0.1}	0.176409	0.9683		0.981116	0.155298	*0.000291	*0.000196
D	{1}	0.114175	0.830601	0.981116		0.308912	*0.000406	*0.000150
E	{10}	*0.017626	0.338711	0.155298	0.308912		*0.000890	*0.000180
F	{100}	*0.000166	*0.000378	*0.000291	*0.000406	*0.000890076160430		*0.000177
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000180	*0.000177	

Newman-Keuls test; MN_B_C4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.126000	.9280000	.9300000	.9450000	.8930000	.7760000	.3990000
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000196099281311		0.923956	0.692868	0.110719	*0.000185	*0.000196
C	{0.1}	*0.000180	0.923956		0.477589	0.205359	*0.000203	*0.000150
D	{1}	*0.000176	0.692868	0.477589		0.098128	*0.000154	*0.000158
E	{10}	*0.000150	0.110719	0.205359	0.098128		*0.000220	*0.000180
F	{100}	*0.000158	*0.000185	*0.000203	*0.000154	*0.000220417976379		*0.000176
G	{500}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000180	*0.000176	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.126000	1.092000	1.125000	1.103000	1.079000	.8410000	.5180000
A	{0}		0.496867	0.966937	0.604509	0.319384	*0.000158	*0.000174
B	{0.01	0.496867		0.368763	0.648628	0.59075	*0.000180	*0.000196
C	{0.1}	0.966937	0.368763		0.367467	0.253324	*0.000150	*0.000158
D	{1}	0.604509	0.648628	0.367467		0.579103	*0.000196	*0.000150
E	{10}	0.319384	0.59075	0.253324	0.579103		*0.000176	*0.000180
F	{100}	*0.000158	*0.000180	*0.000150	*0.000196	*0.000176272777175		*0.000176
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.126000	1.032000	1.047000	1.040000	.9790000	.4540000	0.000000
A	{0}		0.061537	*0.034256	0.056157	*0.004979	*0.000158	*0.000174
B	{0.01	*0.0615373253822327		0.897124	0.815668	0.137751	*0.000180	*0.000196
C	{0.1}	0.034257	0.897124		0.838332	0.227056	*0.000150	*0.000158
D	{1}	0.056157	0.815668	0.838332		0.201554	*0.000196	*0.000150
E	{10}	*0.004979	0.137751	0.227056	0.201554		0*.000176	*0.000180
F	{100}	*0.000158	*0.000180	*0.000150	*0.000196	*0.000176668167114		*0.000176
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; FE_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.4630000	.4050000	.4040000	.4040000	.4030000	.3290000	.1510000
A	{0}		0.070935	0.238219	0.151571	0.305255	*0.005248	*0.000174
B	{0.01	0.070935		0.999448	0.973721	0.999894	0.132053	*0.000160
C	{0.1}	0.238219	0.999448		1	0.973721	0.059001	*0.000197
D	{1}	0.151571	0.973721	1		0.999448	0.098509	*0.000152
E	{10}	0.305255	0.999894	0.973721	0.999448		*0.025866	*0.000181
F	{100}	*0.005248	0.132053	0.059001	0.098509	*0.025866925716400		*0.000200
G	{500}	*0.000174	*0.000160	*0.000197	*0.000152	*0.000181	*0.000200	

Newman-Keuls test; MN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.4630000	.4220000	.4340000	.4290000	.4060000	.3400000	.1840000
A	{0}		0.612042	0.394687	0.571029	0.450131	*0.022272	*0.000178
B	{0.01	0.612042		0.930195	0.83524	0.635548	0.063979	*0.000209
C	{0.1}	0.394687	0.930195		0.881886	0.830788	0.080877	*0.000176
D	{1}	0.571029	0.83524	0.881886		0.769339	0.073296	*0.000174
E	{10}	0.450131	0.635548	0.830788	0.769339		0.065499	*0.000197
F	{100}	*0.022272	0.063979	0.080877	0.073296	0.065499		*0.000475
G	{500}	*0.000178	*0.000209	*0.000176	*0.000174	*0.000197	*0.000475	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.4630000	.3890000	.3900000	.3990000	.4100000	.3350000	.1530000
A	{0}		0.298777	0.230635	0.217925	0.16661	*0.032138	*0.000177
B	{0.01	0.298777		0.978532	0.959266	0.937025	0.159293	*0.000207
C	{0.1}	0.230635	0.978532		0.807977	0.847826	0.31425	*0.000248
D	{1}	0.217925	0.959266	0.807977		0.766528	0.330641	*0.000212
E	{10}	0.16661	0.937025	0.847826	0.766528		0.287255	*0.000204
F	{100}	*0.032138	0.159293	0.31425	0.330641	0.287255		*0.000345
G	{500}	*0.000177	*0.000207	*0.000248	*0.000212	*0.000204	*0.000345	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.4630000	.4350000	.4360000	.4350000	.4190000	.4000000	0.000000
A	{0}		0.731878	0.334872	0.567403	0.504852	0.245051	*0.000174
B	{0.01	0.731878		0.999335	1	0.563418	0.420888	*0.000196
C	{0.1}	0.334872	0.999335		0.971142	0.921059	0.677134	*0.000158
D	{1}	0.567403	1	0.971142		0.826656	0.580745	*0.000150
E	{10}	0.504852	0.563418	0.921059	0.826656		0.493733	*0.000180
F	{100}	0.245051	0.420888	0.677134	0.580745	0.493733		*0.000176
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176	

APPENDIX 35: ANOVA: *Boergesenia forbesii* : Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.126000	{0.01 mg/L} .9980000	{0.1 mg/L} .9950000	{1 mg/L} .9800000	{10 mg/L} .9380000	{100 mg/L} .5930000	{500 mg/L} 0.000000
A	{0}		*0.000181	*0.000192	*0.000201	*0.000151	*0.000158	*0.000174
B	{0.01	*0.000181		0.876389	0.618144	*0.030690	*0.000150	*0.000158
C	{0.1}	*0.000192	0.876389		0.441251	*0.023821	*0.000196	*0.000150
D	{1}	*0.000201	0.618144	0.441251		*0.043566	*0.000180	*0.000196
E	{10}	*0.000151	*0.030690	*0.023821	*0.043566		*0.000176	*0.000180
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176		*0.000176
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .4630000	{0.01 mg/L} .4420000	{0.1 mg/L} .4400000	{1 mg/L} .4390000	{10 mg/L} .4360000	{100 mg/L} .3570000	{500 mg/L} 0.000000
A	{0}		0.420764	0.643869	0.779942	0.820125	*0.009535	*0.000174
B	{0.01	0.420764		0.938233	0.99236	0.995154	*0.032162	*0.000158
C	{0.1}	0.643869	0.938233		0.969184	0.986434	*0.025073	*0.000150
D	{1}	0.779942	0.99236	0.969184		0.907443	*0.015443	*0.000196
E	{10}	0.820125	0.995154	0.986434	0.907443		*0.007657	*0.000180
F	{100}	*0.009535	*0.032162	*0.025073	*0.015443	*0.007657		*0.000176
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.126000	{0.01 mg/L} .9470000	{0.1 mg/L} .9860000	{1 mg/L} .9920000	{10 mg/L} .9380000	{100 mg/L} .4170000	{500 mg/L} 0.000000
A	{0}		*0.000196	*0.000197	*0.000186	*0.000151	*0.000158	*0.000174
B	{0.01	*0.000196		0.081281	0.111901	0.671234	*0.000180	*0.000196
C	{0.1}	*0.000197	0.081281		0.776834	0.086921	*0.000196	*0.000150
D	{1}	*0.000186	0.111901	0.776834		0.086567	*0.000150	*0.000158
E	{10}	*0.000151	0.671234	0.086921	0.086567		*0.000176	*0.000180
F	{100}	*0.000158	*0.000180	*0.000196	*0.000150	*0.000176		*0.000176
G	{500}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000180	*0.000176	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .4630000	{0.01 mg/L} .4090000	{0.1 mg/L} .4140000	{1 mg/L} .4360000	{10 mg/L} .4310000	{100 mg/L} .2090000	{500 mg/L} 0.000000
A	{0}		*0.001154	*0.001745	*0.020787	*0.020512	*0.000158	*0.000174
B	{0.01	*0.001154		0.636685	0.085648	0.120525	*0.000176	*0.000180
C	{0.1}	*0.001745	0.636685		0.120525	0.122931	*0.000180	*0.000196
D	{1}	*0.020787	0.085648	0.120525		0.636685	*0.000150	*0.000158
E	{10}	*0.020512	0.120525	0.122931	0.636685		*0.000196	*0.000150
F	{100}	*0.000158	*0.000176	*0.000180	*0.000150	*0.000196		*0.000176
G	{500}	*0.000174	*0.000180	*0.000196	*0.000158	*0.000150	*0.000176	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.126000	{0.01 mg/L} .9110000	{0.1 mg/L} 1.047000	{1 mg/L} 1.032000	{10 mg/L} .9610000	{100 mg/L} .9180000	{500 mg/L} .7740000
A	{0}		*0.000158	*0.000179	*0.000181	*0.000196	*0.000150	*0.000174
B	{0.01	*0.000158		*0.000150	*0.000196	*0.001579	0.542314	*0.000176
C	{0.1}	*0.000179	*0.000150978565216		0.202084	*0.000183	*0.000196	*0.000158
D	{1}	*0.000181	*0.000196	0.202084		*0.000188	*0.000180	*0.000150
E	{10}	*0.000196	*0.001579	*0.000183	*0.000188767910003		*0.001969	*0.000196
F	{100}	*0.000150	0.542314	*0.000196	*0.000180	*0.001969456672668		*0.000180
G	{500}	*0.000174	*0.000176	*0.000158	*0.000150	*0.000196	*0.000180	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .4630000	{0.01 mg/L} .3610000	{0.1 mg/L} .3220000	{1 mg/L} .2690000	{10 mg/L} .1950000	{100 mg/L} .1190000	{500 mg/L} .0790000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		*0.000643	*0.000180	*0.000196	*0.000150	*0.000158
C	{0.1}	*0.000180	*0.000643610954284		*0.000195	*0.000180	*0.000196	*0.000150
D	{1}	*0.000196	*0.000180	*0.000195443630218		*0.000176	*0.000180	*0.000196
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176846981048		80.00018	*0.000180
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	80.00018		*0.000547
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000547	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 1.126000	{0.01 mg/L} .9610000	{0.1 mg/L} .9560000	{1 mg/L} .9340000	{10 mg/L} .7530000	{100 mg/L} .6010000	{500 mg/L} .2010000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	0.000177		0.65724	0.06814	*0.000196	*0.000150	*0.000158
C	{0.1}	0.000181	0.65724		0.065936	*0.000180	*0.000196	*0.000150
D	{1}	0.000196	0.06814	0.065936		*0.000176	*0.000180	*0.000196
E	{10}	0.000151	*0.000196	*0.000180	*0.000176668167114		*0.000176	*0.000180
F	{100}	0.000158	*0.000150	*0.000196	*0.000180	*0.000176681671142		*0.000176
G	{500}	0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .4630000	{0.01 mg/L} .2230000	{0.1 mg/L} .0690000	{1 mg/L} .0640000	{10 mg/L} 0.000000	{100 mg/L} 0.000000	{500 mg/L} 0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000174	*0.000158	*0.000150
B	{0.01	*0.000176		*0.000176	*0.000180	*0.000158	*0.000150	*0.000196
C	{0.1}	*0.000180	*0.000176668167114		0.550263	*0.000153	*0.000197	*0.000181
D	{1}	*0.000196	*0.000180	0.550263		*0.000199	*0.000182	*0.000177
E	{10}	*0.000174	*0.000158	*0.000153	*0.000199		1	1
F	{100}	*0.000158	*0.000150	*0.000197	*0.000182	1		1
G	{500}	*0.000150	*0.000196	*0.000181	*0.000177	1	1	

APPENDIX 35: ANOVA: *Boergesenia forbesii* : Growth (Carotenoid)

* = Significant difference : p<0.05

ANOVA-CAR-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0386100	.0238500	.0094300	.0086900	.0071310	.0062500	.0010810
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114	*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000150978565216064
C	{0.1}	*0.000180	*0.000176727771759	0.613689	0.276247	0.165762	*0.000484168529510498	
D	{1}	*0.000196	*0.000180	0.613689		0.295007	0.238645	*0.000725746154785156
E	{10}	*0.000150	*0.000196	0.276247	0.295007		0.548602	*0.00242489576339722
F	{100}	*0.000158	*0.000150	0.165762	0.238645	0.548602		*0.00300264358520508
G	{500}	*0.000174	*0.000158	*0.000484	*0.000725	*0.002424	*0.00300264358520508	

Newman-Keuls test; CO_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0386100	.0220700	.0225200	.0218000	.0211000	.0209000	.0022200
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180900096893	0.776404	0.864607	0.809374	0.873852	*0.000150978565216064	
C	{0.1}	*0.000176	0.776404	0.889274	0.797788	0.83148	*0.000158190727233887	
D	{1}	*0.000196	0.864607	0.889274		0.659262	0.833206	*0.00019603967666626
E	{10}	*0.000150	0.809374	0.797788	0.659262		0.899502	*0.000180900096893311
F	{100}	*0.000158	0.873852	0.83148	0.833206	0.899502		*0.000176668167114258
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176668167114258	

Newman-Keuls test; CR_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0386100	.0260400	.0272800	.0265200	.0271000	.0071400	.0027200
A	{0}		*0.000151	*0.000176	*0.000196	*0.000180	*0.000158	*0.000174045562744141
B	{0.01	*0.000151038169860	0.75509	0.706234	0.679317	*0.000176	*0.000180900096893311	
C	{0.1}	*0.000176	0.75509	0.817471	0.887418	*0.000150	*0.000158190727233887	
D	{1}	*0.000196	0.706234	0.817471		0.649174	*0.000180	*0.00019603967666626
E	{10}	*0.000180	0.679317	0.887418	0.649174		*0.000196	*0.000150978565216064
F	{100}	*0.000158	*0.000176	*0.000150	*0.000180	*0.000196	*0.000158	*0.00338530540466309
G	{500}	*0.000174	*0.000180	*0.000158	*0.000196	*0.000150	*0.00338530540466309	

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0386100	.0177900	.0174300	.0169700	.0148000	.0090000	.0016390
A	{0}		*0.000187	*0.000206	*0.000237	*0.000177	*0.000159	*0.000174045562744141
B	{0.01	*0.000187397003173	0.913641	0.965813	0.795637	0.104529	*0.00240743160247803	
C	{0.1}	*0.000206	0.913641	0.889792	0.704522	0.088568	*0.00211077928543091	
D	{1}	*0.000237	0.965813	0.889792		0.516067	0.068285	*0.001819908618927
E	{10}	*0.000177	0.795637	0.704522	0.516067		0.096658	*0.00336694717407227
F	{100}	*0.000159	0.104529	0.088568	0.068285	0.096658		*0.0403372645378113
G	{500}	*0.000174	*0.002407	*0.002110	*0.001819	*0.003366	*0.0403372645378113	

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0417400	.0340600	.0337400	.0343300	.0248900	.0146400	.0034970
A	{0}		*0.000214	*0.000237	*0.000194	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000214040279388	0.794401	0.825918	*0.000183	*0.000196	*0.000150978565216064	
C	{0.1}	*0.000237	0.794401	0.877213	*0.000178	*0.000180	*0.00019603967666626	
D	{1}	*0.000194	0.825918	0.877213		*0.000199	*0.000150	*0.000158190727233887
E	{10}	*0.000150	*0.000183	*0.000178	*0.000199973583221	*0.000176	*0.000180900096893311	
F	{100}	*0.000158	*0.000196	*0.000180	*0.000150	*0.000176846981048	*0.000176727771759033	
G	{500}	*0.000174	*0.000150	*0.000196	*0.000158	*0.000180	*0.000176727771759033	

Newman-Keuls test; CO_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0417400	.0330200	.0345100	.0319700	.0243300	.0115500	.000000
A	{0}		*0.000201	*0.000242	*0.000205	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000201463699340	0.277021	0.438757	*0.000202	*0.000196	*0.000150978565216064	
C	{0.1}	*0.000242	0.277021	0.167376	*0.000200	*0.000150	*0.000158190727233887	
D	{1}	*0.000205	0.438757	0.167376		*0.000211	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000202	*0.000200	*0.000211894512176	*0.000176	*0.000180900096893311	
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176727771759	*0.000176846981048584	
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176846981048584	

Newman-Keuls test; CR_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0417400	.0268800	.0268100	.0270800	.0268800	.0170300	.0027100
A	{0}		*0.000361	*0.000399	*0.000207	*0.000263	*0.000158	*0.000174045562744141
B	{0.01	*0.000361502170562	0.978131	0.996545	1	*0.004014	*0.000196992811035	
C	{0.1}	*0.000399	0.978131	0.999564	0.999618	*0.001682	*0.000180959701538086	
D	{1}	*0.000207	0.996545	0.999564	0.937353	*0.009321	*0.000158309365234 37	
E	{10}	*0.000263	1	0.999618	0.937353	*0.007163	*0.000151157379150391	
F	{100}	*0.000158	*0.004014	*0.001682	*0.009321	*0.007163703441619	*0.000216543674468994	
G	{500}	*0.000174	*0.000196	*0.000180	*0.000158	*0.000151	*0.000216543674468994	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0417400	.0322400	.0327800	.0325400	.0300800	.0191400	.0028540
A	{0}		*0.000199	*0.000177	*0.000183	*0.000151	*0.000158	*0.000174045562744141
B	{0.01	*0.000199675559997	0.896107	0.807164	0.09485	*0.000180	*0.000196396766626	
C	{0.1}	*0.000177	0.896107	0.845137	0.160271	*0.000150	*0.000158190727233887	
D	{1}	*0.000183	0.807164	0.845137	0.138962	*0.000196	*0.000150978565216064	
E	{10}	*0.000151	0.09485	0.160271	0.138962	*0.000176	*0.000180900096893311	
F	{100}	*0.000158	*0.000180	*0.000150	*0.000196	*0.000176727771759	*0.000176668167114258	
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176668167114258	

APPENDIX 35: ANOVA: *Boergesenia forbesii* : Growth (Carotenoid)

* = Significant difference : p<0.05

ANOVA-CAR-BOE

Newman-Keuls test; FE_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0386100	{0.01 mg/L} .0272600	{0.1 mg/L} .0271700	{1 mg/L} .0272400	{10 mg/L} .0250500	{100 mg/L} .0159100	{500 mg/L} .0027260
A	{0}		*0.000176	*0.000197	*0.000181	*0.000151	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176846981048	0.997656	0.988565	0.395187	*0.000153	*0.000158190727233887	
C	{0.1}	*0.000197	0.997656		0.959701	0.140646	*0.000181	*0.00019603967666626
D	{1}	*0.000181	0.988565	0.959701		0.272504	*0.000197	*0.000150978565216064
E	{10}	*0.000151	0.395187	0.140646	0.272504		*0.000181	*0.000180900096893311
F	{100}	*0.000158	*0.000153	*0.000181	*0.000197	*0.000181853771209		*0.000176727771759033
G	{500}	*0.000174	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176727771759033	

Newman-Keuls test; FE_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0417400	{0.01 mg/L} .0261200	{0.1 mg/L} .0239400	{1 mg/L} .0237400	{10 mg/L} .0239300	{100 mg/L} .0125200	{500 mg/L} .0022390
A	{0}		*0.000176	*0.000180	*0.000150	*0.000196	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176668167114	0.091836	0.091836	0.242681	0.199555	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	0.091836		0.984999	0.993593	*0.000196	*0.000150978565216064
D	{1}	*0.000150	0.242681	0.984999		0.876979	*0.000176	*0.000180900096893311
E	{10}	*0.000196	0.199555	0.993593	0.876979		*0.000180	*0.00019603967666626
F	{100}	*0.000158	*0.000150	*0.000196	*0.000176	*0.000180959701538		*0.000176846981048584
G	{500}	*0.000174	*0.000158	*0.000150	*0.000180	*0.000196	*0.000176846981048584	

Newman-Keuls test; MN_B_C4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0386100	{0.01 mg/L} .0169300	{0.1 mg/L} .0170000	{1 mg/L} .0173300	{10 mg/L} .0168100	{100 mg/L} .0087000	{500 mg/L} .0018120
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000196039676666	0.972962	0.978723	0.953548	*0.003166	*0.000204145908355713	
C	{0.1}	*0.000180	0.972962		0.872632	0.995222	*0.005295	*0.0001717209815979
D	{1}	*0.000176	0.978723	0.872632		0.993808	*0.005913	*0.000172734260559082
E	{10}	*0.000150	0.953548	0.995222	0.993808		*0.001418	*0.000184953212738037
F	{100}	*0.000158	*0.003166	*0.005295	*0.005913	*0.001418411731		*0.00437235832214355
G	{500}	*0.000174	*0.000204	*0.000171	*0.000172	*0.000184	*0.00437235832214355	

Newman-Keuls test; MN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0417400	{0.01 mg/L} .0312100	{0.1 mg/L} .0323100	{1 mg/L} .0318700	{10 mg/L} .0300700	{100 mg/L} .0197300	{500 mg/L} .0031340
A	{0}		*0.000196	*0.000177	*0.000181	*0.000151	*0.000158	*0.000174045562744141
B	{0.01}	*0.000196754932403	0.640953	0.640953	0.592337	0.359966	*0.000180	*0.00019603967666626
C	{0.1}	*0.000177	0.640953		0.720359	0.288024	*0.000150	*0.000158190727233887
D	{1}	*0.000181	0.592337	0.720359		0.322987	*0.000196	*0.000150978565216064
E	{10}	*0.000151	0.359966	0.288024	0.322987		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000180	*0.000150	*0.000196	*0.000176846981048		*0.000176668167114258
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176668167114258	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0386100	{0.01 mg/L} .0366800	{0.1 mg/L} .0380200	{1 mg/L} .0373000	{10 mg/L} .0259600	{100 mg/L} .0162600	{500 mg/L} .0036710
A	{0}		0.829187	0.798543	0.833971	*0.000673	*0.000158	*0.000174045562744141
B	{0.01}	0.829187		0.827085	0.788554	*0.000473	*0.000181	*0.00019603967666626
C	{0.1}	0.798543	0.827085		0.75556	*0.000716	*0.000151	*0.000158190727233887
D	{1}	0.833971	0.788554	0.75556		*0.000672	*0.000196	*0.000150978565216064
E	{10}	*0.000673	*0.000473	*0.000716	*0.000672161579132		*0.000904	*0.000180959701538086
F	{100}	*0.000158	*0.000181	*0.000151	*0.000196	*0.000904738903045		*0.000234425067901611
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000234425067901611	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0417400	{0.01 mg/L} .0245600	{0.1 mg/L} .0246700	{1 mg/L} .0256300	{10 mg/L} .0268400	{100 mg/L} .0164800	{500 mg/L} .0023850
A	{0}		*0.000150	*0.000196	*0.000180	*0.000176	*0.000158	*0.000174045562744141
B	{0.01}	*0.000150978565216	0.920353	0.920353	0.593828	0.196522	*0.000177	*0.000180900096893311
C	{0.1}	*0.000196	0.920353		0.388757	0.146043	*0.000183	*0.00019603967666626
D	{1}	*0.000180	0.593828	0.388757		0.28103	*0.000197	*0.000150978565216064
E	{10}	*0.000176	0.196522	0.146043	0.28103		*0.000151	*0.000158190727233887
F	{100}	*0.000158	*0.000177	*0.000183	*0.000197	*0.000151157379150		*0.000176668167114258
G	{500}	*0.000174	*0.000180	*0.000196	*0.000150	*0.000158	*0.000176668167114258	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0386100	{0.01 mg/L} .0228800	{0.1 mg/L} .0226100	{1 mg/L} .0222900	{10 mg/L} .0119200	{100 mg/L} .0021650	{500 mg/L} 0.000000
A	{0}		*0.003876	*0.008807	*0.013624	*0.000442	*0.000165	*0.000177860260009766
B	{0.01}	*0.003876566886901	0.953395	0.990779	0.11867	*0.003436	*0.002058207988739	
C	{0.1}	*0.008807	0.953395		0.944764	0.07989	*0.002547	*0.00165832042694092
D	{1}	*0.013624	0.990779	0.944764		*0.038192	*0.001630	*0.00128316879272461
E	{10}	*0.000442	0.11867	0.07989	*0.038192033767700		*0.049256	*0.0487569570541382
F	{100}	*0.000165	*0.003436	*0.002547	*0.001630	*0.049256861209869		0.640111
G	{500}	*0.000177	*0.002058	*0.001658	*0.001283	*0.048756	0.640111	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0417400	{0.01 mg/L} .0323100	{0.1 mg/L} .0318300	{1 mg/L} .0325400	{10 mg/L} .0223900	{100 mg/L} .0032270	{500 mg/L} 0.000000
A	{0}		*0.000180	*0.000196	*0.000176	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000180900096893	0.596145	0.596145	0.798817	*0.000180	*0.000196	*0.000150978565216064
C	{0.1}	*0.000196	0.596145		0.707724	*0.000176	*0.000180	*0.00019603967666626
D	{1}	*0.000176	0.798817	0.707724		*0.000196	*0.000150	*0.000158190727233887
E	{10}	*0.000150	*0.000180	*0.000176	*0.000196034967666		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000196	*0.000180	*0.000150	*0.000176681671142		*0.00279152393341064
G	{500}	*0.000174	*0.000150	*0.000196	*0.000158	*0.000180	*0.00279152393341064	

APPENDIX 35: ANOVA: *Boergesenia forbesii* : Growth (Carotenoid)

* = Significant difference : p<0.05

ANOVA-CAR-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0386100	{0.01 mg/L} .0211400	{0.1 mg/L} .0211400	{1 mg/L} .0207300	{10 mg/L} .0108800	{100 mg/L} .0020910	{500 mg/L} 0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180900096893		1	0.813363	*0.000253	*0.000196	*0.000150978565216064
C	{0.1}	*0.000176	1		0.968709	*0.000343	*0.000150	*0.000158190727233887
D	{1}	*0.000196	0.813363	0.968709		*0.000213	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000253	*0.000343	*0.000213265419006		*0.000302	*0.000213503837585449
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000302791595458		0.239857
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000213	0.239857	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0417400	{0.01 mg/L} .0279100	{0.1 mg/L} .0336100	{1 mg/L} .0337200	{10 mg/L} .0333500	{100 mg/L} .0033430	{500 mg/L} 0.000000
A	{0}		*0.000152	*0.000555	*0.000326	*0.000727	*0.000158	*0.000174045562744141
B	{0.01	*0.000152230262756		*0.007653	*0.011885	*0.004119	*0.000176	*0.000180900096893311
C	{0.1}	*0.000555	*0.007653653621673		0.945588	0.871783	*0.000196	*0.000150978565216064
D	{1}	*0.000326	*0.011885	0.945588		0.970377	*0.000150	*0.000158190727233887
E	{10}	*0.000727	*0.004119	0.871783	0.970377		*0.000180	*0.00019603967666626
F	{100}	*0.000158	*0.000176	*0.000196	*0.000150	*0.000180900096893		0.05292
G	{500}	*0.000174	*0.000180	*0.000150	*0.000158	*0.000196	0.05292	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0386100	{0.01 mg/L} .0179100	{0.1 mg/L} .0212800	{1 mg/L} .0213200	{10 mg/L} .0112900	{100 mg/L} .0021320	{500 mg/L} 0.000000
A	{0}		*0.000197	*0.000191	*0.000180	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000197589397430	0.198241	0.383863	*0.019037	*0.000217	*0.000210	*0.000210821628570557
C	{0.1}	*0.000191	0.198241		0.98754	*0.003608	*0.000201	*0.000152945518493652
D	{1}	*0.000180	0.383863	0.98754		*0.006245	*0.000167	*0.000160813331604004
E	{10}	*0.000150	*0.019037	*0.003608	*0.006245076656341		*0.002668	*0.00141686201095581
F	{100}	*0.000158	*0.000217	*0.000201	*0.000167	*0.002668499946594		0.407281
G	{500}	*0.000174	*0.000210	*0.000152	*0.000160	*0.001416	0.407281	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0417400	{0.01 mg/L} .0299300	{0.1 mg/L} .0306500	{1 mg/L} .0328000	{10 mg/L} .0224800	{100 mg/L} .0033020	{500 mg/L} 0.000000
A	{0}		*0.000196	*0.000180	*0.000177	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196099281311		0.546625	0.066395	*0.000187	*0.000180	*0.00019603967666626
C	{0.1}	*0.000180	0.546625		0.086343	*0.000190	*0.000196	*0.000150978565216064
D	{1}	*0.000177	0.066395	0.086343		*0.000196	*0.000150	*0.000158190727233887
E	{10}	*0.000150	*0.000187	*0.000190	*0.000196695327758		*0.000176	*0.0001809000968
F	{100}	*0.000158	*0.000180	*0.000196	*0.000150	*0.000176681671142		*0.0134091377258301
G	{500}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000180	*0.0134091377258301	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0386100	{0.01 mg/L} .0228100	{0.1 mg/L} .0348600	{1 mg/L} .0335700	{10 mg/L} .0280200	{100 mg/L} .0247000	{500 mg/L} .0065600
A	{0}		*0.000516	0.178244	0.173804	*0.006444	*0.001081	*0.000174045562744141
B	{0.01	*0.000516235828399		*0.003551	*0.005723	0.156464	0.486782	*0.00019448995590 21
C	{0.1}	0.178244	*0.003551423549652		0.633431	0.053091	*0.008729	*0.000158190727233887
D	{1}	0.173804	*0.005723	0.633431		0.054616	*0.012430	*0.000150978565216064
E	{10}	*0.006444	0.156464	0.053091	0.054616		0.230053	*0.0001983642578125
F	{100}	*0.001081	0.486782	*0.008729	*0.012430	0.230053		*0.000193774700164795
G	{500}	*0.000174	*0.000194	*0.000158	*0.000150	*0.000198	*0.000193774700164795	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0417400	{0.01 mg/L} .0025100	{0.1 mg/L} .0025800	{1 mg/L} .0023200	{10 mg/L} .0013100	{100 mg/L} .0005000	{500 mg/L} .0000800
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180900096893		0.87017	0.658157	*0.032054	*0.001604	*0.000510275363922119
C	{0.1}	*0.000176	0.87017		0.812411	*0.040442	*0.001778	*0.00053107738494873
D	{1}	*0.000196	0.658157	0.812411		*0.030763	*0.001992	*0.000706195831298828
E	{10}	*0.000150	*0.032054	*0.040442	*0.030763983726501		0.074515	*0.0280104875564575
F	{100}	*0.000158	*0.001604	*0.001778	*0.001992	0.074515		0.334556
G	{500}	*0.000174	*0.000510	*0.000531	*0.000706	*0.028010	0.334556	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0386100	{0.01 mg/L} .0274400	{0.1 mg/L} .0263900	{1 mg/L} .0255500	{10 mg/L} .0133900	{100 mg/L} .0099600	{500 mg/L} .0017180
A	{0}		*0.000824	*0.000987	*0.001017	*0.000151	*0.000158	*0.000174045562744141
B	{0.01	*0.000824213027954		0.68973	0.74792	*0.000603	*0.000210	*0.000158309936523437
C	{0.1}	*0.000987	0.68973		0.749205	*0.000630	*0.000264	*0.000151157379150391
D	{1}	*0.001017	0.74792	0.749205		*0.000477	*0.000250	*0.000196337699890137
E	{10}	*0.000151	*0.000603	*0.000630	*0.000477492809295		0.204216	*0.00140011310577393
F	{100}	*0.000158	*0.000210	*0.000264	*0.000250	0.204216		*0.00656580924987793
G	{500}	*0.000174	*0.000158	*0.000151	*0.000196	*0.001400	*0.00656580924987793	

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .0417400	{0.01 mg/L} .0007700	{0.1 mg/L} .0002800	{1 mg/L} .0001500	{10 mg/L} .0001390	{100 mg/L} .0002030	{500 mg/L} 0.000000
A	{0}		*0.000176	*0.000180	*0.000150	*0.000158	*0.000196	*0.000174045562744141
B	{0.01	*0.000176668167114		0.172358	0.304999	0.384432	0.25283	0.271897
C	{0.1}	*0.000180	0.172358		0.923355	0.975236	0.824531	0.919445
D	{1}	*0.000150	0.304999	0.923355		0.974825	0.878679	0.89942
E	{10}	*0.000158	0.384432	0.975236	0.974825		0.980839	0.689503
F	{100}	*0.000196	0.25283	0.824531	0.878679	0.980839		0.931682
G	{500}	*0.000174	0.271897	0.919445	0.89942	0.689503	0.931682	

APPENDIX 36: ANOVA: *Boergesenia forbesii* : Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		11.94830	10.59100	8.743000	5.472000	4.763000	3.350000	.7333000
A	{0}		0.192457	*0.015603	*0.000247	*0.000180	*0.000160	*0.000174
B	{0.01}	0.192457		0.083415	*0.000540	*0.000384	*0.000178	*0.000158
C	{0.1}	*0.015603	0.083415		*0.005400	*0.003532	*0.000613	*0.000155
D	{1}	*0.000247	*0.000540	*0.005400	7172584533	0.486231	0.117251	*0.001610
E	{10}	*0.000180	*0.000384	*0.003532	0.486231		0.175926	*0.003220
F	{100}	*0.000160	*0.000178	*0.000613	0.117251	0.175926		*0.019530
G	{500}	*0.000174	*0.000158	*0.000155	*0.001610	*0.003220	*0.019530	

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		15.93100	6.910000	9.491000	9.336000	8.306000	4.038000	.9350000
A	{0}		*0.000151	*0.000185	*0.000200	*0.000201	*0.000158	*0.000174
B	{0.01}	*0.000151	6342163085	0.086211	0.068257	0.180842	*0.011841	*0.000253
C	{0.1}	*0.000185	0.086211		0.878051	0.47484	*0.000749	*0.000160
D	{1}	*0.000200	0.068257	0.878051		0.316342	*0.000692	*0.000153
E	{10}	*0.000201	0.180842	0.47484	0.316342		*0.002082	*0.000204
F	{100}	*0.000158	*0.011841	*0.000749	*0.000692	*0.002082	4074745178	*0.007511
G	{500}	*0.000174	*0.000253	*0.000160	*0.000153	*0.000204	*0.007511	

Newman-Keuls test; CO_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		11.94830	11.98700	12.09700	11.64900	11.87400	8.786000	.6515000
A	{0}		0.969541	0.98777	0.951213	0.941388	*0.029570	*0.000150
B	{0.01}	0.969541		0.913304	0.985832	0.992927	*0.040685	*0.000158
C	{0.1}	0.98777	0.913304		0.990465	0.995856	*0.044848	*0.000174
D	{1}	0.951213	0.985832	0.990465		0.823778	*0.012052	*0.000180
E	{10}	0.941388	0.992927	0.995856	0.823778		*0.019561	*0.000196
F	{100}	*0.029570	*0.040685	*0.044848	*0.012052	*0.019561	1119270325	*0.000176
G	{500}	*0.000150	*0.000158	*0.000174	*0.000180	*0.000196	*0.000176	

Newman-Keuls test; CO_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		15.93100	7.601000	6.514000	6.332000	5.840000	3.897000	.9999000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01}	*0.000176	8469810485	0.291287	0.42852	0.324025	*0.015943	*0.000263
C	{0.1}	*0.000180	0.291287		0.857028	0.778685	0.080853	*0.000688
D	{1}	*0.000196	0.42852	0.857028		0.627374	0.067145	*0.000662
E	{10}	*0.000150	0.324025	0.778685	0.627374		0.070237	*0.000798
F	{100}	*0.000158	*0.015943	0.080853	0.067145	0.070237		*0.011266
G	{500}	*0.000174	*0.000263	*0.000688	*0.000662	*0.000798	*0.011266	

Newman-Keuls test; CR_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		11.94830	14.58200	14.79700	14.66600	14.76000	7.088000	.1438900
A	{0}		*0.018893	0.077079	*0.039692	0.056796	*0.000386	*0.000180
B	{0.01}	*0.018893	0630683895	0.996289	0.933754	0.982474	*0.000184	*0.000196
C	{0.1}	0.077079	0.996289		0.990507	0.970878	*0.000170	*0.000174
D	{1}	*0.039692	0.933754	0.990507		0.925882	*0.000201	*0.000150
E	{10}	0.056796	0.982474	0.970878	0.925882		*0.000159	*0.000158
F	{100}	*0.000386	*0.000184	*0.000170	*0.000201	*0.000159	9261932325	*0.000179
G	{500}	*0.000180	*0.000196	*0.000174	*0.000150	*0.000158	*0.000179	

Newman-Keuls test; CR_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		15.93100	14.98100	14.95800	15.01900	15.07500	7.099000	1.509900
A	{0}		0.562588	0.662037	0.432636	0.252144	*0.000158	*0.000174
B	{0.01}	0.562588		0.974976	0.95857	0.99065	*0.000180	*0.000196
C	{0.1}	0.662037	0.974976		0.996102	0.998445	*0.000176	*0.000180
D	{1}	0.432636	0.95857	0.996102		0.938907	*0.000196	*0.000150
E	{10}	0.252144	0.99065	0.998445	0.938907		*0.000150	*0.000158
F	{100}	*0.000158	*0.000180	*0.000176	*0.000196	*0.000150	9785652160	*0.000177
G	{500}	*0.000174	*0.000196	*0.000180	*0.000150	*0.000158	*0.000177	

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		11.94830	11.18300	10.06900	10.22400	9.914000	6.284000	.6783000
A	{0}		0.452926	0.273359	0.225574	0.292445	*0.000728	*0.000174
B	{0.01}	0.452926		0.515621	0.349711	0.589233	*0.001800	*0.000158
C	{0.1}	0.273359	0.515621		0.878051	0.878051	*0.005111	*0.000196
D	{1}	0.225574	0.349711	0.878051		0.947771	*0.006789	*0.000151
E	{10}	0.292445	0.589233	0.878051	0.947771		*0.002708	*0.000180
F	{100}	*0.000728	*0.001800	*0.005111	*0.006789	*0.002708	6734771728	*0.000223
G	{500}	*0.000174	*0.000158	*0.000196	*0.000151	*0.000180	*0.000223	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		15.93100	15.10400	15.13200	15.13200	14.10400	7.146000	1.515100
A	{0}		0.671157	0.527943	0.288752	0.141153	*0.000158	*0.000174
B	{0.01}	0.671157		0.969854	0.999244	0.18921	*0.000180	*0.000196
C	{0.1}	0.527943	0.969854		1	0.358269	*0.000196	*0.000150
D	{1}	0.288752	0.999244	1		0.508678	*0.000150	*0.000158
E	{10}	0.141153	0.18921	0.358269	0.508678		*0.000176	*0.000180
F	{100}	*0.000158	*0.000180	*0.000196	*0.000150	*0.000176	7277717596	*0.000177
G	{500}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000180	*0.000177	

APPENDIX 36: ANOVA: *Boergesenia forbesii* : Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-BOE

Newman-Keuls test; FE_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		11.94830	12.15600	8.997000	8.349000	7.618000	4.278000	.7009000
A	{0}		0.837108	*0.010129	*0.007294	*0.003292	*0.000159	*0.000158
B	{0.01}	0.837108		*0.017060	*0.008720	*0.003405	*0.000166	*0.000174
C	{0.1}	*0.010129	*0.017060		0.523901	0.371661	*0.001664	*0.000153
D	{1}	*0.007294	*0.008720	0.523901		0.473047	*0.002983	*0.000201
E	{10}	*0.003292	*0.003405	0.371661	0.473047		*0.004724	*0.000190
F	{100}	*0.000159	*0.000166	*0.001664	*0.002983	*0.004724		*0.002991
G	{500}	*0.000158	*0.000174	*0.000153	*0.000201	*0.000190	*0.002991	

Newman-Keuls test; MN_B_C4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		11.94830	6.177000	9.181000	9.011000	8.492000	5.563000	1.142300
A	{0}		*0.000486	*0.014540	*0.026133	*0.016944	*0.000311	*0.000174
B	{0.01}	*0.000486		*0.039804	*0.031832	*0.034995	0.545601	*0.000604
C	{0.1}	*0.014540	*0.039804		0.86636	0.770138	*0.018723	*0.000164
D	{1}	*0.026133	*0.031832	0.86636		0.60878	*0.017213	*0.000156
E	{10}	*0.016944	*0.034995	0.770138	0.60878		*0.026551	*0.000205
F	{100}	*0.000311	0.545601	*0.018723	*0.017213	*0.026551		*0.000683
G	{500}	*0.000174	*0.000604	*0.000164	*0.000156	*0.000205	*0.000683	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		11.94830	7.192000	7.233000	7.658000	6.899000	5.182000	.6233000
A	{0}		*0.001564	*0.000968	*0.000835	*0.001400	*0.000237	*0.000174
B	{0.01}	*0.001564		0.967705	0.886259	0.77194	0.141962	*0.000238
C	{0.1}	*0.000968	0.967705		0.674657	0.939644	0.21047	*0.000223
D	{1}	*0.000835	0.886259	0.674657		0.868418	0.146715	*0.000202
E	{10}	*0.001400	0.77194	0.939644	0.868418		0.105226	*0.000217
F	{100}	*0.000237	0.141962	0.21047	0.146715	0.105226		*0.000560
G	{500}	*0.000174	*0.000238	*0.000223	*0.000202	*0.000217	*0.000560	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		11.94830	11.83200	12.94300	13.17200	12.84800	1.325600	.6036000
A	{0}		0.908353	0.586633	0.616229	0.379453	*0.000180	*0.000196
B	{0.01}	0.908353		0.683092	0.665396	0.573786	*0.000176	*0.000180
C	{0.1}	0.586633	0.683092		0.820704	0.925096	*0.000150	*0.000158
D	{1}	0.616229	0.665396	0.820704		0.9431	*0.000158	*0.000174
E	{10}	0.379453	0.573786	0.925096	0.9431		*0.000196	*0.000150
F	{100}	*0.000180	*0.000176	*0.000150	*0.000158	*0.000196		0.478411
G	{500}	*0.000196	*0.000180	*0.000158	*0.000174	*0.000150	0.478411	

Newman-Keuls test; FE_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		15.93100	11.18300	13.97500	10.80200	9.984000	5.697000	.3991000
A	{0}		*0.000919	0.068583	*0.000874	*0.000392	*0.000158	*0.000174
B	{0.01}	*0.000919		*0.013853	0.706518	0.46696	*0.000549	*0.000150
C	{0.1}	0.068583	*0.013853		*0.016605	*0.006168	*0.000153	*0.000158
D	{1}	*0.000874	0.706518	*0.016605		0.423102	*0.000550	*0.000196
E	{10}	*0.000392	0.46696	*0.006168	0.423102		*0.000839	*0.000180
F	{100}	*0.000158	*0.000549	*0.000153	*0.000550	*0.000839		*0.000263
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000263	

Newman-Keuls test; MN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		15.93100	10.01300	12.19800	12.34000	12.57900	4.449000	.6022000
A	{0}		*0.000404	*0.010026	*0.007411	*0.004616	*0.000158	*0.000174
B	{0.01}	*0.000404		*0.044803	0.081662	0.088535	*0.000227	*0.000180
C	{0.1}	*0.010026	*0.044803		0.888216	0.922275	*0.000182	*0.0001960
D	{1}	*0.007411	0.081662	0.888216		0.813031	*0.000199	*0.000150
E	{10}	*0.004616	0.088535	0.922275	0.813031		*0.000154	*0.000158
F	{100}	*0.000158	*0.000227	*0.000182	*0.000199	*0.000154		*0.001805
G	{500}	*0.000174	*0.000180	*0.000196	*0.000150	*0.000158	*0.001805	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		15.93100	12.63600	13.04200	12.79100	10.49200	4.997000	.8631000
A	{0}		*0.023018	*0.011446	*0.017696	*0.000764	*0.000158	*0.000174
B	{0.01}	*0.023018		0.912267	0.878051	*0.048395	*0.000183	*0.000196
C	{0.1}	*0.011446	0.912267		0.803839	0.091081	*0.000155	*0.000158
D	{1}	*0.017696	0.878051	0.803839		0.085865	*0.000199	*0.000150
E	{10}	*0.000764	*0.048395	0.091081	0.085865		*0.000235	*0.000180
F	{100}	*0.000158	*0.000183	*0.000155	*0.000199	*0.000235		*0.001079
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.001079	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		15.93100	10.53400	10.90100	11.45100	9.745000	1.256500	.7220000
A	{0}		*0.000610	*0.000607	*0.000626	*0.000304	*0.000158	*0.000174
B	{0.01}	*0.000610		0.716778	0.633844	0.439353	*0.000180	*0.000196
C	{0.1}	*0.000607	0.716778		0.587759	0.491336	*0.000196	*0.000150
D	{1}	*0.000626	0.633844	0.587759		0.349604	*0.000150	*0.000158
E	{10}	*0.000304	0.439353	0.491336	0.349604		*0.000176	*0.000180
F	{100}	*0.000158	*0.000180	*0.000196	*0.000150	*0.000176		0.598216
G	{500}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000180	0.598216	

APPENDIX 36: ANOVA: *Boergesenia forbesii* : Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 11.94830	{0.01 mg/L} 13.46800	{0.1 mg/L} 14.38400	{1 mg/L} 14.58200	{10 mg/L} 13.18800	{100 mg/L} 1.517400	{500 mg/L} .1470900
A	{0}		0.305956	0.111253	0.112364	0.231526	*0.000176(	*0.000180
B	{0.01	0.305956		0.371126	0.515621	0.781768	*0.000196(	*0.000150
C	{0.1}	0.111253	0.371126		0.84462	0.468642	*0.000150(	*0.000158
D	{1}	0.112364	0.515621	0.84462		0.515741	*0.000158:	*0.000174
E	{10}	0.231526	0.781768	0.468642	0.515741		*0.000180(	*0.000196
F	{100}	*0.000176(	*0.000196(	*0.000150(	*0.000158:	*0.000180900096893		0.188485
G	{500}	*0.000180(	*0.000150(	*0.000158:	*0.000174(	*0.000196(	0.188485	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 15.93100	{0.01 mg/L} 15.16500	{0.1 mg/L} 15.16500	{1 mg/L} 15.13200	{10 mg/L} 14.13200	{100 mg/L} 1.522100	{500 mg/L} .1515100
A	{0}		0.483147	0.257372	0.617979	0.091983	*0.000158:	*0.000174
B	{0.01	0.483147		1	0.960262	0.281095	*0.000196(	*0.000150
C	{0.1}	0.257372	1		0.998655	0.413688	*0.000150(	*0.000158
D	{1}	0.617979	0.960262	0.998655		0.145542	*0.000180(	*0.000196
E	{10}	0.091983	0.281095	0.413688	0.145542		*0.000176(	*0.000180
F	{100}	*0.000158:	*0.000196(	*0.000150(	*0.000180(	*0.000176668167114		0.053132
G	{500}	*0.000174(	*0.000150(	*0.000158:	*0.000196(	*0.000180(	0.053132	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 11.94830	{0.01 mg/L} 15.17400	{0.1 mg/L} 14.86400	{1 mg/L} 13.27000	{10 mg/L} 7.511000	{100 mg/L} 1.456800	{500 mg/L} .9434000
A	{0}		*0.026239:	*0.027235(	0.203673	*0.000666(	*0.000180(	*0.000196
B	{0.01	*0.026239216327667		0.759149	0.169385	*0.000167:	*0.000158:	*0.000174
C	{0.1}	*0.027235(	0.759149		0.130156	*0.000205:	*0.000150(	*0.000158
D	{1}	0.203673	0.169385	0.130156		*0.000287(	*0.000196(	*0.000150
E	{10}	*0.000666(	*0.000167:	*0.000205:	*0.000287950038909		*0.000195(	*0.000201
F	{100}	*0.000180(	*0.000158:	*0.000150(	*0.000196(	*0.000195802580871		0.612614
G	{500}	*0.000196(	*0.000174(	*0.000158:	*0.000150(	*0.000201:	0.612614	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 15.93100	{0.01 mg/L} 14.50700	{0.1 mg/L} 13.54800	{1 mg/L} 13.20900	{10 mg/L} 12.88600	{100 mg/L} 1.332200	{500 mg/L} .1129600
A	{0}		0.071898	*0.014875(	*0.010885(	*0.007213:	*0.000158:	*0.000174
B	{0.01	0.071898		0.210803	0.213574	0.166389	*0.000150(	*0.000158
C	{0.1}	*0.014875(	0.210803		0.650123	0.645833	*0.000196(	*0.000150
D	{1}	*0.010885(	0.213574	0.650123		0.665498	*0.000180(	*0.000196
E	{10}	*0.007213:	0.166389	0.645833	0.665498		*0.000176(	*0.000180
F	{100}	*0.000158:	*0.000150(	*0.000196(	*0.000180(	*0.000176668167114		*0.117687
G	{500}	*0.000174(	*0.000158:	*0.000150(	*0.000196(	*0.000180(	*0.117687	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 11.94830	{0.01 mg/L} 13.53800	{0.1 mg/L} 14.81900	{1 mg/L} 14.44100	{10 mg/L} 13.51900	{100 mg/L} 7.038000	{500 mg/L} 1.435100
A	{0}		0.276427	0.074117	0.100741	0.135406	*0.000365(	*0.000180
B	{0.01	0.276427		0.422099	0.377757	0.985077	*0.000244(	*0.000150
C	{0.1}	0.074117	0.422099		0.708712	0.570819	*0.000168(	*0.000174
D	{1}	0.100741	0.377757	0.708712		0.630799	*0.000172(	*0.000158
E	{10}	0.135406	0.985077	0.570819	0.630799		*0.000205(	*0.000196
F	{100}	*0.000365(	*0.000244(	*0.000168(	*0.000172(	*0.000205039978027		*0.000224
G	{500}	*0.000180(	*0.000150(	*0.000174(	*0.000158:	*0.000196(	*0.000224	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 15.93100	{0.01 mg/L} 14.06000	{0.1 mg/L} 14.65200	{1 mg/L} 14.83100	{10 mg/L} 14.49700	{100 mg/L} 9.058000	{500 mg/L} .1171000
A	{0}		0.067344	0.144624	0.104818	0.154737	*0.000158:	*0.000174
B	{0.01	0.067344		0.628818	0.627367	0.502085	*0.000177:	*0.000180
C	{0.1}	0.144624	0.628818		0.781947	0.810539	*0.000196(	*0.000150
D	{1}	0.104818	0.627367	0.781947		0.859696	*0.000151(	*0.000158
E	{10}	0.154737	0.502085	0.810539	0.859696		*0.000181:	*0.000196
F	{100}	*0.000158:	*0.000177:	*0.000196(	*0.000151(	*0.000181317329406		*0.000176
G	{500}	*0.000174(	*0.000180(	*0.000150(	*0.000158:	*0.000196(	*0.000176	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 11.94830	{0.01 mg/L} 10.82100	{0.1 mg/L} 12.55600	{1 mg/L} 12.85700	{10 mg/L} 12.04900	{100 mg/L} 5.172000	{500 mg/L} 1.460500
A	{0}		0.05849	0.523191	0.378693	0.856676	*0.000180(	*0.000196
B	{0.01	0.05849		*0.030637:	*0.016399(	0.09794	*0.000176(	*0.000180
C	{0.1}	0.523191	*0.030637264251709		0.590875	0.369807	*0.000150(	*0.000158
D	{1}	0.378693	*0.016399(	0.590875		0.330971	*0.000158:	*0.000174
E	{10}	0.856676	0.09794	0.369807	0.330971		*0.000196(	*0.000150
F	{100}	*0.000180	*0.000176(	*0.000150(	*0.000158:	*0.000196039676666		*0.000181

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 15.93100	{0.01 mg/L} 12.97900	{0.1 mg/L} 14.17800	{1 mg/L} 14.93000	{10 mg/L} 14.89700	{100 mg/L} 6.269200	{500 mg/L} .1055800
A	{0}		0.005785	0.095688	0.168308	0.3202	*0.000158:	*0.000174
B	{0.01	0.005785		0.103763	0.057298	*0.036680(	*0.000176:	*0.000180
C	{0.1}	0.095688	0.103763		0.534466	0.314344	*0.000180(	*0.000196
D	{1}	0.168308	0.057298	0.534466		0.962579	*0.000150(	*0.000158
E	{10}	0.3202	*0.036680(	0.314344	0.962579		*0.000196(	*0.000150
F	{100}	*0.000158:	*0.000176:	*0.000180(	*0.000150(	*0.000196039676666		*0.000176

G {500} *0.0001960 *0.0001800 *0.0001580 *0.0001740 *0.0001500 *0.000181

G {500} *0.0001740 *0.0001800 *0.0001960 *0.0001581 *0.0001500 *0.000176

APPENDIX 36: ANOVA: *Boergesenia forbesii* : Biochemical composition (Protein)

* = Significant difference : p<0.05

ANOVA-PROTEIN-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	6.888000	7.322000	7.499000	7.303000	6.227000	3.725000
A	{0}		*0.000196(	*0.000158:	*0.000174(	*0.000150(	*0.000180(	*0.000364363193511963
B	{0.01	*0.000196039676666		0.512871	0.415434	0.298708	0.10768	*0.000181674957275391
C	{0.1}	*0.000158:	0.512871		0.652416	0.961399	0.055675	*0.000151395797729492
D	{1}	*0.000174(	0.415434	0.652416		0.867872	*0.035237(	*0.000158309936523437
E	{10}	*0.000150(	0.298708	0.961399	0.867872		*0.035710:	*0.000196337699890137
F	{100}	*0.000180(	0.10768	*0.055675(	*0.035237(	0.03571		*0.000185251235961914
G	{500}	*0.000364:	*0.000181(	*0.000151:	*0.000158:	*0.000196:	*0.000185251235961914	

Newman-Keuls test; CO_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	8.294000	8.274000	9.106000	8.966000	7.840000	.6759000
A	{0}		*0.000196(	*0.000180(	*0.000158:	*0.000150(	*0.000176(	*0.00311261415481567
B	{0.01	*0.000196039676666		0.950897	0.056657	0.05337	0.354925	*0.000150978565216064
C	{0.1}	*0.000180(	0.950897		0.084885	0.110922	0.194439	*0.00019603967666626
D	{1}	*0.000158:	0.056657	0.084885		0.666955	*0.010238(	*0.000174045562744141
E	{10}	*0.000150(	0.05337	0.110922	0.666955		*0.015452(	*0.000158190727233887
F	{100}	*0.000176(	0.354925	0.194439	*0.010238(	*0.015452623367309(		*0.000180900096893311
G	{500}	*0.003112(	*0.000150(	*0.000196(	*0.000174(	*0.000158:	*0.000180900096893311	

Newman-Keuls test; CR_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	5.023000	5.107000	5.351000	5.065000	2.511000	.5964000
A	{0}		*0.000353(	*0.000569:	*0.000434(	*0.000498(	0.250192	0.052757
B	{0.01	*0.000353991985321(		0.988493	0.939958	0.943117	*0.000806(	*0.000201702117919922
C	{0.1}	*0.000569:	0.988493		0.679043	0.943117	*0.002625(	*0.000169456005096436
D	{1}	*0.000434(	0.939958	0.679043		0.874668	*0.001873(	*0.000180900096893311
E	{10}	*0.000498(	0.943117	0.943117	0.874668		*0.001695(	*0.000159919261932373
F	{100}	0.250192	*0.000806(	*0.002625(	*0.001873(	*0.001695394515991(		0.01333
G	{500}	0.052757	*0.000201:	*0.000169(	*0.000180(	*0.000159(	0.01333	

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	5.385000	5.821000	5.779000	5.721000	3.502000	.5359000
A	{0}		*0.000236(	*0.000224:	*0.000204(	*0.000229:	*0.011416(	*0.0434092283248901
B	{0.01	*0.000236570835113(		0.872952	0.77732	0.569879	*0.005818(	*0.000197350978851318
C	{0.1}	*0.000224:	0.872952		0.943117	0.983686	*0.009495(	*0.000175058417053222
D	{1}	*0.000204(	0.77732	0.943117		0.921503	*0.007199:	*0.000159084796905518
E	{10}	*0.000229:	0.569879	0.983686	0.921503		*0.004879(	*0.000151753425598145
F	{100}	*0.011416(	*0.005818(	*0.009495(	*0.007199:	*0.004879772663116(		*0.000559628009796143
G	{500}	*0.043409:	*0.000197:	*0.000175(	*0.000159(	*0.000151:	*0.000559628009796143	

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	7.762000	7.754000	8.042000	7.972000	3.123000	.6743000
A	{0}		*0.000196(	*0.000180(	*0.000158:	*0.000150(	0.065755	*0.000346839427947998
B	{0.01	*0.000196039676666		0.982176	0.708484	0.557677	*0.000180(	*0.000150978565216064
C	{0.1}	*0.000180(	0.982176		0.842257	0.809782	*0.000176(	*0.00019603967666626
D	{1}	*0.000158:	0.708484	0.842257		0.844258	*0.000150(	*0.000174045562744141
E	{10}	*0.000150(	0.557677	0.809782	0.844258		*0.000196(	*0.000158190727233887
F	{100}	0.065755	*0.000180(	*0.000176(	*0.000150(	*0.000196039676666		*0.000190436840057373
G	{500}	*0.000346(	*0.000150(	*0.000196(	*0.000174(	*0.000158:	*0.000190436840057373	

Newman-Keuls test; CO_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	8.669000	7.824000	7.773000	7.515000	3.185000	.6642000
A	{0}		*0.000158:	*0.000150(	*0.000196(	*0.000180(	0.103864	*0.00138086080551147
B	{0.01	*0.000158190727233(		0.073599	0.136544	0.080647	*0.000150(	*0.000174045562744141
C	{0.1}	*0.000150(	0.073599		0.908809	0.763175	*0.000196(	*0.000158190727233887
D	{1}	*0.000196(	0.136544	0.908809		0.564231	*0.000180(	*0.000150978565216064
E	{10}	*0.000180(	0.080647	0.763175	0.564231		*0.000176:	*0.00019603967666626
F	{100}	0.103864	*0.000150(	*0.000196(	*0.000180(	*0.000176727771759(		*0.000295937061309814
G	{500}	*0.001380(	*0.000174(	*0.000158:	*0.000150(	*0.000196(	*0.000295937061309814	

Newman-Keuls test; CR_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	6.953000	6.894000	6.552000	6.143000	3.196000	.7412000
A	{0}		*0.000158:	*0.000150(	*0.000196(	*0.000181:	0.099225	*0.00188601016998291
B	{0.01	*0.000158250331878(		0.894527	0.637855	0.289946	*0.000152(	*0.000174045562744141
C	{0.1}	*0.000150(	0.894527		0.4465	0.232375	*0.000197:	*0.000158190727233887
D	{1}	*0.000196(	0.637855	0.4465		0.364788	*0.000183:	*0.000150978565216064
E	{10}	*0.000181:	0.289946	0.232375	0.364788		*0.000181(	*0.00019603967666626
F	{100}	0.099225	*0.000152(	*0.000197:	*0.000183:	*0.000181674957275(		*0.000332117080688477
G	{500}	*0.001886(	*0.000174(	*0.000158:	*0.000150(	*0.000196(	*0.000332117080688477	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	7.361000	7.325000	7.263000	6.297000	3.000000	.7286000
A	{0}		*0.000158:	*0.000150(	*0.000196(	*0.000180(	0.164548	*0.000836491584777832
B	{0.01	*0.000158190727233(		0.928218	0.966289	0.070848	*0.000150(	*0.000174045562744141
C	{0.1}	*0.000150(	0.928218		0.876671	*0.049581(	*0.000196(	*0.000158190727233887
D	{1}	*0.000196(	0.966289	0.876671		*0.027385(	*0.000180(	*0.000150978565216064
E	{10}	*0.000180(	0.070848	*0.049581(	*0.027385711669921(		*0.000176(	*0.00019603967666626
F	{100}	0.164548	*0.000150(	*0.000196(	*0.000180(	*0.000176846981048(		*0.000291109085083008
G	{500}	*0.000836(	*0.000174(	*0.000158:	*0.000150(	*0.000196(	*0.000291109085083008	

APPENDIX 36: ANOVA: *Boergesenia forbesii* : Biochemical composition (Protein)

* = Significant difference : p<0.05

ANOVA-PROTEIN-BOE

Newman-Keuls test; FE_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	5.905000	6.090000	5.511000	5.292000	2.561000	.5721000
A	{0}		*0.000188	*0.000183	*0.000262	*0.000254	0.219233	0.048797
B	{0.01	*0.000188		0.753442	0.506154	0.551986	*0.000415	*0.000158846378326416
C	{0.1}	*0.000183	0.753442		0.587052	0.529676	*0.000349	*0.000174641609191895
D	{1}	*0.000262	0.506154	0.587052		0.710196	*0.000580	*0.000152826309204102
E	{10}	*0.000254	0.551986	0.529676	0.710196		*0.000472	*0.000198185443878174
F	{100}	0.219233	*0.000415	*0.000349	*0.000580	*0.000472187995910		*0.0104150772094727
G	{500}	0.048797	*0.000158	*0.000174	*0.000152	*0.000198	*0.0104150772094727	

Newman-Keuls test; MN_B_C4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	5.704000	5.950000	6.384000	5.174000	3.477000	.6031000
A	{0}		*0.000231	*0.000184	*0.000167	*0.000287	*0.012426	0.053901
B	{0.01	*0.000231		0.676577	0.484825	0.374235	*0.004754	*0.000152051448822021
C	{0.1}	*0.000184	0.676577		0.464725	0.395285	*0.003841	*0.000158727169036865
D	{1}	*0.000167	0.484825	0.464725		0.201872	*0.001543	*0.000174224376678467
E	{10}	*0.000287	0.374235	0.395285	0.201872		*0.010912	*0.000199496746063232
F	{100}	*0.012426	*0.004754	*0.003841	*0.001543	*0.010912716388702		*0.000695466995239258
G	{500}	0.053901	*0.000152	*0.000158	*0.000174	*0.000199	*0.000695466995239258	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	5.813000	5.368000	5.452000	4.527000	2.847000	.5460000
A	{0}		*0.000225	*0.000302	*0.000290	*0.001075	0.096563	*0.0448535084724426
B	{0.01	*0.000225		0.726334	0.541895	0.163529	*0.001306	*0.000175178050994873
C	{0.1}	*0.000302	0.726334		0.886491	0.167302	*0.001872	*0.000153720378875732
D	{1}	*0.000290	0.541895	0.886491		0.277116	*0.002554	*0.000161111354827881
E	{10}	*0.001075	0.163529	0.167302	0.277116		*0.011562	*0.000221610069274902
F	{100}	0.096563	*0.001306	*0.001872	*0.002554	*0.011562108993530		*0.00373399257659912
G	{500}	*0.044853	*0.000175	*0.000153	*0.000161	*0.000221	*0.00373399257659912	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	6.275000	6.586000	7.165000	5.955000	.7073000	.6485000
A	{0}		*0.000183	*0.000197	*0.000151	*0.000178	*0.074920	0.142334
B	{0.01	*0.000183		0.598613	0.302379	0.588185	*0.000196	*0.000151157379150391
C	{0.1}	*0.000197	0.598613		0.332964	0.533652	*0.000150	*0.000158309936523437
D	{1}	*0.000151	0.302379	0.332964		0.201872	*0.000158	*0.000174045562744141
E	{10}	*0.000178	0.588185	0.533652	0.201872		*0.000180	*0.000196397304534912
F	{100}	0.074921	*0.000196	*0.000150	*0.000158	*0.000180959701538		0.920424
G	{500}	0.142334	*0.000151	*0.000158	*0.000174	*0.000196	0.920424	

Newman-Keuls test; FE_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	5.284000	5.653000	5.989000	5.973000	3.040000	.5595000
A	{0}		*0.000773	*0.000547	*0.000437	*0.000358	0.30932	*0.00656747817993164
B	{0.01	*0.000773		0.537001	0.631157	0.482519	*0.001926	*0.000198423862457275
C	{0.1}	*0.000547	0.537001		0.83472	0.591748	*0.001523	*0.000152289867401123
D	{1}	*0.000437	0.631157	0.83472		0.978599	*0.001484	*0.000174880027770996
E	{10}	*0.000358	0.482519	0.591748	0.978599		*0.001080	*0.000158727169036865
F	{100}	0.30932	*0.001926	*0.001523	*0.001484	*0.001080930233001		*0.00228577852249146
G	{500}	*0.006567	*0.000198	*0.000152	*0.000174	*0.000158	*0.00228577852249146	

Newman-Keuls test; MN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	6.124000	6.594000	5.861000	5.267000	3.577000	.6292000
A	{0}		*0.000217	*0.000175	*0.000284	*0.000539	0.055024	*0.00578939914703369
B	{0.01	*0.000217		0.407387	0.640064	0.295487	*0.002079	*0.000158309936523437
C	{0.1}	*0.000175	0.407387		0.401299	0.12023	*0.000768	*0.000174105167388916
D	{1}	*0.000284	0.640064	0.401299		0.298537	*0.002753	*0.000151216983795166
E	{10}	*0.000539	0.295487	0.12023	0.298537		*0.008436	*0.000197231769561768
F	{100}	0.055024	*0.002079	*0.000768	*0.002753	*0.008436501026153		*0.000429809093475342
G	{500}	*0.005789	*0.000158	*0.000174	*0.000151	*0.000197	*0.000429809093475342	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	5.191000	5.704000	5.838000	5.687000	2.553000	.5981000
A	{0}		*0.000987	*0.000625	*0.000599	*0.000513	0.829451	*0.00746554136276245
B	{0.01	*0.000987		0.661109	0.689512	0.409243	*0.000621	*0.000199735164642334
C	{0.1}	*0.000625	0.661109		0.821602	0.977262	*0.000641	*0.000159859657287598
D	{1}	*0.000599	0.689512	0.821602		0.963857	*0.000617	*0.000175595283508301
E	{10}	*0.000513	0.409243	0.977262	0.963857		*0.000421	*0.000152289867401123
F	{100}	0.829451	*0.000621	*0.000641	*0.000617	*0.000421404838562		*0.0124158859525293
G	{500}	*0.007465	*0.000199	*0.000159	*0.000175	*0.000152	*0.0124158859525293	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	6.183000	6.544000	6.636000	6.880000	3.897000	.6149000
A	{0}		*0.000209	*0.000214	*0.000181	*0.000173	*0.024376	*0.00789481401443481
B	{0.01	*0.000209		0.545743	0.722626	0.639255	*0.001674	*0.000196158885955811
C	{0.1}	*0.000214	0.545743		0.876944	0.83472	*0.001379	*0.000150978565216064
D	{1}	*0.000181	0.722626	0.876944		0.681947	*0.001849	*0.000158250331878662
E	{10}	*0.000173	0.639255	0.83472	0.681947		*0.001350	*0.000174105167388916
F	{100}	*0.024376	*0.001674	*0.001379	*0.001849	*0.001350224018096		*0.000330150127410889
G	{500}	*0.007894	*0.000196	*0.000150	*0.000158	*0.000174	*0.000330150127410889	

APPENDIX 36: ANOVA: *Boergesenia forbesii* : Biochemical composition (Protein)

* = Significant difference : p<0.05

ANOVA-PROTEIN-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	5.653000	5.805000	6.082000	5.687000	3.149000	.6972000
A	{0}		*0.000200(	*0.000200(	*0.000183(	*0.000233(	*0.037105(	0.072599
B	{0.01}	*0.000200629234313(		0.962686	0.878071	0.953956	*0.000823(	*0.000196993350982666
C	{0.1}	*0.000200(	0.962686		0.638854	0.841072	*0.002186(	*0.000159621238708496
D	{1}	*0.000183(	0.878071	0.638854		0.776344	*0.001433(	*0.000174820423126221
E	{10}	*0.000233(	0.953956	0.841072	0.776344		*0.001778(	*0.0001524686813
F	{100}	*0.037105(	*0.000823(	*0.002186(	*0.001433(	*0.001778542995452(		*0.00231975317001343
G	{500}	0.072599	*0.000196(	*0.000159(	*0.000174(	*0.000152(	*0.00231975317001343	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	7.328000	7.135000	7.387000	7.535000	3.213000	.7076000
A	{0}		*0.000196(	*0.000180(	*0.000150(	*0.000158(	*0.031041(	*0.000286638736724854
B	{0.01}	*0.000196039676666(		0.566262	0.860123	0.806252	*0.000180(	*0.000150978565216064
C	{0.1}	*0.000180(	0.566262		0.728498	0.626261	*0.000176(	*0.00019603967666626
D	{1}	*0.000150(	0.860123	0.728498		0.65929	*0.000196(	*0.000158190727233887
E	{10}	*0.000158(	0.806252	0.626261	0.65929		*0.000150(	*0.000174045562744141
F	{100}	*0.031041(	*0.000180(	*0.000176(	*0.000196(	*0.000150978565216(		*0.000183701515197754
G	{500}	*0.000286(	*0.000150(	*0.000196(	*0.000158(	*0.000174(	*0.000183701515197754	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	6.250000	6.258000	6.132000	3.401000	.7249000	.0794700
A	{0}		*0.000201(	*0.000167(	*0.000184(	*0.016047(	0.079128	*0.0238288640975952
B	{0.01}	*0.000201582908630(		0.989248	0.841072	*0.000740(	*0.000151(	*0.000158190727233887
C	{0.1}	*0.000167(	0.989248		0.974192	*0.001228(	*0.000158(	*0.000174105167388916
D	{1}	*0.000184(	0.841072	0.974192		*0.000472(	*0.000196(	*0.000150978565216064
E	{10}	*0.016047(	*0.000740(	*0.001228(	*0.000472187995910(		*0.001178(	*0.000438036534454346
F	{100}	0.079128	*0.000151(	*0.000158(	*0.000196(	*0.001178562641143(		0.282421
G	{500}	*0.023828(	*0.000158(	*0.000174(	*0.000150(	*0.000438(	0.282421	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	7.529000	7.913000	8.039000	8.476000	4.031000	.8389000
A	{0}		*0.000180(	*0.000196(	*0.000150(	*0.000158(	*0.002255(	*0.00245589017868042
B	{0.01}	*0.000180900096893(		0.383658	0.475345	0.165974	*0.000176(	*0.00019603967666626
C	{0.1}	*0.000196(	0.383658		0.772284	0.408283	*0.000180(	*0.000150978565216064
D	{1}	*0.000150(	0.475345	0.772284		0.323363	*0.000196(	*0.000158190727233887
E	{10}	*0.000158(	0.165974	0.408283	0.323363		*0.000150(	*0.000174045562744141
F	{100}	*0.002255(	*0.000176(	*0.000180(	*0.000196(	*0.000150978565216(		*0.000184535980224609
G	{500}	*0.002455(	*0.000196(	*0.000150(	*0.000158(	*0.000174(	*0.000184535980224609	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	7.703000	7.725000	8.039000	7.930000	3.664000	.7840000
A	{0}		*0.000180(	*0.000196(	*0.000158(	*0.000150(	*0.000183(	*0.00256764888763428
B	{0.01}	*0.000180900096893(		0.938622	0.637252	0.703071	*0.000176(	*0.00019603967666626
C	{0.1}	*0.000196(	0.938622		0.517602	0.476572	*0.000180(	*0.000150978565216064
D	{1}	*0.000158(	0.637252	0.517602		0.703212	*0.000150(	*0.000174045562744141
E	{10}	*0.000150(	0.703071	0.476572	0.703212		*0.000196(	*0.000158190727233887
F	{100}	*0.000183(	*0.000176(	*0.000180(	*0.000150(	*0.000196039676666(		*0.000180900096893311
G	{500}	*0.002567(	*0.000196(	*0.000150(	*0.000174(	*0.000158(	*0.000180900096893311	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	8.076000	7.966000	8.036000	8.364000	3.840000	.7675000
A	{0}		*0.000150(	*0.000196(	*0.000180(	*0.000196(	*0.000158(	*0.00298739476226807
B	{0.01}	*0.000150978565216(		0.957723	0.92027	0.474699	*0.000196(	*0.000158190727233887
C	{0.1}	*0.000180(	0.957723		0.860919	0.743313	*0.000176(	*0.00019603967666626
D	{1}	*0.000196(	0.92027	0.860919		0.687106	*0.000180(	*0.000150978565216064
E	{10}	*0.000158(	0.474699	0.743313	0.687106		*0.000150(	*0.000174045562744141
F	{100}	*0.002987(	*0.000196(	*0.000176(	*0.000180(	*0.000150978565216(		*0.000182569026947021
G	{500}	*0.000981(	*0.000158(	*0.000196(	*0.000150(	*0.000174(	*0.000182569026947021	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		1.818400	8.000000	7.810000	7.588000	6.866000	4.059000	.7541000
A	{0}		*0.000158(	*0.000150(	*0.000196(	*0.000180(	*0.000209(	*0.0150389075279236
B	{0.01}	*0.000158190727233(		0.628103	0.544721	*0.045654(	*0.000150(	*0.000174045562744141
C	{0.1}	*0.000150(	0.628103		0.571995	0.066627	*0.000196(	*0.000158190727233887
D	{1}	*0.000196(	0.544721	0.571995		0.08081	*0.000180(	*0.000150978565216064
E	{10}	*0.000180(	*0.045654(	0.066627	0.08081		*0.000178(	*0.00019603967666626
F	{100}	*0.000209(	*0.000150(	*0.000196(	*0.000180(	*0.000178217887878(		*0.000181257724761963

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.425000	8.554000	7.765000	7.639000	6.947000	3.955000	.8154000
A	{0}		*0.000158(	*0.000150(	*0.000196(	*0.000180(	*0.003663(	*0.00259876251220703
B	{0.01}	*0.000158190727233(		0.092484	0.126877	*0.011815(	*0.000150(	*0.000174045562744141
C	{0.1}	*0.000150(	0.092484		0.777307	0.183122	*0.000196(	*0.000158190727233887
D	{1}	*0.000196(	0.126877	0.777307		0.135533	*0.000181(	*0.000150978565216064
E	{10}	*0.000180(	*0.011815(	0.183122	0.135533		*0.000180(	*0.00019603967666626
F	{100}	*0.003663(	*0.000150(	*0.000196(	*0.000181(	*0.000180840492248(		*0.000187516212463379

G {500} *0.015038 *0.000174 *0.000158 *0.000150 *0.000196 *0.000181257724761963

G {500} *0.002598 *0.000174 *0.000158 *0.000150 *0.000196 *0.000187516212463379

APPENDIX 36: ANOVA: *Boergesenia forbesii* : Biochemical composition (Lipid)

* = Significant difference : p<0.05

ANOVA-LIPID-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	3.110000	3.035000	2.372000	2.268000	.6660000	.1030000
A	{0}		*0.000182	*0.000183	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000182	390213012	0.352715	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000183	0.352715		*0.000176	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.000176	8469810485	0.20381	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000196	*0.000180	0.20381		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	6681671142	*0.000178515911102295
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000178	515911102295

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	5.100000	1.840000	1.274800	1.045100	.3858400	.1297400
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	*0.000176	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	*0.000176	6681671142	*0.000178	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.000178	4565306457	*0.010918	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000196	*0.000180	*0.010918	6172485352	*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	8469810485	*0.00564903020858765
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000564	903020858765

Newman-Keuls test; CO_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	3.936400	3.509000	.4630000	.2930000	.1850000	.0496000
A	{0}		*0.000813	0.097497	*0.000180	*0.000196	*0.000150	*0.000158190727233887
B	{0.01	*0.000813	0073547363	*0.000242	*0.000196	*0.000150	*0.000158	*0.000174045562744141
C	{0.1}	0.097497	*0.000242	2928810119	*0.000176	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000180	*0.000196	*0.000176	6681671142	*0.028967	*0.003758	*0.00307097930902803
E	{10}	*0.000196	*0.000150	*0.000180	*0.028967	4401283264	0.144232	*0.00961560010910034
F	{100}	*0.000150	*0.000158	*0.000196	*0.003758	0.144232		0.07294
G	{500}	*0.000158	*0.000174	*0.000150	*0.000370	*0.009615	0.07294	

Newman-Keuls test; CO_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	2.045100	2.030700	1.112800	.4106600	.2177400	.0368920
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	0.839909	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	0.839909		*0.000176	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.000176	6681671142	*0.000176	7277717590	*0.00019603967666626
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176	7277717590	*0.015519	*0.000436365604400635
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.015519	9765615905	*0.0216861367225647
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000436	*0.021686	1367225647

Newman-Keuls test; CR_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	2.358000	2.963000	2.787000	2.197000	1.554000	.1890000
A	{0}		*0.000196	*0.000177	*0.000180	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196	0396766662	*0.000189	*0.000340	0.080261	*0.000180	*0.00019603967666626
C	{0.1}	*0.000177	*0.000189	0659332275	0.058375	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000180	*0.000340	0.058375		*0.000192	*0.000196	*0.000150978565216064
E	{10}	*0.000150	0.080261	*0.000196	*0.000192	4037933345	*0.000177	*0.000180900096893311
F	{100}	*0.000158	*0.000180	*0.000150	*0.000196	*0.000177	6814460754	*0.000176668167114258
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176	668167114258

Newman-Keuls test; CR_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	2.109700	1.635900	1.387700	1.291800	.2250000	.0399380
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	*0.000197	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	*0.000197	3509788513	*0.006841	*0.001744	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.006841	5999412536	0.239593	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000196	*0.001744	0.239593		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	6681671142	*0.0327717661857605
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.032771	7661857605

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	1.951800	.6090000	.1920000	.1740000	.0930000	.0148000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	*0.000176	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	*0.000176	6681671142	*0.000176	*0.000181	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.000176	8469810485	0.72026	0.146218	*0.0137507915496826
E	{10}	*0.000150	*0.000196	*0.000181	0.72026		0.122342	*0.0156412124633789
F	{100}	*0.000158	*0.000150	*0.000196	0.146218	0.122342		0.134688
G	{500}	*0.000174	*0.000158	*0.000150	*0.013750	*0.015641	0.134688	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	5.268600	5.968100	3.591400	2.873800	.5844000	.0135400
A	{0}		*0.000185	*0.025605	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000185	5492591857	*0.000406	*0.000176	*0.000180	*0.000196	*0.000150978565216064
C	{0.1}	*0.025605	*0.000406	0864448547	*0.000180	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000196	*0.000176	*0.000180	9000968933	*0.000355	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000180	*0.000196	*0.000355	3032875061	*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176	6681671142	*0.00155085325241089
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.001550	85325241089

APPENDIX 36: ANOVA: *Boergesenia forbesii* : Biochemical composition (Lipid)

* = Significant difference : p<0.05

ANOVA-LIPID-BOE

Newman-Keuls test; FE_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	3.858000	2.482000	2.358000	2.132000	.1920000	.0354000
A	{0}		*0.012158	*0.000176	*0.000180	*0.000196	*0.000150	*0.000158190727233887
B	{0.01}	*0.012158		*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
C	{0.1}	*0.000176	*0.000180		0.13434	*0.001513	*0.000196	*0.000150978565216064
D	{1}	*0.000180	*0.000196	0.13434		*0.011859	*0.000180	*0.000196039676666626
E	{10}	*0.000196	*0.000150	*0.001513	*0.011859		*0.000176	*0.000180900096893311
F	{100}	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176		0.064496
G	{500}	*0.000158	*0.000174	*0.000150	*0.000196	*0.000180	0.064496	

Newman-Keuls test; FE_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	2.146700	2.256400	1.304600	1.049200	.3385000	.0481730
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000180		0.181839	*0.000176	*0.000180	*0.000196	*0.000150978565216064
C	{0.1}	*0.000176	0.181839		*0.000180	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000196	*0.000176	*0.000180		*0.005711	*0.000180	*0.000196039676666626
E	{10}	*0.000150	*0.000180	*0.000196	*0.005711		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176		*0.002442359924316
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.002442	

Newman-Keuls test; MN_B_C4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	2.550000	2.171000	1.850000	1.489000	.1920000	.0233500
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176		*0.000403	*0.000181	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	*0.000403		*0.001179	*0.000181	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000181	*0.001179		*0.000536	*0.000180	*0.000196039676666626
E	{10}	*0.000150	*0.000196	*0.000181	*0.000536		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176		*0.048328816890716
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.048328	

Newman-Keuls test; MN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	2.583500	3.248200	2.910700	2.436900	.7220000	.0370040
A	{0}		*0.000196	*0.000176	*0.000180	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000196		*0.000181	*0.001044	0.081479	*0.000180	*0.000196039676666626
C	{0.1}	*0.000176	*0.000181		*0.000843	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000180	*0.001044	*0.000843		*0.000248	*0.000196	*0.000150978565216064
E	{10}	*0.000150	0.081479	*0.000196	*0.000248		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000180	*0.000150	*0.000196	*0.000176		*0.000176846981048584
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	5.291000	4.330000	2.903000	2.223000	1.870000	.5291000
A	{0}		*0.000180	*0.000176	*0.000176	*0.000180	*0.000196	*0.000150978565216064
B	{0.01}	*0.000180		*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
C	{0.1}	*0.000176	*0.000176		*0.000180	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000176	*0.000196	*0.000180		*0.000177	*0.000180	*0.000196039676666626
E	{10}	*0.000180	*0.000150	*0.000196	*0.000177		*0.001146	*0.000180900096893311
F	{100}	*0.000196	*0.000158	*0.000150	*0.000180	*0.001146		*0.000176684981671142
G	{500}	*0.000150	*0.000174	*0.000158	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	2.780100	2.608091	2.195200	1.063880	.7130100	.0992800
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176		*0.032988	*0.000181	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	*0.032988		*0.000221	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000181	*0.000221		*0.000176	*0.000180	*0.000196039676666626
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176		*0.000420	*0.000180900096893311
F	{100}	*0.000158	*0.000180	*0.000150	*0.000196	*0.000420		*0.000176846981048584
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	6.529000	6.338000	5.577000	1.165400	.7751000	.0971000
A	{0}		*0.000196	*0.000180	*0.000176	*0.000176	*0.000180	*0.000196039676666626
B	{0.01}	*0.000196		*0.028114	*0.000180	*0.000150	*0.000158	*0.000174045562744141
C	{0.1}	*0.000180	*0.028114		*0.000176	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000176	*0.000180	*0.000176		*0.000180	*0.000196	*0.000150978565216064
E	{10}	*0.000176	*0.000150	*0.000196	*0.000180		*0.000346	*0.000180900096893311
F	{100}	*0.000180	*0.000158	*0.000150	*0.000196	*0.000346		*0.000176846981048584
G	{500}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	4.067500	3.295300	2.129200	1.450850	.1360590	.0159074
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176		*0.000180	*0.000196	*0.000150	*0.000158	*0.000158190727233887
C	{0.1}	*0.000180	*0.000180		*0.000176	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.000176		*0.000176	*0.000180	*0.000196039676666626
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176		0.146175
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	0.146175	

APPENDIX 36: ANOVA: *Boergesenia forbesii* : Biochemical composition (Lipid)

* = Significant difference : p<0.05

ANOVA-LIPID-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	6.773000	6.476000	4.858000	3.713000	.9003000	.0128950
A	{0}		*0.000150	*0.000196	*0.000180	0.322098	*0.000176	*0.000180900096893311
B	{0.01	*0.000150978565216	*0.002064	*0.000180	*0.000196	*0.000158	*0.000174045562744141	
C	{0.1}	*0.000196	*0.002064824104309	*0.000176	*0.000180	*0.000150	*0.000158190727233887	
D	{1}	*0.000180	*0.000180	*0.000176668167114	*0.000176	*0.000196	*0.000150978565216064	
E	{10}	0.322098	*0.000196	*0.000180	*0.000176668167114	*0.000180	*0.00019603967666626	
F	{100}	*0.000176	*0.000158	*0.000150	*0.000196	*0.000180900096893	*0.000176668167114258	
G	{500}	*0.000180	*0.000174	*0.000158	*0.000150	*0.000196	*0.000176668167114258	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	3.555700	3.048000	2.079000	1.328100	.3497400	.0309120
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114	*0.003505	*0.000180	*0.000196	*0.000150	*0.000158190727233887	
C	{0.1}	*0.000180	*0.003505825996398	*0.000181	*0.000180	*0.000196	*0.000150978565216064	
D	{1}	*0.000196	*0.000180	*0.000181972980499	*0.000289	*0.000180	*0.00019603967666626	
E	{10}	*0.000150	*0.000196	*0.000180	*0.000289678573608	*0.000181	*0.000180959701538086	
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000181317329406	*0.0439982414245605	
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.0439982414245605	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	6.206000	4.531000	3.412000	1.145100	.8191000	.0667900
A	{0}		*0.000180	*0.000176	*0.013359	*0.000180	*0.000196	*0.000150978565216064
B	{0.01	*0.000180900096893	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141	
C	{0.1}	*0.000176	*0.000176668167114	*0.000180	*0.000196	*0.000150	*0.000158190727233887	
D	{1}	*0.013359	*0.000196	*0.000180900096893	*0.000176	*0.000180	*0.00019603967666626	
E	{10}	*0.000180	*0.000150	*0.000196	*0.000176668167114	*0.001058	*0.000180900096893311	
F	{100}	*0.000196	*0.000158	*0.000150	*0.000180	*0.001058459281921	*0.000176727771759033	
G	{500}	*0.000150	*0.000174	*0.000158	*0.000196	*0.000180	*0.000176727771759033	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	3.989500	4.151700	4.266500	3.446000	.9409100	.0462560
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196039676666	0.278927	0.168638	*0.002204	*0.000180	*0.00019603967666626	
C	{0.1}	*0.000180	0.278927	0.438665	*0.000777	*0.000196	*0.000150978565216064	
D	{1}	*0.000176	0.168638	0.438665	*0.000461	*0.000150	*0.000158190727233887	
E	{10}	*0.000150	*0.002204	*0.000777	*0.000461876392364	*0.000176	*0.000180900096893311	
F	{100}	*0.000158	*0.000180	*0.000196	*0.000150	*0.000176668167114	*0.00019228458404541	
G	{500}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000180	*0.00019228458404541	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	7.649000	9.657000	2.380000	2.248000	1.437000	.1047000
A	{0}		*0.000176	*0.000180	*0.000176	*0.000180	*0.000196	*0.000150978565216064
B	{0.01	*0.000176668167114	*0.000176	*0.000180	*0.000196	*0.000150	*0.000158190727233887	
C	{0.1}	*0.000180	*0.000176668167114	*0.000196	*0.000150	*0.000158	*0.000174045562744141	
D	{1}	*0.000176	*0.000180	*0.000196039676666	0.292476	*0.000182	*0.00019603967666626	
E	{10}	*0.000180	*0.000196	*0.000150	0.292476		*0.000181	*0.000180900096893311
F	{100}	*0.000196	*0.000150	*0.000158	*0.000182	*0.000181972980499	*0.000176668167114258	
G	{500}	*0.000150	*0.000158	*0.000174	*0.000196	*0.000180	*0.000176668167114258	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	2.552800	2.128200	1.529100	1.180300	.1049200	.0108644
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114	*0.010714	*0.000188	*0.000196	*0.000150	*0.000158190727233887	
C	{0.1}	*0.000180	*0.010714650154113	*0.001098	*0.000203	*0.000196	*0.000150978565216064	
D	{1}	*0.000196	*0.000188	*0.001098453998565	*0.029676	*0.000180	*0.00019603967666626	
E	{10}	*0.000150	*0.000196	*0.000203	*0.029676139354705	*0.000177	*0.000181794166564941	
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000177800655364	0.524266	
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000181	0.524266	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.633000	11.48900	11.80100	9.488000	5.142000	1.862000	.2132000
A	{0}		*0.000196	*0.000150	*0.000180	*0.000176	*0.000176	*0.000180900096893311
B	{0.01	*0.000196039676666	0.071117	*0.000176	*0.000180	*0.000150	*0.000158190727233887	
C	{0.1}	*0.000150	0.071117	*0.000180	*0.000196	*0.000158	*0.000174045562744141	
D	{1}	*0.000180	*0.000176	*0.000180900096893	*0.000176	*0.000196	*0.000150978565216064	
E	{10}	*0.000176	*0.000180	*0.000196	*0.000176668167114	*0.000180	*0.00019603967666626	
F	{100}	*0.000176	*0.000150	*0.000158	*0.000196	*0.000180900096893	*0.000176668167114258	

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		6.328000	1.669700	1.555400	1.488000	.7148000	.3497400	.0491890
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114	0.23844	0.159508	*0.000196	*0.000150	*0.000158190727233887	
C	{0.1}	*0.000180	0.23844	0.479766	*0.000180	*0.000196	*0.000150978565216064	
D	{1}	*0.000196	0.159508	0.479766	*0.000176	*0.000180	*0.00019603967666626	
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176846981048	*0.001639	*0.000187695026397705	
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.001639485359191	*0.00608831644058228	

G {500} *0.0001809 *0.0001581 *0.000174(*0.0001509 *0.000196(*0.000176668167114258

G {500} *0.000174(*0.0001582 *0.0001509 *0.000196(*0.000187(*0.00608831644058228

APPENDIX 37: ANOVA: *Boergesenia forbesii* : DNA Damage (RAPD-Appearance of new band)

* = Significant difference : p<0.05

ANOVA-APPEARANCE-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	88.06000	77.39000	72.78000	71.94000	58.72000	22.17000
A	{0}		*0.000174(	*0.000158:	*0.000151(	*0.000196(	*0.000181:	*0.008554
B	{0.01	*0.0001740455627441		0.162391	0.12314	0.163288	*0.008814:	*0.000159
C	{0.1}	*0.000158:	0.162391		0.534285	0.736547	0.089795	*0.000169
D	{1}	*0.000151(	0.12314	0.534285		0.909301	0.16322	*0.00021708
E	{10}	*0.000196(	0.163288	0.736547	0.909301		0.089081	*0.00019305
F	{100}	*0.000181:	*0.008814:	0.089795	0.16322	0.089081		*0.000332
G	{500}	*0.008554:	*0.000159(	*0.000169(	*0.000217(	*0.000193(	*0.000332	

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	62.29000	67.99000	58.79000	47.55000	42.06000	39.42000
A	{0}		*0.000268(	*0.000220(	*0.000300:	*0.001034(	*0.001539:	*0.001036
B	{0.01	*0.0002686977386474		0.553862	0.715168	0.290871	0.18441	0.162942
C	{0.1}	*0.000220(	0.553862		0.601329	0.177928	0.094181	0.076451
D	{1}	*0.000300:	0.715168	0.601329		0.251484	0.211789	0.213058
E	{10}	*0.001034(	0.290871	0.177928	0.251484		0.56838	0.670001
F	{100}	*0.001539:	0.18441	0.094181	0.211789	0.56838		0.782922
G	{500}	*0.001036:	0.162943	0.076451	0.213058	0.670001	0.782922	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	62.68000	53.75000	50.31000	41.27000	35.34000	33.01000
A	{0}		*0.000365(	*0.000908-	*0.001172:	*0.003935(	*0.006955(	*0.004330
B	{0.01	*0.0003650188446044		0.371238	0.428759	0.166821	0.083477	0.072625
C	{0.1}	*0.000908-	0.371238		0.727267	0.42272	0.269886	0.255232
D	{1}	*0.001172:	0.428759	0.727267		0.365549	0.299242	0.318107
E	{10}	*0.003935(	0.166821	0.42272	0.365549		0.549399	0.676386
F	{100}	*0.006955(	0.083477	0.269886	0.299242	0.549399		0.813074
G	{500}	*0.004330:	0.072626	0.255232	0.318107	0.676386	0.813074	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	68.80000	68.89000	60.74000	57.50000	40.70000	0.000000
A	{0}		*0.000295(	*0.000356(	*0.000537(	*0.000618(	*0.004851	1
B	{0.01	*0.0002959966659545		0.993437	0.458909	0.548328	0.078679	*0.000248
C	{0.1}	*0.000356(	0.993437		0.72664	0.708765	0.111024	*0.000293
D	{1}	*0.000537(	0.458909	0.72664		0.764028	0.176944	*0.000443
E	{10}	*0.000618(	0.548328	0.708765	0.764028		0.134714	*0.000396
F	{100}	*0.004851(	0.078679	0.111024	0.176944	0.134714		*0.001935
G	{500}	1	*0.000248(	*0.000293(	*0.000443:	*0.000396:	*0.001935	

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	89.11000	91.28000	76.56000	74.78000	64.78000	57.72000
A	{0}		*0.000158:	*0.000174(	*0.000151:	*0.000196:	*0.000181(	*0.000178
B	{0.01	*0.0001581907272338		0.79108	0.140608	0.2107	*0.039967(	*0.011614
C	{0.1}	*0.000174(	0.79108		0.195246	0.215557	*0.035847-	*0.009707
D	{1}	*0.000151:	0.140608	0.195246		0.827922	0.335862	0.1346
E	{10}	*0.000196:	0.2107	0.215557	0.827922		0.233644	0.120773
F	{100}	*0.000181(	*0.039967(	*0.035847-	0.335862	0.233644		0.394392
G	{500}	*0.000178(	*0.011614(	*0.009707(	0.134601	0.120774	0.394392	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	46.94000	67.41000	32.59000	31.55000	19.35000	15.18000
A	{0}		*0.000363(	*0.000175(	*0.005071:	*0.004324:	0.052925	0.061922
B	{0.01	*0.0003636479377746		*0.016181-	0.075715	0.135053	*0.011594:	*0.006205
C	{0.1}	*0.000175(	*0.016181468963623		*0.001139(	*0.001574(	*0.000261(	*0.000218
D	{1}	*0.005071:	0.075715	*0.0011399984359741		0.8915	0.215195	0.138585
E	{10}	*0.004324:	0.135053	*0.001574(	0.8915		0.125198	0.107979
F	{100}	0.052925	*0.011594:	*0.000261(	0.215195	0.125198		0.586005
G	{500}	0.061923	*0.006205:	*0.000218(	0.138586	0.107979	0.586005	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	33.29000	53.55000	44.61000	35.75000	32.04000	30.60000
A	{0}		*0.004334(	*0.000287(	*0.000797(	*0.003720(	*0.003262:	*0.001821
B	{0.01	*0.0043345689773559		0.092093	0.351017	0.760001	0.876536	0.938332
C	{0.1}	*0.000287(	0.092093		0.276471	0.096237	0.100033	0.096355
D	{1}	*0.000797(	0.351017	0.276471		0.280609	0.413723	0.424256
E	{10}	*0.003720(	0.760001	0.096237	0.280609		0.886347	0.912994
F	{100}	*0.003262:	0.876536	0.100033	0.413723	0.886347		0.85796
G	{500}	*0.001821(	0.938333	0.096355	0.424257	0.912995	0.85796	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	37.67000	40.48000	44.05000	40.48000	31.72000	0.000000
A	{0}		*0.005801(	*0.005019:	*0.004554(	*0.006925:	*0.010953	1
B	{0.01	*0.0058019757270813		0.766541	0.900117	0.950939	0.531771	*0.003255
C	{0.1}	*0.005019:	0.766541		0.92214	1	0.622432	*0.003329
D	{1}	*0.004554(	0.900117	0.92214		0.706264	0.678796	*0.003480
E	{10}	*0.006925:	0.950939	1	0.706264		0.782056	*0.005019
F	{100}	*0.010953(	0.531771	0.622432	0.678796	0.782056		*0.004304
G	{500}	1	*0.003255:	*0.003329(	*0.003480:	*0.005019:	*0.004304	

APPENDIX 37: ANOVA: *Boergesenia forbesii* : DNA Damage (RAPD-Appearance of new band)

* = Significant difference : p<0.05

ANOVA-APPEARANCE-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	32.03000	29.00000	25.11000	13.33000	0.000000	0.000000
A	{0}		*0.000174(	*0.000158:	*0.000150!	*0.000259	1	1
B	{0.01	*0.0001740455627441	0.166306	0.166306	*0.012855!	*0.000196-	*0.000158:	*0.000150
C	{0.1}	*0.000158:	0.166306		0.081885	*0.000184(	*0.000150!	*0.000196
D	{1}	*0.000150!	*0.012855!	0.081885		*0.000221;	*0.000196(	*0.000180
E	{10}	*0.000259(	*0.000196-	*0.000184(	*0.000221729278564-		*0.000211:	*0.000186
F	{100}	1	*0.000158:	*0.000150!	*0.000196(	*0.000211		1
G	{500}	1	*0.000150!	*0.000196(	*0.000180!	*0.000186	1	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	64.91000	63.61000	59.37000	55.56000	47.07000	0.000000
A	{0}		*0.000343!	*0.000317;	*0.000395;	*0.000506;	*0.000977(	1
B	{0.01	*0.0003439188003540	0.897573	0.897573	0.843517	0.782227	0.410594	*0.000284
C	{0.1}	*0.000317;	0.897573		0.675237	0.701578	0.374547	*0.000262
D	{1}	*0.000395;	0.843517	0.675237		0.706393	0.449426	*0.000350
E	{10}	*0.000506;	0.782227	0.701578	0.706393		0.405963	*0.000336
F	{100}	*0.000977(	0.410594	0.374547	0.449426	0.405963		*0.000460
G	{500}	1	*0.000284-	*0.000262;	*0.000350!	*0.000336;	*0.000460	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	29.84000	39.72000	34.26000	28.28000	26.38000	0.000000
A	{0}		*0.000716(	*0.000210;	*0.000337:	*0.000792(	*0.000787	1
B	{0.01	*0.0007166266441345	0.195265	0.195265	0.426178	0.77666	0.799966	*0.000550
C	{0.1}	*0.000210;	0.195265		0.328455	0.193842	0.15265	*0.000184
D	{1}	*0.000337:	0.426178	0.328455		0.524277	0.48459	*0.000276
E	{10}	*0.000792(	0.77666	0.193842	0.524277		0.729885	*0.000489
F	{100}	*0.000787(	0.799966	0.15265	0.48459	0.729885		*0.000390
G	{500}	1	*0.000550(	*0.000184;	*0.000276(	*0.000489!	*0.000390	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	39.61000	31.56000	20.27000	19.58000	17.05000	0.000000
A	{0}		*0.000174(	*0.000158:	*0.000151(	*0.000196(	*0.000181	1
B	{0.01	*0.0001740455627441	*0.001447!	*0.000180!	*0.000196(	*0.000150!	*0.000158	
C	{0.1}	*0.000158:	*0.0014479756355285	*0.000227(	*0.000262;	*0.000209!	*0.000150	
D	{1}	*0.000151(	*0.000180!	*0.0002276897430415	0.736649	0.277415	*0.000196	
E	{10}	*0.000196(	*0.000196(	*0.000262;	0.736649	0.22888	*0.000180	
F	{100}	*0.000181;	*0.000150!	*0.000209!	0.277415	0.22888	*0.000176	
G	{500}	1	*0.000158:	*0.000150!	*0.000196(	*0.000180!	*0.000176	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	36.32000	38.39000	35.27000	32.65000	27.60000	23.33000
A	{0}		*0.000158:	*0.000174(	*0.000150!	*0.000196(	*0.000180!	*0.000176
B	{0.01	*0.0001581907272335	0.388086	0.658308	0.286348	*0.010277!	*0.000663	
C	{0.1}	*0.000174(	0.388086		0.395916	0.108781	*0.003033;	*0.000301
D	{1}	*0.000150!	0.658308	0.395916		0.278433	*0.013719;	*0.000920
E	{10}	*0.000196(	0.286348	0.108781	0.278433		*0.047467;	*0.003559
F	{100}	*0.000180!	*0.010277!	*0.003033;	*0.013719;	*0.0474677085876465		0.087456
G	{500}	*0.000176;	*0.000663(	*0.000301(	*0.000920!	*0.003559-	0.087456	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	35.57000	31.74000	31.38000	31.68000	28.33000	23.27000
A	{0}		*0.000229;	*0.000329-	*0.000274!	*0.000273!	*0.000313!	*0.000505
B	{0.01	*0.0002292394638061	0.45434	0.833656	0.719876	0.605074	0.19745	
C	{0.1}	*0.000329-	0.45434		0.997183	0.990676	0.900996	0.46329
D	{1}	*0.000274!	0.833656	0.997183		0.952866	0.549807	0.26613
E	{10}	*0.000273!	0.719876	0.990676	0.952866		0.782504	0.364519
F	{100}	*0.000313!	0.605074	0.900996	0.549807	0.782504		0.326494
G	{500}	*0.000505!	0.19745	0.463291	0.26613	0.364519	0.326494	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	47.99000	45.72000	41.17000	39.74000	41.43000	40.00000
A	{0}		*0.000175-	*0.000160;	*0.000200(	*0.000177;	*0.000158!	*0.000184
B	{0.01	*0.0001754164695739	0.674901	0.585154	0.636395	0.451267	0.573898	
C	{0.1}	*0.000160;	0.674901		0.673887	0.789119	0.43171	0.70696
D	{1}	*0.000200(	0.585154	0.673887		0.960818	0.961664	0.828488
E	{10}	*0.000177;	0.636395	0.789119	0.960818		0.98833	0.961664
F	{100}	*0.000158!	0.451267	0.43171	0.961664	0.98833		0.960818
G	{500}	*0.000184(	0.573898	0.70696	0.828489	0.961664	0.960818	

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	47.27000	50.13000	47.27000	44.09000	42.77000	41.43000
A	{0}		*0.000237!	*0.000251!	*0.000281(	*0.000307-	*0.000265-	*0.000215
B	{0.01	*0.0002379417419433	0.91724	1	0.665531	0.809022	0.84817	
C	{0.1}	*0.000251!	0.91724		0.697252	0.83514	0.840982	0.825809
D	{1}	*0.000281(	1	0.697252		0.898828	0.922342	0.922857
E	{10}	*0.000307-	0.665531	0.83514	0.898828		0.857249	0.927951
F	{100}	*0.000265-	0.809022	0.840982	0.922342	0.857249		0.855112
G	{500}	*0.000215;	0.84817	0.825809	0.922857	0.927951	0.855112	

APPENDIX 37: ANOVA: *Boergesenia forbesii* : DNA Damage (RAPD-Disappearance of band)

* = Significant difference : p<0.05

ANOVA-DISAPPEARANCE-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	45.60000	53.20000	58.86000	64.90000	53.96000	45.20000
A	{0}		*0.006927	*0.003947	*0.003636	*0.002077	*0.005335	*0.00289583206176758
B	{0.01}	*0.006927		0.551833	0.71596	0.550332	0.78388	0.974977
C	{0.1}	*0.003947	0.551833		0.893413	0.784894	0.952327	0.799862
D	{1}	*0.003636	0.71596	0.893413		0.635518	0.7002	0.805694
E	{10}	*0.002077	0.550332	0.784894	0.635518		0.662466	0.622678
F	{100}	*0.005335	0.78388	0.952327	0.7002	0.662466		0.894275
G	{500}	*0.002895	0.974977	0.799862	0.805694	0.622678	0.894275	

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	43.52000	55.63000	57.21000	62.13000	63.29000	66.31000
A	{0}		*0.002498	*0.000999	*0.001387	*0.001027	*0.001180	*0.00103354454040527
B	{0.01}	*0.002498		0.319899	0.4916	0.417623	0.473469	0.419071
C	{0.1}	*0.000999	0.319899		0.894986	0.846372	0.913011	0.888539
D	{1}	*0.001387	0.4916	0.894986		0.681637	0.864013	0.864422
E	{10}	*0.001027	0.417623	0.846372	0.681637		0.922807	0.932925
F	{100}	*0.001180	0.473469	0.913011	0.864013	0.922807		0.800853
G	{500}	*0.001033	0.419071	0.888539	0.864422	0.932925	0.800853	

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	63.77000	68.26000	71.57000	70.37000	77.28000	60.36000
A	{0}		*0.000184	*0.000198	*0.000161	*0.000154	*0.000175	*0.000179290771484375
B	{0.01}	*0.000184		0.606571	0.797111	0.724142	0.529002	0.695142
C	{0.1}	*0.000198	0.606571		0.920698	0.808119	0.719034	0.632714
D	{1}	*0.000161	0.797111	0.920698		0.890131	0.513777	0.686509
E	{10}	*0.000154	0.724142	0.808119	0.890131		0.702573	0.651615
F	{100}	*0.000175	0.529002	0.719034	0.513777	0.702573		0.39607
G	{500}	*0.000179	0.695142	0.632714	0.686509	0.651615	0.39607	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	56.13000	60.82000	69.05000	87.58000	86.01000	86.01000
A	{0}		*0.000202	*0.000209	*0.000206	*0.000174	*0.000151	*0.000158965587615967
B	{0.01}	*0.000202		0.626071	0.380877	*0.044780	*0.030467	*0.0449697375297546
C	{0.1}	*0.000209	0.626071		0.396728	0.081403	*0.044887	0.07583
D	{1}	*0.000206	0.380877	0.396728		0.24546	0.093187	0.204826
E	{10}	*0.000174	*0.044780	0.081403	0.24546		0.984868	0.869996
F	{100}	*0.000151	*0.030467	*0.044887	0.093187	0.984868		1
G	{500}	*0.000158	*0.044969	0.07583	0.204826	0.869996	1	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	39.00000	40.82000	41.04000	41.73000	41.76000	46.78000
A	{0}		*0.000408	*0.000605	*0.000966	*0.001198	*0.001621	*0.000802516937255859
B	{0.01}	*0.000408		0.82422	0.965272	0.985996	0.996677	0.920697
C	{0.1}	*0.000605	0.82422		0.978658	0.993025	0.99945	0.942879
D	{1}	*0.000966	0.965272	0.978658		0.932893	0.995676	0.889844
E	{10}	*0.001198	0.985996	0.993025	0.932893		0.997183	0.807257
F	{100}	*0.001621	0.996677	0.99945	0.995676	0.997183		0.542367
G	{500}	*0.000802	0.920697	0.942879	0.889844	0.807257	0.542367	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	29.84000	40.34000	46.40000	52.58000	58.94000	54.41000
A	{0}		*0.002391	*0.000635	*0.000412	*0.000236	*0.000210	*0.000240564346313477
B	{0.01}	*0.002391		0.210471	0.132284	0.056211	*0.026141	0.054221
C	{0.1}	*0.000635	0.210471		0.461371	0.307371	0.193956	0.332167
D	{1}	*0.000412	0.132284	0.461371		0.452715	0.426743	0.588029
E	{10}	*0.000236	0.056211	0.307371	0.452715		0.71202	0.822451
F	{100}	*0.000210	*0.026141	0.193956	0.426743	0.71202		0.580233
G	{500}	*0.000240	0.054221	0.332167	0.588029	0.822451	0.580233	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	53.13000	52.99000	56.88000	59.72000	62.50000	87.50000
A	{0}		*0.000890	*0.000434	*0.000904	*0.000863	*0.000787	*0.000186681747436523
B	{0.01}	*0.000890		0.990171	0.739285	0.824086	0.830532	0.050335
C	{0.1}	*0.000434	0.990171		0.934261	0.927411	0.906268	0.06589
D	{1}	*0.000904	0.739285	0.934261		0.800858	0.868259	0.063771
E	{10}	*0.000863	0.824086	0.927411	0.800858		0.804982	0.060329
F	{100}	*0.000787	0.830532	0.906268	0.868259	0.804982		*0.0400729775428772
G	{500}	*0.000186	0.050335	0.06589	0.063771	0.060329	*0.0400729775428772	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	50.09000	51.29000	56.10000	58.95000	65.86000	100.0000
A	{0}		*0.000190	*0.000211	*0.000216	*0.000175	*0.000163	*0.000174045562744141
B	{0.01}	*0.000190		0.882837	0.737163	0.689956	0.326495	*0.000374197959899902
C	{0.1}	*0.000211	0.882837		0.556772	0.613518	0.303288	*0.000354468822479248
D	{1}	*0.000216	0.737163	0.556772		0.726652	0.460285	*0.000575244267272 95
E	{10}	*0.000175	0.689956	0.613518	0.726652		0.40169	*0.000558853149414062
F	{100}	*0.000163	0.326495	0.303288	0.460285	0.40169		*0.000911891460418701
G	{500}	*0.000174	*0.000374	*0.000354	*0.000575	*0.000558	*0.000911891460418701	

APPENDIX 37: ANOVA: *Boergesenia forbesii* : DNA Damage (RAPD-Disappearance of band)

* = Significant difference : p<0.05

ANOVA-DISAPPEARANCE-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	61.74000	63.59000	67.14000	83.13000	81.67000	100.0000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000158	*0.000150	*0.000174045562744141
B	{0.01	*0.000176	27771759	0.77641	0.681902	*0.032691	*0.033714	*0.000505030155181885
C	{0.1}	*0.000180	0.77641		0.587164	*0.037761	*0.033606	*0.00056612491607666
D	{1}	*0.000196	0.681902	0.587164		0.061685	*0.039254	*0.000913023948669434
E	{10}	*0.000158	*0.032691	*0.037761	0.061685		0.822577	*0.0194856524467468
F	{100}	*0.000150	*0.033714	*0.033606	*0.039254	0.822577		*0.031207263469696
G	{500}	*0.000174	*0.000505	*0.000566	*0.000913	*0.019485	*0.031207263469696	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	67.03000	72.36000	73.12000	80.47000	82.49000	100.0000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	27771759	0.471597	0.471597	0.681867	0.285583	0.255044	*0.0047033429145813
C	{0.1}	*0.000180	0.471597		0.91757	0.514461	0.515767	*0.0132217407226562
D	{1}	*0.000196	0.681867	0.91757		0.324841	0.417649	*0.0106955766677856
E	{10}	*0.000150	0.285583	0.514461	0.324841		0.78331	*0.0420170426368713
F	{100}	*0.000158	0.255044	0.515767	0.417649	0.78331		*0.0291904211044312
G	{500}	*0.000174	*0.004703	*0.013221	*0.010695	*0.042017	*0.0291904211044312	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	67.67000	73.00000	75.68000	79.72000	81.83000	100.0000
A	{0}		*0.000182	*0.000187	*0.000204	*0.000157	*0.000164	*0.000174283981323242
B	{0.01	*0.000182	6286315917	0.606848	0.714265	0.642415	0.638098	0.058359
C	{0.1}	*0.000187	0.606848		0.795151	0.787765	0.818945	0.110503
D	{1}	*0.000204	0.714265	0.795151		0.695931	0.818415	0.122239
E	{10}	*0.000157	0.642415	0.787765	0.695931		0.837975	0.14794
F	{100}	*0.000164	0.638098	0.818945	0.818415	0.837975		0.094359
G	{500}	*0.000174	0.058359	0.110503	0.122239	0.14794	0.094359	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	66.18000	68.43000	68.78000	68.93000	72.32000	100.0000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000151	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	27771759	0.759639	0.759639	0.931219	0.980408	0.909551	*0.00384187698364258
C	{0.1}	*0.000180	0.759639		0.962073	0.99741	0.947847	*0.00487148761749268
D	{1}	*0.000196	0.931219	0.962073		0.983802	0.876687	*0.00352704524993896
E	{10}	*0.000151	0.980408	0.99741	0.983802		0.645489	*0.00206863880157471
F	{100}	*0.000158	0.909551	0.947847	0.876687	0.645489		*0.00196027755737305
G	{500}	*0.000174	*0.003841	*0.004871	*0.003527	*0.002068	*0.00196027755737305	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	80.91000	83.06000	84.37000	85.03000	85.34000	92.54000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	0.731805	0.841664	0.906582	0.948178	0.44512
C	{0.1}	*0.000180	0.731805		0.834394	0.945244	0.981928	0.554025
D	{1}	*0.000196	0.841664	0.834394		0.916131	0.986472	0.5606
E	{10}	*0.000150	0.906582	0.945244	0.916131		0.960606	0.460333
F	{100}	*0.000158	0.948178	0.981928	0.986472	0.960606		0.261063
G	{500}	*0.000174	0.44512	0.554025	0.5606	0.460333	0.261063	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	70.02000	71.93000	78.14000	78.76000	79.76000	79.20000
A	{0}		*0.000177	*0.000181	*0.000196	*0.000151	*0.000175	*0.000159263610839844
B	{0.01	*0.000177	0853996276	0.831558	0.831558	0.635924	0.756151	0.87108	0.831799
C	{0.1}	*0.000181	0.831558		0.492482	0.72367	0.896335	0.841597
D	{1}	*0.000196	0.635924	0.492482		0.944973	0.997746	0.992125
E	{10}	*0.000151	0.756151	0.72367	0.944973		0.992988	0.960983
F	{100}	*0.000175	0.87108	0.896335	0.997746	0.992988		0.950292
G	{500}	*0.000159	0.831799	0.841597	0.992125	0.960983	0.950292	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	63.24000	68.06000	78.90000	79.50000	86.91000	81.93000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000176	8469810485	0.524938	0.121788	0.171011	0.057437	0.139483
C	{0.1}	*0.000180	0.524938		0.164624	0.299714	0.134552	0.281235
D	{1}	*0.000196	0.121788	0.164624		0.93654	0.704607	0.912137
E	{10}	*0.000150	0.171011	0.299714	0.93654		0.587255	0.747271
F	{100}	*0.000174	0.057437	0.134552	0.704607	0.587255		0.511486
G	{500}	*0.000158	0.139483	0.281235	0.912137	0.747271	0.511486	

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	65.36000	68.90000	69.45000	75.29000	82.68000	83.25000
A	{0}		*0.000177	*0.000181	*0.000197	*0.000151	*0.000158	*0.000174343585968018
B	{0.01	*0.000177	2642135620	0.681389	0.681389	0.879724	0.650661	0.292768	0.3318
C	{0.1}	*0.000181	0.681389		0.949055	0.734462	0.393113	0.464521
D	{1}	*0.000197	0.879724	0.949055		0.500442	0.291503	0.391914
E	{10}	*0.000151	0.650661	0.734462	0.500442		0.396198	0.62314
F	{100}	*0.000158	0.292768	0.393113	0.291503	0.396198		0.947204
G	{500}	*0.000174	0.3318	0.464521	0.391914	0.62314	0.947204	

APPENDIX 37: ANOVA: *Boergesenia forbesii* : DNA Damage (RAPD-Similarity of band)

* = Significant difference : p<0.05

ANOVA-SIMILIAR-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	47.60000	58.56000	46.95000	38.35000	38.33000	35.49000
A	{0}		*0.000254(	*0.000453(	*0.000313:	*0.000188:	*0.000203(	*0.00020754337310791
B	{0.01	*0.000254034996032:		0.228407	0.94159	0.551203	0.715101	0.642147
C	{0.1}	*0.000453(	0.228407		0.40028	0.139763	0.19395	0.148118
D	{1}	*0.000313:	0.94159	0.40028		0.33976	0.594363	0.567601
E	{10}	*0.000188:	0.551203	0.139763	0.33976		0.998294	0.942491
F	{100}	*0.000203(	0.715101	0.19395	0.594363	0.998294		0.749024
G	{500}	*0.000207!	0.642147	0.148118	0.567601	0.942491	0.749024	

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	49.42000	36.57000	37.44000	33.14000	30.43000	29.48000
A	{0}		*0.000269:	*0.000235(	*0.000203-	*0.000191!	*0.000187(	*0.000207662582397461
B	{0.01	*0.000269174575805(		0.392074	0.928539	0.723939	0.798001	0.877224
C	{0.1}	*0.000235(	0.392074		0.928539	0.723939	0.798001	0.877224
D	{1}	*0.000203-	0.228629	0.928539		0.89439	0.880715	0.91467
E	{10}	*0.000191!	0.354509	0.723939	0.89439		0.780044	0.922185
F	{100}	*0.000187(	0.316863	0.798001	0.880715	0.780044		0.92199
G	{500}	*0.000207(	0.342676	0.877224	0.91467	0.922185	0.92199	

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	32.64000	25.25000	22.05000	19.58000	18.69000	0.000000
A	{0}		*0.000176(	*0.000180!	*0.000196(	*0.000150!	*0.000158:	*0.000174045562744141
B	{0.01	*0.000176668167114:		0.072662	*0.036804-	*0.018825(	*0.018219!	*0.000160574913024902
C	{0.1}	*0.000180!	0.072662		0.414698	0.325246	0.348543	*0.000228404998779297
D	{1}	*0.000196(	*0.036804-	0.414698		0.526941	0.659447	*0.000414907932281494
E	{10}	*0.000150!	*0.018825(	0.325246	0.526941		0.818586	*0.000554859638214111
F	{100}	*0.000158:	*0.018219!	0.348543	0.659447	0.818586		*0.000383317470550537
G	{500}	*0.000174(	*0.000160!	*0.000228-	*0.000414!	*0.000554!	*0.000383317470550537	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	43.87000	39.18000	30.95000	14.20000	13.99000	13.99000
A	{0}		*0.000187!	*0.000192-	*0.000199!	*0.000151:	*0.000174:	*0.000158309936523437
B	{0.01	*0.000187933444976!		0.602731	0.33553	*0.021167:	*0.040804:	*0.0301489233970642
C	{0.1}	*0.000192-	0.602731		0.365946	*0.033237!	0.078968	0.054411
D	{1}	*0.000199!	0.33553	0.365946		0.078008	0.261653	0.168055
E	{10}	*0.000151:	*0.021167:	*0.033237!	0.078008		0.999723	0.981414
F	{100}	*0.000174:	*0.040804:	0.078968	0.261653	0.999723		1
G	{500}	*0.000158:	*0.030148!	0.054411	0.168055	0.981414	1	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	67.50000	56.46000	54.51000	50.98000	48.61000	48.76000
A	{0}		*0.006139:	*0.001988:	*0.002491:	*0.002010-	*0.002433(	*0.00190210342407227
B	{0.01	*0.006139218807220-		0.29053	0.422052	0.387356	0.451759	0.378241
C	{0.1}	*0.001988:	0.29053		0.849024	0.850522	0.931935	0.868251
D	{1}	*0.002491:	0.422052	0.849024		0.730709	0.934375	0.836904
E	{10}	*0.002010-	0.387356	0.850522	0.730709		0.969943	0.828442
F	{100}	*0.002433(	0.451759	0.931935	0.934375	0.969943		0.9884
G	{500}	*0.001902:	0.378241	0.868251	0.836904	0.828442	0.9884	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	70.16000	59.67000	53.60000	47.43000	47.91000	45.59000
A	{0}		*0.006075-	*0.001835:	*0.001071!	*0.000737-	*0.000601:	*0.000712990760803223
B	{0.01	*0.006075441837310:		0.273949	0.206203	0.1543	0.119562	0.144353
C	{0.1}	*0.001835:	0.273949		0.520699	0.560808	0.430519	0.562015
D	{1}	*0.001071!	0.206203	0.520699		0.784427	0.546765	0.820282
E	{10}	*0.000737-	0.1543	0.560808	0.784427		0.959289	0.844644
F	{100}	*0.000601:	0.119562	0.430519	0.546765	0.959289		0.965803
G	{500}	*0.000712!	0.144353	0.562015	0.820282	0.844644	0.965803	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	46.87000	53.73000	49.28000	45.95000	37.50000	12.50000
A	{0}		*0.000703(	*0.000526(	*0.000594:	*0.000840:	*0.000364:	*0.000176310539245605
B	{0.01	*0.000703096389770!		0.773791	0.812413	0.927812	0.624489	*0.0181533694267273
C	{0.1}	*0.000526(	0.773791		0.66195	0.861741	0.503873	*0.0104115605354309
D	{1}	*0.000594:	0.812413	0.66195		0.940572	0.646923	*0.0173161029815674
E	{10}	*0.000840:	0.927812	0.861741	0.940572		0.410594	*0.0123043656349182
F	{100}	*0.000364:	0.624489	0.503873	0.646923	0.410594		*0.0250999927520752
G	{500}	*0.000176:	*0.018153:	*0.010411!	*0.017316:	*0.012304:	*0.0250999927520752	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	40.13000	38.06000	34.56000	31.22000	27.33000	0.000000
A	{0}		*0.000177-	*0.000182-	*0.000197!	*0.000152:	*0.000158:	*0.000174045562744141
B	{0.01	*0.000177443027496:		0.795976	0.762028	0.675018	0.503421	*0.00186324119567871
C	{0.1}	*0.000182-	0.795976		0.662653	0.666453	0.538741	*0.0021173357963562
D	{1}	*0.000197!	0.762028	0.662653		0.677087	0.636492	*0.00310641527175903
E	{10}	*0.000152:	0.675018	0.666453	0.677087		0.62804	*0.00379973649978638
F	{100}	*0.000158:	0.503421	0.538741	0.636492	0.62804		*0.00381875038146973
G	{500}	*0.000174(	*0.001863-	*0.002117:	*0.003106-	*0.003799:	*0.00381875038146973	

APPENDIX 37: ANOVA: *Boergesenia forbesii* : DNA Damage (RAPD-Similarity of band)

* = Significant difference : p<0.05

ANOVA-SIMILIAR-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	38.26000	47.80000	49.45000	45.00000	38.33000	0.000000
A	{0}		*0.000503	*0.000614	*0.000384	*0.000698	*0.000398	*0.000174462795257568
B	{0.01	*0.000503	3242015838	0.791535	0.81054	0.793045	0.994786	*0.00246274471282959
C	{0.1}	*0.000614	0.791535		0.875127	0.789815	0.637417	*0.00305503606796265
D	{1}	*0.000384	0.81054	0.875127		0.902973	0.707067	*0.0031699538230896
E	{10}	*0.000698	0.793045	0.789815	0.902973		0.52786	*0.00329691171646118
F	{100}	*0.000398	0.994786	0.637417	0.707067	0.52786		*0.00615400075912476
G	{500}	*0.000174	*0.002462	*0.003055	*0.003169	*0.003296	*0.00615400075912476	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	37.66000	32.24000	30.72000	22.32000	15.00000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	7873764038	0.45414	0.597203	0.176783	*0.041455	*0.00126034021377563
C	{0.1}	*0.000180	0.45414		0.83222	0.362825	0.112821	*0.00339430570602417
D	{1}	*0.000196	0.597203	0.83222		0.252556	0.099925	*0.00331985950469971
E	{10}	*0.000150	0.176783	0.362825	0.252556		0.316004	*0.0175970196723938
F	{100}	*0.000158	*0.041455	0.112821	0.099925	0.316004		0.051367
G	{500}	*0.000174	*0.001260	*0.003394	*0.003319	*0.017597	0.051367	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	37.98000	32.40000	28.38000	26.08000	24.23000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000151	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	8469810485	0.457956	0.411112	0.395356	0.370142	*0.00161981582641602
C	{0.1}	*0.000180	0.457956		0.591045	0.670365	0.684936	*0.00442367792129517
D	{1}	*0.000196	0.411112	0.591045		0.757727	0.839155	*0.00806772708892822
E	{10}	*0.000151	0.395356	0.670365	0.757727		0.803957	*0.0082288384437561
F	{100}	*0.000158	0.370142	0.684936	0.839155	0.803957		*0.0052497386932373
G	{500}	*0.000174	*0.001619	*0.004423	*0.008067	*0.008228	*0.0052497386932373	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	37.16000	29.92000	21.40000	21.23000	0.000000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000176	6681671142	0.246775	*0.048794	0.078142	*0.000396	*0.000314652919769287
C	{0.1}	*0.000180	0.246775		0.176821	0.343072	*0.001648	*0.00114202499389648
D	{1}	*0.000196	*0.048794	0.176821		0.977869	*0.014420	*0.00815367698669434
E	{10}	*0.000150	0.078142	0.343072	0.977869		*0.008606	*0.00337809324264526
F	{100}	*0.000174	*0.000396	*0.001648	*0.014420	*0.008606	4930525	1
G	{500}	*0.000158	*0.000314	*0.001142	*0.008153	*0.003378	1	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	16.89000	16.94000	15.64000	14.97000	12.97000	7.460000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180	9000968933	0.987684	0.698022	0.817873	0.611646	0.062889
C	{0.1}	*0.000176	0.987684		0.911297	0.922561	0.719122	0.081424
D	{1}	*0.000196	0.698022	0.911297		0.834975	0.68145	0.087981
E	{10}	*0.000150	0.817873	0.922561	0.834975		0.536446	0.077078
F	{100}	*0.000158	0.611646	0.719122	0.68145	0.536446		0.102707
G	{500}	*0.000174	0.062889	0.081424	0.087981	0.077078	0.102707	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	24.89000	28.08000	21.86000	21.24000	20.24000	17.89000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180	9000968933	0.155597	0.17588	0.233349	0.174317	*0.0361680388450623
C	{0.1}	*0.000176	0.155597		*0.028019	*0.028061	*0.017423	*0.00319910049438477
D	{1}	*0.000196	0.17588	*0.028019	6070671082	0.774838	0.731328	0.284698
E	{10}	*0.000150	0.233349	*0.028061	0.774838		0.645273	0.287687
F	{100}	*0.000158	0.174317	*0.017423	0.731328	0.645273		0.287465
G	{500}	*0.000174	*0.036168	*0.003199	0.284698	0.287687	0.287465	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	44.67000	31.94000	28.13000	23.27000	18.79000	17.15000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	*0.010877	*0.005087	*0.001237	*0.000401	*0.000335752964019775
C	{0.1}	*0.000180	*0.010877	4304389954	0.393661	0.147984	*0.039275	*0.0288403630256653
D	{1}	*0.000196	*0.005087	0.393661		0.280418	0.1139	0.097002
E	{10}	*0.000150	*0.001237	0.147984	0.280418		0.31822	0.360611
F	{100}	*0.000158	*0.000401	*0.039275	0.1139	0.31822		0.710514
G	{500}	*0.000174	*0.000335	*0.028840	0.097002	0.360611	0.710514	

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	34.64000	31.10000	30.55000	24.71000	17.32000	16.75000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176	6681671142	0.468918	0.673017	0.204189	*0.018982	*0.0207734107971191
C	{0.1}	*0.000180	0.468918		0.909642	0.395342	0.050776	0.059648
D	{1}	*0.000196	0.673017	0.909642		0.239582	*0.036779	0.050387
E	{10}	*0.000150	0.204189	0.395342	0.239582		0.142475	0.249125
F	{100}	*0.000158	*0.018982	0.050776	*0.036779	0.142475		0.906373
G	{500}	*0.000174	*0.020773	0.059648	0.050387	0.249125	0.906373	

APPENDIX 37: ANOVA: *Boergesenia forbesii* : DNA Damage (RAPD-Intensity of band)

* = Significant difference : p<0.05

ANOVA-INTENSITY-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	126.1800	125.8400	117.8000	115.5900	112.2200	104.8400
A	{0}		0.36987	0.323638	0.594869	0.583818	0.582333	0.695016
B	{0.01}	0.36987		0.978072	0.771277	0.817094	0.775601	0.515441
C	{0.1}	0.323638	0.978072		0.516902	0.68048	0.6798	0.444335
D	{1}	0.594869	0.771277	0.516902		0.857664	0.89012	0.711344
E	{10}	0.583818	0.817094	0.68048	0.857664		0.784588	0.655578
F	{100}	0.582333	0.775601	0.6798	0.89012	0.784588		0.55141
G	{500}	0.695016	0.515441	0.444335	0.711344	0.655578	0.55141	

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	87.88000	88.81000	97.61000	114.2900	109.6000	96.01400
A	{0}		0.802257	0.74171	0.831051	0.417952	0.397175	0.930459
B	{0.01}	0.802257		0.933852	0.812374	0.265928	0.400717	0.744267
C	{0.1}	0.74171	0.933852		0.708608	0.249464	0.364982	0.522814
D	{1}	0.831051	0.812374	0.708608		0.453426	0.53478	0.886699
E	{10}	0.417952	0.265928	0.249464	0.453426		0.676101	0.484942
F	{100}	0.397175	0.400717	0.364982	0.53478	0.676101		0.615269
G	{500}	0.930459	0.744267	0.522814	0.886699	0.484942	0.615269	

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	105.5200	121.1800	129.1300	133.1100	153.5400	172.8100
A	{0}		0.674098	0.258942	0.153286	0.128749	*0.009903	*0.00100469589233398
B	{0.01}	0.674098		0.243096	0.193695	0.186145	*0.015944	*0.00151687860488892
C	{0.1}	0.258942	0.243096		0.546107	0.631889	0.100151	*0.00947326421737671
D	{1}	0.153286	0.193695	0.546107		0.761406	0.175337	*0.0199939012527466
E	{10}	0.128749	0.186145	0.631889	0.761406		0.13423	*0.0205560922622681
F	{100}	*0.009903	*0.015944	0.100151	0.175337	0.13423		0.155961
G	{500}	*0.001004	*0.001516	*0.009473	*0.019993	*0.020556	0.155961	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	102.1600	115.4800	117.5500	96.62000	96.83000	92.60000
A	{0}		0.849449	0.373995	0.424379	0.950968	0.780685	0.909258
B	{0.01}	0.849449		0.252667	0.377982	0.958645	0.882968	0.907969
C	{0.1}	0.373995	0.252667		0.855653	0.470328	0.374059	0.364495
D	{1}	0.424379	0.377982	0.855653		0.454388	0.382574	0.337176
E	{10}	0.950968	0.958645	0.470328	0.454388		0.985357	0.724221
F	{100}	0.780685	0.882968	0.374059	0.382574	0.985357		0.92439
G	{500}	0.909258	0.907969	0.364495	0.337176	0.724221	0.92439	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	119.2900	130.1700	144.3100	143.0300	157.1300	168.9600
A	{0}		0.225791	0.153274	0.072261	0.05786	*0.021172	*0.0066990852355957
B	{0.01}	0.225791		0.486613	0.387477	0.294766	0.149752	0.051577
C	{0.1}	0.153274	0.486613		0.631642	0.412518	0.326621	0.135063
D	{1}	0.072261	0.387477	0.631642		0.934276	0.413937	0.270288
E	{10}	0.05786	0.294766	0.412518	0.934276		0.633227	0.35813
F	{100}	*0.021172	0.149752	0.326621	0.413937	0.633227		0.450088
G	{500}	*0.006699	0.051577	0.135063	0.270288	0.35813	0.450088	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	102.1100	107.1500	107.3300	119.9900	116.6800	90.05000
A	{0}		0.898296	0.898904	0.968056	0.813346	0.837435	0.548862
B	{0.01}	0.898296		0.760307	0.944628	0.801653	0.805141	0.741625
C	{0.1}	0.898904	0.760307		0.991405	0.856493	0.828445	0.720511
D	{1}	0.968056	0.944628	0.991405		0.719873	0.572922	0.819925
E	{10}	0.813346	0.801653	0.856493	0.719873		0.841073	0.539936
F	{100}	0.837435	0.805141	0.828445	0.572922	0.841073		0.585322
G	{500}	0.548862	0.741625	0.720511	0.819925	0.539936	0.585322	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	133.4000	138.7300	151.5600	159.9000	177.5400	0.000000
A	{0}		*0.028546	*0.033492	*0.009943	*0.004858	*0.000775	*0.000178217887878418
B	{0.01}	*0.028546		0.154994	0.702608	0.403474	0.256964	*0.040792	*0.000180959701538086
C	{0.1}	*0.033492	0.154994		0.702608	0.403474	0.256964	*0.040792	*0.000196099281311035
D	{1}	*0.009943	0.702608	0.702608		0.403474	0.256964	*0.040792	*0.000150978565216064
E	{10}	*0.004858	0.403474	0.403474	0.403474		0.256964	*0.040792	*0.000158190727233887
F	{100}	*0.000775	*0.040792	*0.040792	*0.040792	*0.040792		*0.000174045562744141
G	{500}	*0.000178	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	101.4900	126.1200	127.9800	109.6200	87.89000	0.000000
A	{0}		0.905707	0.195363	0.212219	0.721151	0.343101	*0.000181913375854492
B	{0.01}	0.905707		0.149744	0.18629	0.520757	0.528315	*0.000197887420654297
C	{0.1}	0.195363	0.149744		0.882427	0.202528	0.051666	*0.000158309936523437
D	{1}	0.212219	0.18629	0.882427		0.326075	0.052887	*0.000174224376678467
E	{10}	0.721151	0.520757	0.202528	0.326075		0.331183	*0.000151932239532471
F	{100}	0.343101	0.528315	0.051666	0.052887	0.331183		*0.000179052352905273
G	{500}	*0.000181	*0.000197	*0.000158	*0.000174	*0.000151	*0.000179052352905273	

APPENDIX 37: ANOVA: *Boergesenia forbesii* : DNA Damage (RAPD-Intensity of band)

* = Significant difference : p<0.05

ANOVA-INTENSITY-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	124.7400	162.9500	159.6400	153.2300	150.3700	0.000000
A	{0}		0.070127	*0.002285	*0.002608	*0.004313	*0.003682	*0.000177145004272461
B	{0.01}	0.070127		0.0585	0.064489	0.095621	0.061681	*0.000180959701538086
C	{0.1}	*0.002285	0.0585		0.796918	0.726478	0.75333	*0.000174045562744141
D	{1}	*0.002608	0.064489	0.796918		0.619321	0.747333	*0.000158190727233887
E	{10}	*0.004313	0.095621	0.726478	0.619321		0.823997	*0.000150978565216064
F	{100}	*0.003682	0.061681	0.75333	0.747333	0.823997		*0.00019603967666626
G	{500}	*0.000177	*0.000180	*0.000174	*0.000158	*0.000150	*0.00019603967666626	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	100.1600	103.1000	97.75000	95.68000	94.47000	0.000000
A	{0}		0.98724	0.946028	0.820835	0.898139	0.940157	*0.000150978565216064
B	{0.01}	0.98724		0.767415	0.967005	0.966621	0.975365	*0.000158309936523437
C	{0.1}	0.946028	0.767415		0.945328	0.937529	0.943889	*0.000174105167388916
D	{1}	0.820835	0.967005	0.945328		0.83493	0.93979	*0.000196099281311035
E	{10}	0.898139	0.966621	0.937529	0.83493		0.903056	*0.000180959701538086
F	{100}	0.940157	0.975365	0.943889	0.93979	0.903056		*0.000176727771759033
G	{500}	*0.000150	*0.000158	*0.000174	*0.000196	*0.000180	*0.000176727771759033	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	110.6200	116.9200	136.3100	139.7700	145.2000	0.000000
A	{0}		0.25255	0.174857	*0.005587	*0.004143	*0.001964	*0.000176668167114258
B	{0.01}	0.25255		0.49066	*0.030219	*0.025219	*0.012087	*0.000180900096893311
C	{0.1}	0.174857	0.49066		*0.046995	0.05485	*0.030280	*0.00019603967666626
D	{1}	*0.005587	*0.030219	*0.046995759010314	0.703328	0.589381	*0.000150978565216064	
E	{10}	*0.004143	*0.025219	0.05485	0.703328		0.551566	*0.000158190727233887
F	{100}	*0.001964	*0.012087	*0.030280	0.589381	0.551566		*0.000174045562744141
G	{500}	*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	87.18000	99.41000	110.8200	100.3400	74.04000	0.000000
A	{0}		0.367761	0.949644	0.483042	0.97105	0.057139	*0.000150978565216064
B	{0.01}	0.367761		0.203232	0.127985	0.498665	0.173505	*0.000180959701538086
C	{0.1}	0.949644	0.203232		0.609863	0.994427	*0.037729	*0.00019603967666626
D	{1}	0.483042	0.127985	0.609863		0.271876	*0.013096	*0.000174045562744141
E	{10}	0.97105	0.498665	0.994427	0.271876		0.077561	*0.000158190727233887
F	{100}	0.057139	0.173505	*0.037729	*0.013096	0.077561		*0.000176906585693359
G	{500}	*0.000150	*0.000180	*0.000196	*0.000174	*0.000158	*0.000176906585693359	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	81.75000	84.28000	83.67000	92.81000	116.5800	93.22000
A	{0}		0.506777	0.444878	0.524592	0.766577	0.128144	0.519139
B	{0.01}	0.506777		0.96713	0.854198	0.707345	0.051516	0.794109
C	{0.1}	0.444878	0.96713		0.953474	0.419362	*0.046937	0.665847
D	{1}	0.524592	0.854198	0.953474		0.654087	0.056618	0.788617
E	{10}	0.766577	0.707345	0.419362	0.654087		0.140664	0.968799
F	{100}	0.128144	0.051516	*0.046937	0.056618	0.140664		0.092258
G	{500}	0.519139	0.794109	0.665847	0.788617	0.968799	0.092258	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	88.30000	90.39000	104.7300	86.69000	86.28000	84.81000
A	{0}		0.549334	0.395993	0.673046	0.629013	0.72353	0.735179
B	{0.01}	0.549334		0.851757	0.464631	0.885539	0.981593	0.98843
C	{0.1}	0.395993	0.851757		0.414519	0.93957	0.981409	0.985172
D	{1}	0.673046	0.464631	0.414519		0.49558	0.563688	0.55921
E	{10}	0.629013	0.885539	0.93957	0.49558		0.970851	0.984043
F	{100}	0.72353	0.981593	0.981409	0.563688	0.970851		0.895442
G	{500}	0.735179	0.98843	0.985172	0.55921	0.984043	0.895442	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	131.5300	174.3900	125.3300	117.1800	114.5200	100.7400
A	{0}		0.44446	*0.007500	0.566427	0.734277	0.666046	0.965297
B	{0.01}	0.44446		*0.022211	0.715334	0.672198	0.73999	0.386185
C	{0.1}	*0.007500	*0.022211849689483	*0.027034	*0.018691	*0.020753	*0.00621998310089111	
D	{1}	0.566427	0.715334	*0.027034163475036	0.632231	0.795918	0.476195	
E	{10}	0.734277	0.672198	*0.018691	0.632231		0.87548	0.596528
F	{100}	0.666046	0.73999	*0.020753	0.795918	0.87548		0.421966
G	{500}	0.965297	0.386185	*0.006219	0.476195	0.596528	0.421966	

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	85.45000	91.92000	98.01000	131.2100	119.1900	101.3600
A	{0}		0.551819	0.740626	0.856872	0.052211	0.214371	0.901911
B	{0.01}	0.551819		0.559652	0.494792	*0.011545	0.066869	0.596401
C	{0.1}	0.740626	0.559652		0.582671	*0.026528	0.141718	0.819035
D	{1}	0.856872	0.494792	0.582671		0.05465	0.249993	0.948837
E	{10}	0.052211	*0.011545	*0.026528	0.05465		0.285564	*0.0385624766349792
F	{100}	0.214371	0.066869	0.141718	0.249993	0.285564		0.121862
G	{500}	0.901911	0.596401	0.819035	0.948837	*0.038562	0.121862	

APPENDIX 37: ANOVA: *Boergesenia forbesii* : DNA Damage (RAPD-Genomic Template Stability)

* = Significant difference : p<0.05

ANOVA-GTS-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	25.00000	23.57000	15.43000	15.00000	14.15000	12.81000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114		0.62612	*0.012880	*0.017044	*0.014700	*0.00855427980422974
C	{0.1}	*0.000180	0.62612		*0.013348	*0.025050	*0.024924	*0.0155842900276184
D	{1}	*0.000196	*0.012880	*0.013348579406738		0.883149	0.896978	0.798404
E	{10}	*0.000150	*0.017044	*0.025050	0.883149		0.771545	0.730913
F	{100}	*0.000158	*0.014700	*0.024924	0.896978	0.771545		0.647847
G	{500}	*0.000174	*0.008554	*0.015584	0.798404	0.730913	0.647847	

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	20.00000	13.33000	10.60000	10.04000	7.780000	7.780000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000176668167114		*0.001827	*0.000385	*0.000417	*0.000202	*0.000187575817108154
C	{0.1}	*0.000180	*0.001827239902343		0.135149	0.172106	*0.041101	*0.027779221534729
D	{1}	*0.000196	*0.000385	0.135149		0.749835	0.390188	0.262862
E	{10}	*0.000150	*0.000417	0.172106	0.749835		0.4114	0.210362
F	{100}	*0.000174	*0.000202	*0.041101	0.390188	0.4114		1
G	{500}	*0.000158	*0.000187	*0.027779	0.262862	0.210362	1	

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	14.29000	14.29000	14.06000	12.50000	4.760000	4.640000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180900096893		1	0.901916	0.602182	*0.000837	*0.00105977058410645
C	{0.1}	*0.000176	1		0.99142	0.764027	*0.001173	*0.00143605470657349
D	{1}	*0.000196	0.901916	0.99142		0.408584	*0.000604	*0.000912487506866455
E	{10}	*0.000150	0.602182	0.764027	0.408584		*0.000982	*0.00213634967803955
F	{100}	*0.000158	*0.000837	*0.001173	*0.000604	*0.000982820987701		0.948749
G	{500}	*0.000174	*0.001059	*0.001436	*0.000912	*0.002136	0.948749	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	17.19000	16.67000	15.63000	4.760000	3.130000	1.560000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114		0.800477	*0.724997	*0.000300	*0.000195	*0.000171065330505371
C	{0.1}	*0.000180	0.800477		*0.614393	*0.000271	*0.000232	*0.00017249584197998
D	{1}	*0.000196	*0.724997	*0.614393055438995		*0.000256	*0.000235	*0.000218093395233154
E	{10}	*0.000150	*0.000300	*0.000271	*0.000256955623626		0.432809	0.28381
F	{100}	*0.000158	*0.000195	*0.000232	*0.000235	0.432809		0.449567
G	{500}	*0.000174	*0.000171	*0.000172	*0.000218	0.28381	0.449567	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	27.15000	22.26000	20.15000	19.83000	16.78000	14.98000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114		0.172585	0.135038	0.184746	0.056494	*0.029161274433136
C	{0.1}	*0.000180	0.172585		0.545096	0.759191	0.404094	0.257439
D	{1}	*0.000196	0.135038	0.545096		0.926481	0.594376	0.452324
E	{10}	*0.000150	0.184746	0.759191	0.926481		0.385161	0.354933
F	{100}	*0.000158	0.056494	0.404094	0.594376	0.385161		0.605055
G	{500}	*0.000174	*0.029161	0.257439	0.452324	0.354933	0.605055	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	32.04000	26.21000	22.28000	21.94000	20.56000	8.190000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114		*0.002093	*0.000214	*0.000242	*0.000172	*0.000158190727233887
C	{0.1}	*0.000180	*0.002093553543090		*0.022633	*0.036587	*0.011665	*0.000150978565216064
D	{1}	*0.000196	*0.000214	*0.022633254528045		0.827757	0.516748	*0.000196397304534912
E	{10}	*0.000150	*0.000242	*0.036587	0.827757		0.383305	*0.000181019306182861
F	{100}	*0.000158	*0.000172	*0.011665	0.516748	0.383305		*0.000176906585693359
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000181	*0.000176906585693359	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	13.33000	13.13000	12.50000	10.00000	5.000000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114		0.903777	0.86708	0.216285	*0.001317	*0.000163257122039795
C	{0.1}	*0.000180	0.903777		0.703792	0.167298	*0.001118	*0.000155329704284668
D	{1}	*0.000196	0.86708	0.703792		0.145822	*0.001206	*0.000201225280761719
E	{10}	*0.000150	0.216285	0.167298	0.145822		*0.008292	*0.000238299369812012
F	{100}	*0.000158	*0.001317	*0.001118	*0.001206	*0.008292853832244		*0.00829285383224487
G	{500}	*0.000174	*0.000163	*0.000155	*0.000201	*0.000238	*0.00829285383224487	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	22.84000	18.92000	16.53000	13.53000	3.920000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114		0.121982	*0.047100	*0.007662	*0.000156	*0.000158429145812988
C	{0.1}	*0.000180	0.121982		0.332514	0.094716	*0.000275	*0.000156998634338379
D	{1}	*0.000196	*0.047100	0.332514		0.228254	*0.000460	*0.000219345092773437
E	{10}	*0.000150	*0.007662	0.094716	0.228254		*0.001367	*0.000316202640533447
F	{100}	*0.000158	*0.000156	*0.000275	*0.000460	*0.001367866992950		*0.121982216835022
G	{500}	*0.000174	*0.000158	*0.000156	*0.000219	*0.000316	*0.121982216835022	

APPENDIX 37: ANOVA: *Boergesenia forbesii* : DNA Damage (RAPD-Genomic Template Stability)

* = Significant difference : p<0.05

ANOVA-GTS-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	19.65000	16.13000	14.74000	14.17000	10.50000	0.000000
A	{0}		*0.000176(	*0.000180	*0.000196(	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114		0.510896	0.624196	0.723608	0.435273	*0.0206270813941956
C	{0.1}	*0.000180	0.510896		0.793855	0.9256	0.707211	0.05224
D	{1}	*0.000196(	0.624196	0.793855		0.914643	0.701464	0.05798
E	{10}	*0.000150	0.723608	0.9256	0.914643		0.493353	*0.0416486859321594
F	{100}	*0.000158	0.435273	0.707211	0.701464	0.493353		0.063866
G	{500}	*0.000174(	*0.020627(	0.05224	0.05798	*0.041648(	0.063866	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	11.11000	12.18000	11.11000	9.720000	5.210000	0.000000
A	{0}		*0.000196(	*0.000176(	*0.000180	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196039676666		0.934502	1	0.654927	0.164537	*0.0124580264091492
C	{0.1}	*0.000176(	0.934502		0.730451	0.849454	0.204805	*0.0133931636810303
D	{1}	*0.000180	1	0.730451		0.892249	0.256724	*0.0187110304832458
E	{10}	*0.000150	0.654927	0.849454	0.892249		0.160555	*0.0168429613113403
F	{100}	*0.000158	0.164537	0.204805	0.256724	0.160555		0.10901
G	{500}	*0.000174(	*0.012458(	*0.013393	*0.018711(	*0.016842	0.10901	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	17.85000	17.79000	11.54000	10.18000	5.210000	0.000000
A	{0}		*0.000176(	*0.000180	*0.000196(	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176668167114		0.982499	0.079469	0.053239	*0.002582	*0.00025862455368042
C	{0.1}	*0.000180	0.982499		*0.034671	*0.032402	*0.001809	*0.000224769115447998
D	{1}	*0.000196(	0.079469	*0.034671366214752		0.618523	0.078413	*0.00358504056930542
E	{10}	*0.000150	0.053239	*0.032402	0.618523		0.083902	*0.00517374277114868
F	{100}	*0.000158	*0.002582	*0.001809	0.078413	0.083902		0.071419
G	{500}	*0.000174(	*0.000258(	*0.000224	*0.003585(	*0.005173	0.071419	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	16.98000	17.29000	20.50000	12.22000	11.21000	0.000000
A	{0}		*0.000196(	*0.000180	*0.000176(	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196039676666		0.920815	0.500203	0.142045	0.17914	*0.000539541244506836
C	{0.1}	*0.000180	0.920815		0.311782	0.255427	0.238548	*0.000602960586547852
D	{1}	*0.000176(	0.500203	0.311782		0.071804	0.05766	*0.000255584716796875
E	{10}	*0.000150	0.142045	0.255427	0.071804		0.746221	*0.00366926193237305
F	{100}	*0.000158	0.17914	0.238548	0.05766	0.746221		*0.00269687175750732
G	{500}	*0.000174(	*0.000539	*0.000602	*0.000255	*0.003669	*0.00269687175750732	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	11.28000	17.16000	19.94000	9.730000	5.890000	3.680000
A	{0}		*0.000196(	*0.000180	*0.000176(	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000196039676666		0.252581	0.219476	0.757802	0.533111	0.440127
C	{0.1}	*0.000180	0.252581		0.581598	0.317089	0.148258	0.098169
D	{1}	*0.000176(	0.219476	0.581598		0.209568	0.08023	*0.0482534766197205
E	{10}	*0.000150	0.757802	0.317089	0.209568		0.448825	0.45694
F	{100}	*0.000158	0.533111	0.148258	0.08023	0.448825		0.660712
G	{500}	*0.000174(	0.440127	0.098169	*0.048253	0.45694	0.660712	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	14.65000	17.03000	11.87000	10.80000	9.800000	6.800000
A	{0}		*0.000180	*0.000176(	*0.000196(	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180900096893		0.188748	0.12892	0.099625	0.059024	*0.00353658199310303
C	{0.1}	*0.000176(	0.188748		*0.024585	*0.013278	*0.006789	*0.000538170337677002
D	{1}	*0.000196(	0.12892	*0.024585306644439		0.544521	0.471419	*0.0467615127563477
E	{10}	*0.000150	0.099625	*0.013278	0.544521		0.570829	0.085469
F	{100}	*0.000158	0.059024	*0.006789	0.471419	0.570829		0.103546
G	{500}	*0.000174(	*0.003536	*0.000538	*0.046761	0.085469	0.103546	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	17.11000	14.56000	8.270000	9.100000	0.000000	0.000000
A	{0}		*0.000176(	*0.000180	*0.000150	*0.000196(	*0.000174	*0.000158190727233887
B	{0.01	*0.000176668167114		*0.017145	*0.000196	*0.000181	*0.000158	*0.000150978565216064
C	{0.1}	*0.000180	*0.017145335674285		*0.000199	*0.000212	*0.000150	*0.00019603967666626
D	{1}	*0.000150	*0.000196	*0.000199198722833		0.393143	*0.000181	*0.000176846981048584
E	{10}	*0.000196(	*0.000181	*0.000212	0.393143		*0.000196(	*0.000180959701538086
F	{100}	*0.000174(	*0.000158	*0.000150	*0.000181	*0.000196099281311(		1
G	{500}	*0.000158	*0.000150	*0.000196(	*0.000176	*0.000180	1	

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	12.82000	9.420000	8.730000	6.070000	0.000000	0.000000
A	{0}		*0.000176(	*0.000180	*0.000196(	*0.000150	*0.000174	*0.000158190727233887
B	{0.01	*0.000176668167114		*0.001994(	*0.001234	*0.000202	*0.000158	*0.000150978565216064
C	{0.1}	*0.000180	*0.001994037390960		0.449982	*0.005568	*0.000150	*0.00019603967666626
D	{1}	*0.000196(	*0.001234	0.449982		*0.009765	*0.000196(	*0.000180959701538086
E	{10}	*0.000150	*0.000202	*0.005568	*0.009765863418579		*0.000194	*0.000181078910827637
F	{100}	*0.000174(	*0.000158	*0.000150	*0.000196(	*0.000194132328033		1
G	{500}	*0.000158	*0.000150	*0.000196(	*0.000180	*0.000181(	1	

APPENDIX 38: ANOVA: *Boergesenia forbesii* : DNA Damage (AP-Site)

* = Significant difference : p<0.05

ANOVA-AP-SITE-BOE

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		132.4890	188.2850	182.1210	183.1930	189.7920	175.3530	174.5160
A	{0}		*0.0001581	*0.000196(	*0.0001509	*0.000174(	*0.000180	*0.000176
B	{0.01	*0.00015819072723382		0.117005	0.098614	0.608718	*0.002635	*0.002361
C	{0.1}	*0.000196(	0.117005		0.715122	0.077248	*0.033923(	*0.047747
D	{1}	*0.000150	0.098614	0.715122		0.089935	*0.040997	*0.040945
E	{10}	*0.000174(	0.608718	0.077248	0.089935		*0.001587	*0.001345
F	{100}	*0.000180	*0.002635	*0.033923(	*0.040997	*0.0015875697135925		0.775482
G	{500}	*0.000176(	*0.002361	*0.047747(	*0.040945	*0.001345	0.775482	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		158.9210	170.0600	176.9610	180.1940	184.9850	177.5310	174.1310
A	{0}		*0.0085863	*0.0012062	*0.0006036	*0.0002273	*0.0013504	*0.002605
B	{0.01	*0.0085863471031189		0.175949	0.089886	*0.011115(	0.215461	0.281839
C	{0.1}	*0.0012062	0.175949		0.655719	0.169261	0.877787	0.449495
D	{1}	*0.0006036	0.089886	0.655719		0.208893	0.47618	0.375751
E	{10}	*0.0002273	*0.011115(	0.169261	0.208893		0.136919	0.063376
F	{100}	*0.0013504	0.215461	0.877787	0.47618	0.136919		0.628005
G	{500}	*0.002605(	0.281839	0.449496	0.375752	0.063376	0.628005	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		132.4890	138.5030	160.7810	168.2350	170.3280	182.0700	149.9430
A	{0}		0.1692	*0.000225(	*0.0001527	*0.000159(	*0.000174(	*0.002488
B	{0.01	0.1692		*0.000423(	*0.0002111	*0.000168	*0.000158	*0.015541
C	{0.1}	*0.000225(	*0.00042396783828737		0.094018	0.088664	*0.000930	*0.020593
D	{1}	*0.0001527	*0.0002111	0.094018		0.621785	*0.012859	*0.001737
E	{10}	*0.000159(	*0.000168	0.088664	0.621785		*0.013491(	*0.001297
F	{100}	*0.000174(	*0.000158	*0.000930	*0.012859	*0.013491630554199		*0.000159
G	{500}	*0.002488	*0.015541(	*0.020593	*0.001737	*0.001297(	*0.00015980	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		158.9210	115.0850	113.5280	125.2030	192.1370	139.4410	113.5780
A	{0}		*0.000196(	*0.0001581	*0.000180	*0.000176	*0.000268	*0.000150
B	{0.01	*0.00019603967666622		0.905933	*0.015418	*0.000150	*0.000200	0.68711
C	{0.1}	*0.0001581	0.905933		*0.029777(	*0.000174(	*0.000188	0.989418
D	{1}	*0.000180	*0.015418	*0.029777050018310		*0.000196(	*0.001788	*0.017524
E	{10}	*0.000176	*0.000150	*0.000174(	*0.000196039676666		*0.000180	*0.000158
F	{100}	*0.000268	*0.000200	*0.000188	*0.001788	*0.000180900096893		*0.000214
G	{500}	*0.000150	0.68711	0.989419	*0.017524	*0.000158	*0.000214	

APPENDIX 39: ANOVA: *Boergesenia forbesii* : Superoxide dismutase (SOD) activity

* = Significant difference : p<0.05

ANOVA-SOD-BOE

Newman-Keuls test; CD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		61.39300	75.89100	65.53500	62.93600	61.02300	105.3850	81.76900
A	{0}		*0.005282	0.486729	0.668474	0.918038	*0.000158	*0.000513
B	{0.01	*0.005282		*0.010975	*0.006737	*0.006563	*0.000181	0.117827
C	{0.1}	0.486729	*0.010975		0.473393	0.589802	*0.000196	*0.001242
D	{1}	0.668474	*0.006737	0.473393		0.851938	*0.000150	*0.000697
E	{10}	0.918038	*0.006563	0.589802	0.851938		*0.000174	*0.000578
F	{100}	*0.000158	*0.000181	*0.000196	*0.000150	*0.000174		*0.000182
G	{500}	*0.000513	0.117827	*0.001242	*0.000697	*0.000578	*0.000182	

Newman-Keuls test; CU_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		61.39300	47.53700	51.20200	52.81700	113.4050	147.5950	158.7090
A	{0}		*0.000196	*0.000198	*0.000225	*0.000176	*0.000180	*0.000196
B	{0.01	*0.000196		*0.030570	*0.009978	*0.000150	*0.000158	*0.000174
C	{0.1}	*0.000198	*0.030570		0.306847	*0.000196	*0.000150	*0.000158
D	{1}	*0.000225	*0.009978	0.306847		*0.000180	*0.000196	*0.000150
E	{10}	*0.000176	*0.000150	*0.000196	*0.000180		*0.000176	*0.000180
F	{100}	*0.000180	*0.000158	*0.000150	*0.000196	*0.000176		*0.000178
G	{500}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	*0.000178	

Newman-Keuls test; ZN_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		61.39300	102.6400	81.14800	68.45500	46.08200	34.64100	9.833000
A	{0}		*0.000196	*0.000180	*0.000328	*0.000176	*0.000180	*0.000196
B	{0.01	*0.000196		*0.000176	*0.000180	*0.000150	*0.000158	*0.000174
C	{0.1}	*0.000180	*0.000176		*0.000176	*0.000196	*0.000150	*0.000158
D	{1}	*0.000328	*0.000180	*0.000176		*0.000180	*0.000196	*0.000150
E	{10}	*0.000176	*0.000150	*0.000196	*0.000180		*0.000176	*0.000180
F	{100}	*0.000180	*0.000158	*0.000150	*0.000196	*0.000176		*0.000176
G	{500}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; AD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		61.39300	110.5160	119.0730	113.4460	112.0180	74.08700	8.606900
A	{0}		*0.000180	*0.000158	*0.000150	*0.000196	*0.000467	*0.000176
B	{0.01	*0.000180		0.029377	0.533229	0.583945	*0.000176	*0.000196
C	{0.1}	*0.000158	0.029377		0.054364	*0.048597	*0.000150	*0.000174
D	{1}	*0.000150	0.533229	0.054364		0.602419	*0.000196	*0.000158
E	{10}	*0.000196	0.583945	0.048598	0.602419		*0.000180	*0.000150
F	{100}	*0.000467	*0.000176	*0.000150	*0.000196	*0.000180		*0.000180
G	{500}	*0.000176	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	

Newman-Keuls test; CD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		70.42300	60.95400	90.74500	96.05900	91.26200	88.18400	52.72600
A	{0}		*0.000181	*0.000180	*0.000150	*0.000196	*0.000176	*0.000180
B	{0.01	*0.000181		*0.000196	*0.000158	*0.000150	*0.000180	*0.000205
C	{0.1}	*0.000180	*0.000196		*0.005221	0.716666	0.087903	*0.000150
D	{1}	*0.000150	*0.000158	*0.005221		*0.004155	*0.000488	*0.000174
E	{10}	*0.000196	*0.000150	0.716666	*0.004155		0.104908	*0.000158
F	{100}	*0.000176	*0.000180	0.087903	*0.000488	0.104908		*0.000196
G	{500}	*0.000180	*0.000205	*0.000150	*0.000174	*0.000158	*0.000196	

Newman-Keuls test; CU_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		70.42300	73.30000	81.16800	81.24200	95.71400	95.35800	99.25400
A	{0}		0.222873	*0.000958	*0.001568	*0.000158	*0.000150	*0.000174
B	{0.01	0.222873		*0.003763	*0.008995	*0.000151	*0.000196	*0.000158
C	{0.1}	*0.000958	*0.003763		0.974419	*0.000256	*0.000220	*0.000156
D	{1}	*0.001568	*0.008995	0.974419		*0.000211	*0.000190	*0.000199
E	{10}	*0.000158	*0.000151	*0.000256	*0.000211		0.876925	0.138902
F	{100}	*0.000150	*0.000196	*0.000220	*0.000190	0.876925		0.229866
G	{500}	*0.000174	*0.000158	*0.000156	*0.000199	0.138903	0.229866	

Newman-Keuls test; ZN_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		70.42300	59.29000	65.40400	77.23900	98.57800	102.9620	100.6850
A	{0}		*0.000289	*0.020435	*0.003321	*0.000180	*0.000150	*0.000196
B	{0.01	*0.000289		*0.006729	*0.000196	*0.000150	*0.000174	*0.000158
C	{0.1}	*0.020435	*0.006729		*0.000237	*0.000196	*0.000158	*0.000150
D	{1}	*0.003321	*0.000196	*0.000237		*0.000176	*0.000196	*0.000180
E	{10}	*0.000180	*0.000150	*0.000196	*0.000176		0.09118	0.290547
F	{100}	*0.000150	*0.000174	*0.000158	*0.000196	0.09118		0.254963
G	{500}	*0.000196	*0.000158	*0.000150	*0.000180	0.290547	0.254963	

Newman-Keuls test; AD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		70.42300	89.26800	75.50100	71.90900	69.89600	57.17900	46.54600
A	{0}		*0.000203	0.142065	0.562479	0.836448	*0.000465	*0.000196
B	{0.01	*0.000203		*0.000241	*0.000191	*0.000167	*0.000158	*0.000174
C	{0.1}	0.142065	*0.000241		0.17351	0.160764	*0.000177	*0.000158
D	{1}	0.562479	*0.000191	0.17351		0.706833	*0.000383	*0.000151
E	{10}	0.836448	*0.000167	0.160764	0.706833		*0.000324	*0.000180
F	{100}	*0.000465	*0.000158	*0.000177	*0.000383	*0.000324		*0.000954
G	{500}	*0.000196	*0.000174	*0.000158	*0.000151	*0.000180	*0.000954	

APPENDIX 39: ANOVA: *Boergesenia forbesii* : Superoxide dismutase (SOD) activity

* = Significant difference : p<0.05

ANOVA-SOD-BOE

Newman-Keuls test; BD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		61.39300	149.7740	128.5420	119.9200	114.9270	116.3850	109.5430
A	{0}		*0.000174(	*0.000158:	*0.000150!	*0.000180!	*0.000196(	*0.000176
B	{0.01	*0.000174045562744	*0.000188(	*0.000181(	*0.000150!	*0.000196(	*0.000158	
C	{0.1}	*0.000158:	*0.000188052654266	*0.021744:	*0.005579-	*0.007121!	*0.000570	
D	{1}	*0.000150!	*0.000181(	*0.021744191646575!	0.322103	0.307266	*0.034336	
E	{10}	*0.000180!	*0.000150!	*0.005579-	0.322103		0.668814	0.128928
F	{100}	*0.000196(	*0.000196(	*0.007121!	0.307266	0.668814		0.136612
G	{500}	*0.000176(	*0.000158:	*0.000570:	*0.034336!	0.128928	0.136612	

Newman-Keuls test; BD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		70.42300	92.85000	94.49500	93.43800	98.72900	88.59200	83.36500
A	{0}		*0.000196(	*0.000158:	*0.000150!	*0.000174(	*0.000180!	*0.000177
B	{0.01	*0.000196039676666-	0.623187	0.741179	*0.021148(	*0.028665(	*0.000395	
C	{0.1}	*0.000158:	0.623187		0.554374	*0.029428-	*0.020601(	*0.000271
D	{1}	*0.000150!	0.741179	0.554374		*0.022913:	*0.037138:	*0.000429
E	{10}	*0.000174(	*0.021148(	*0.029428-	*0.022913157939910!		*0.000493!	*0.000159
F	{100}	*0.000180!	*0.028665(	*0.020601(	*0.037138:	*0.000493526458740-		*0.009776
G	{500}	*0.000177!	*0.000395-	*0.000271:	*0.000429!	*0.000159;	*0.0097767	

Newman-Keuls test; MC_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		61.39300	178.6880	140.1050	123.9340	130.4340	87.05100	30.25100
A	{0}		*0.000158:	*0.000150!	*0.000180!	*0.000196(	*0.000176(	*0.000176
B	{0.01	*0.000158190727233!	*0.000176(	*0.000196(	*0.000180!	*0.000150!	*0.000174	
C	{0.1}	*0.000150!	*0.000176668167114;	*0.000192!	*0.001184;	*0.000196(	*0.000158	
D	{1}	*0.000180!	*0.000196(	*0.000192999839782;	*0.015277:	*0.000176(	*0.000196	
E	{10}	*0.000196(	*0.000180!	*0.001184;	*0.015277385711669!	*0.000180!	*0.000150	
F	{100}	*0.000176(	*0.000150!	*0.000196(	*0.000176(	*0.000180900096893:	*0.000180	
G	{500}	*0.000176(	*0.000174(	*0.000158:	*0.000196(	*0.000150!	*0.000180	

Newman-Keuls test; MC_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		70.42300	82.73800	90.45400	92.21700	93.90700	68.42500	57.35500
A	{0}		*0.008064;	*0.000642!	*0.000587!	*0.000437!	0.623417	*0.014168
B	{0.01	*0.008064389228820!	0.072962	0.076774	0.059939	*0.007783:	*0.000264	
C	{0.1}	*0.000642!	0.072962		0.66453	0.668435	*0.000548(	*0.000153
D	{1}	*0.000587!	0.076774	0.66453		0.677536	*0.000400!	*0.000159
E	{10}	*0.000437!	0.059939	0.668435	0.677536		*0.000321!	*0.000175
F	{100}	0.623417	*0.007783:	*0.000548(	*0.000400!	*0.000321865081787;	*0.014819	
G	{500}	*0.014168;	*0.000264;	*0.000153(	*0.000159(	*0.000175(	*0.014819	

Newman-Keuls test; DD_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		61.39300	133.6670	122.7610	101.9520	91.04000	62.66000	45.61500
A	{0}		*0.000158:	*0.000150!	*0.000196(	*0.000181!	0.735612	*0.000886
B	{0.01	*0.000158190727233!	*0.010361;	*0.000181(	*0.000196(	*0.000150!	*0.000174	
C	{0.1}	*0.000150!	*0.010361254215240!	*0.000223-	*0.000181;	*0.000196(	*0.000158	
D	{1}	*0.000196(	*0.000181;	*0.000223457813262!	*0.010328-	*0.000180!	*0.000150	
E	{10}	*0.000181!	*0.000196(	*0.000181;	*0.010328471660614	*0.000177;	*0.000196	
F	{100}	0.735612	*0.000150!	*0.000196(	*0.000180!	*0.000177323818206;	*0.001177	
G	{500}	*0.000886;	*0.000174(	*0.000158:	*0.000150!	*0.000196(	*0.001177	

Newman-Keuls test; DD_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		70.42300	89.20800	87.81100	87.52900	89.68500	87.52200	63.41400
A	{0}		*0.000150!	*0.000196(	*0.000180!	*0.000158:	*0.000176;	*0.001546
B	{0.01	*0.000150978565216(	0.442468	0.618818	0.791234	0.776758	*0.000158	
C	{0.1}	*0.000196(	0.442468		0.875586	0.552777	0.985458	*0.000150
D	{1}	*0.000180!	0.618818	0.875586		0.624867	0.99701	*0.000196
E	{10}	*0.000158:	0.791234	0.552777	0.624867		0.738214	*0.000174
F	{100}	*0.000176;	0.776758	0.985458	0.99701	0.738214		*0.000180
G	{500}	*0.001546!	*0.000158:	*0.000150!	*0.000196(	*0.000174(	*0.000180	

Newman-Keuls test; MA_B_D4 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		61.39300	97.97700	103.6290	118.5730	122.6630	113.1570	64.13100
A	{0}		*0.000180!	*0.000196(	*0.000158:	*0.000174(	*0.000150!	0.260152
B	{0.01	*0.000180900096893:	*0.029661;	*0.000196(	*0.000150!	*0.000206!	*0.000176	
C	{0.1}	*0.000196(	*0.029661118984222-	*0.000212!	*0.000198;	*0.001251-	*0.000180	
D	{1}	*0.000158:	*0.000196(	*0.000212311744689!	0.101495	*0.035949!	*0.000150	
E	{10}	*0.000174(	*0.000150!	*0.000198;	0.101495		*0.003168!	*0.000158
F	{100}	*0.000150!	*0.000206!	*0.001251-	*0.035949!	*0.003168344497680(		*0.000196
G	{500}	0.260152	*0.000176(	*0.000180!	*0.000150!	*0.000158:	*0.000196	

Newman-Keuls test; MA_B_D10 (anova-boe.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		70.42300	76.15900	64.09500	63.47800	66.70900	63.50800	51.62800
A	{0}		0.082466	0.133636	0.212937	0.245803	0.156222	*0.000429
B	{0.01	0.082466		*0.007322(	*0.010471;	*0.020844!	*0.007737!	*0.000184
C	{0.1}	0.133636	*0.007322072982788(		0.978027	0.408301	0.850997	*0.005733
D	{1}	0.212937	*0.010471;	0.978027		0.721677	0.992433	*0.001858
E	{10}	0.245803	*0.020844!	0.408301	0.721677		0.562489	*0.001873
F	{100}	0.156222	*0.007737!	0.850997	0.992433	0.562489		*0.004602
G	{500}	*0.000429;	*0.000184(	*0.005733:	*0.001858;	*0.001873!	*0.004602	

APPENDIX 40: ANOVA: *Ventricaria ventricosa* : Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .9120000	{0.01 mg/L} .9380000	{0.1 mg/L} .9160000	{1 mg/L} .8340000	{10 mg/L} .6600000	{100 mg/L} .3260000	{500 mg/L} 0.000000
A	{0}		0.482932	0.858494	*0.003384(	*0.000180!	*0.000196(	*0.000150
B	{0.01}	0.482932		0.334561	*0.001768!	*0.000150!	*0.000158:	*0.000174
C	{0.1}	0.858494	0.334561		*0.006101!	*0.000196(	*0.000150!	*0.000158
D	{1}	*0.003384(	*0.001768!	*0.006101906299591(		*0.000177:	*0.000180!	*0.000196
E	{10}	*0.000180!	*0.000150!	*0.000196(	*0.000177145004272-		*0.000176(	*0.000180
F	{100}	*0.000196(	*0.000158:	*0.000150!	*0.000180!	*0.000176681671142		*0.000176
G	{500}	*0.000150!	*0.000174(	*0.000158:	*0.000196(	*0.000180!	*0.000176	

Newman-Keuls test; CO_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .9120000	{0.01 mg/L} .9690000	{0.1 mg/L} .9470000	{1 mg/L} .8900000	{10 mg/L} .7770000	{100 mg/L} .3240000	{500 mg/L} .2330000
A	{0}		*0.042435-	0.118866	0.313981	*0.000211!	*0.000196(	*0.000150
B	{0.01}	*0.042435228824615!		0.313981	*0.010326(	*0.000151!	*0.000158:	*0.000174
C	{0.1}	0.118866	0.313981		*0.042435-	*0.000198(	*0.000150!	*0.000158
D	{1}	0.313981	*0.010326(	*0.042435228824615!		*0.000260!	*0.000180!	*0.000196
E	{10}	*0.000211!	*0.000151!	*0.000198(	*0.000260531902313-		*0.000176(	*0.000180
F	{100}	*0.000196(	*0.000158:	*0.000150!	*0.000180!	*0.000176681671142		*0.000846
G	{500}	*0.000150!	*0.000174(	*0.000158:	*0.000196(	*0.000180!	*0.000846	

Newman-Keuls test; CR_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .9120000	{0.01 mg/L} 1.023000	{0.1 mg/L} 1.044000	{1 mg/L} 1.077000	{10 mg/L} .9850000	{100 mg/L} .8490000	{500 mg/L} 0.000000
A	{0}		*0.000180!	*0.000196(	*0.000150!	*0.000184!	*0.000225!	*0.000180
B	{0.01}	*0.000180900096893-		0.081507	*0.000868:	*0.004483(	*0.000196(	*0.000150
C	{0.1}	*0.000196(	0.081507		*0.010683!	*0.000472:	*0.000150!	*0.000158
D	{1}	*0.000150!	*0.000868:	*0.010683536529541		*0.000197!	*0.000158:	*0.000174
E	{10}	*0.000184!	*0.004483(	*0.000472:	*0.000197887420654-		*0.000180!	*0.000196
F	{100}	*0.000225!	*0.000196(	*0.000150!	*0.000158:	*0.000180900096893-		*0.000176
G	{500}	*0.000180!	*0.000150!	*0.000158:	*0.000174(	*0.000196(	*0.000176	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .9120000	{0.01 mg/L} .8120000	{0.1 mg/L} .8540000	{1 mg/L} .8940000	{10 mg/L} .5940000	{100 mg/L} .1840000	{500 mg/L} 0.000000
A	{0}		*0.000197-	*0.000845!	0.154952	*0.000150!	*0.000158:	*0.000174
B	{0.01}	*0.000197410583496(		*0.003623(	*0.000193!	*0.000176(	*0.000180!	*0.000196
C	{0.1}	*0.000845!	*0.003623604774475-		*0.004990-	*0.000180!	*0.000196(	*0.000150
D	{1}	0.154952	*0.000193!	*0.004990220069885-		*0.000196(	*0.000150!	*0.000158
E	{10}	*0.000150!	*0.000176(	*0.000180!	*0.000196039676666-		*0.000176(	*0.000180
F	{100}	*0.000158:	*0.000180!	*0.000196(	*0.000150!	*0.000176681671142		*0.000176
G	{500}	*0.000174(	*0.000196(	*0.000150!	*0.000158:	*0.000180!	*0.000176	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .3710000	{0.01 mg/L} .3640000	{0.1 mg/L} .3650000	{1 mg/L} .3560000	{10 mg/L} .3230000	{100 mg/L} .2860000	{500 mg/L} 0.000000
A	{0}		0.832677	0.626667	0.610943	*0.010171-	*0.000206:	*0.000174
B	{0.01}	0.832677		0.935194	0.518015	*0.011370!	*0.000254:	*0.000150
C	{0.1}	0.626667	0.935194		0.740781	*0.017111-	*0.000240:	*0.000158
D	{1}	0.610943	0.518015	0.740781		*0.016216:	*0.000289:	*0.000196
E	{10}	*0.010171-	*0.011370!	*0.017111-	*0.016216337680816-		*0.008509:	*0.000180
F	{100}	*0.000206:	*0.000254:	*0.000240:	*0.000289:	*0.008509159088134-		*0.000176
G	{500}	*0.000174(	*0.000150!	*0.000158:	*0.000196(	*0.000180!	*0.000176	

Newman-Keuls test; CO_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .3710000	{0.01 mg/L} .4220000	{0.1 mg/L} .4130000	{1 mg/L} .4080000	{10 mg/L} .3850000	{100 mg/L} .2100000	{500 mg/L} 0.000000
A	{0}		*0.021404!	*0.046470:	0.052082	0.342678	*0.000176(	*0.000180
B	{0.01}	*0.021404504776001		0.537982	0.599451	0.087453	*0.000158:	*0.000174
C	{0.1}	*0.046470:	0.537982		0.731034	0.157724	*0.000150!	*0.000158
D	{1}	0.052082	0.599451	0.731034		0.128966	*0.000196(	*0.0001509
E	{10}	0.342678	0.087453	0.157724	0.128966		*0.000180!	*0.000196
F	{100}	*0.000176(	*0.000158:	*0.000150!	*0.000196(	*0.000180900096893-		*0.000176
G	{500}	*0.000180!	*0.000174(	*0.000158:	*0.000150!	*0.000196(	*0.000176	

Newman-Keuls test; CR_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .3710000	{0.01 mg/L} .3920000	{0.1 mg/L} .4030000	{1 mg/L} .4040000	{10 mg/L} .3860000	{100 mg/L} .1610000	{500 mg/L} 0.000000
A	{0}		0.259968	0.102031	0.126764	0.259437	*0.000176(	*0.000180
B	{0.01}	0.259968		0.403286	0.624691	0.645535	*0.000196(	*0.000150
C	{0.1}	0.102031	0.403286		0.938735	0.401391	*0.000150!	*0.000158
D	{1}	0.126764	0.624691	0.938735		0.513403	*0.000158:	*0.000174
E	{10}	0.259437	0.645535	0.401391	0.513403		*0.000180!	*0.000196
F	{100}	*0.000176(	*0.000196(	*0.000150!	*0.000158:	*0.000180900096893-		*0.000176
G	{500}	*0.000180!	*0.000150!	*0.000158:	*0.000174(	*0.000196(	*0.000176	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .3710000	{0.01 mg/L} .3390000	{0.1 mg/L} .4040000	{1 mg/L} .3770000	{10 mg/L} .3730000	{100 mg/L} .1050000	{500 mg/L} 0.000000
A	{0}		*0.013400:	*0.048571!	0.857396	0.862038	*0.000180!	*0.000196
B	{0.01}	*0.013400316238403-		*0.000527:	*0.021315-	*0.023867(	*0.000176(	*0.000180
C	{0.1}	*0.048571!	*0.0005271434783935		*0.031492!	*0.039434-	*0.000158:	*0.000174
D	{1}	0.857396	*0.021315-	*0.031492173671722-		0.728501	*0.000150!	*0.000158
E	{10}	0.862038	*0.023867(	*0.039434-	0.728501		*0.000196(	*0.000150
F	{100}	*0.000180!	*0.000176(	*0.000158:	*0.000150!	*0.000196039676666-		*0.000176
G	{500}	*0.000196(	*0.000180!	*0.000174(	*0.000158:	*0.000150!	*0.000176	

APPENDIX 40: ANOVA: *Ventricaria ventricosa* : Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-VEN

Newman-Keuls test; FE_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.9120000	1.068000	1.019000	1.019000	.9990000	.6060000	0.000000
A	{0}		*0.000150	*0.000180	*0.000196	*0.000177	*0.000176	*0.000180
B	{0.01	*0.000150	*0.002662	*0.001081	*0.000386	*0.000158	*0.000174	
C	{0.1}	*0.000180	*0.002662		1	0.110886	*0.000196	*0.000150
D	{1}	*0.000196	*0.001081	1		0.238887	*0.000150	*0.000158
E	{10}	*0.000177	*0.000386	0.110886	0.238887		*0.000180	*0.000196
F	{100}	*0.000176	*0.000158	*0.000196	*0.000150	*0.000180		*0.000176
G	{500}	*0.000180	*0.000174	*0.000150	*0.000158	*0.000196	*0.000176	

Newman-Keuls test; MN_V_C4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.9120000	.9160000	.8850000	.8350000	.7410000	.2240000	0.000000
A	{0}		0.804569	0.110713	*0.000830	*0.000196	*0.000150	*0.000158
B	{0.01	0.804569		0.160125	*0.000962	*0.000150	*0.000158	*0.000174
C	{0.1}	0.110713	0.160125		*0.007184	*0.000180	*0.000196	*0.000150
D	{1}	*0.000830	*0.000962	*0.007184		*0.000204	*0.000180	*0.000196
E	{10}	*0.000196	*0.000150	*0.000180	*0.000204		*0.000176	*0.000180
F	{100}	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176		*0.000176
G	{500}	*0.000158	*0.000174	*0.000150	*0.000196	*0.000180	*0.000176	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.9120000	.8380000	.8900000	.9200000	.9190000	.3550000	0.000000
A	{0}		*0.000186	*0.048080	0.716241	0.501906	*0.000196	*0.000150
B	{0.01	*0.000186	*0.000312	*0.000155	*0.000199	*0.000176	*0.000180	
C	{0.1}	*0.048080	*0.000312		*0.045807	*0.032028	*0.000180	*0.000196
D	{1}	0.716241	*0.000155	*0.045807		0.923042	*0.000158	*0.000174
E	{10}	0.501906	*0.000199	*0.032028	0.923042		*0.000150	*0.000158
F	{100}	*0.000196	*0.000176	*0.000180	*0.000158	*0.000150		*0.000176
G	{500}	*0.000150	*0.000180	*0.000196	*0.000174	*0.000158	*0.000176	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.9120000	.7220000	.9260000	.8160000	.7160000	.0080000	0.000000
A	{0}		*0.000180	0.42815	*0.000229	*0.000196	*0.000150	*0.000158
B	{0.01	*0.000180	*0.000196	*0.000243	0.731786	*0.000180	*0.000196	
C	{0.1}	0.42815	*0.000196	*0.000211	*0.000150	*0.000158	*0.000174	
D	{1}	*0.000229	*0.000243	*0.000211		*0.000284	*0.000196	*0.000150
E	{10}	*0.000196	0.731786	*0.000150	*0.000284		*0.000176	*0.000180
F	{100}	*0.000150	*0.000180	*0.000158	*0.000196	*0.000176		0.648193
G	{500}	*0.000158	*0.000196	*0.000174	*0.000150	*0.000180	0.648193	

Newman-Keuls test; FE_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.3710000	.4210000	.4140000	.3970000	.3390000	.1850000	0.000000
A	{0}		*0.002962	*0.005213	*0.037251	*0.013400	*0.000180	*0.000196
B	{0.01	*0.002962	0.545307	0.120387	*0.000179	*0.000158	*0.000174	
C	{0.1}	*0.005213	0.545307		0.154448	*0.000237	*0.000150	*0.000158
D	{1}	*0.037251	0.120387	0.154448		*0.000560	*0.000196	*0.000150
E	{10}	*0.013400	*0.000179	*0.000237	*0.000560		*0.000176	*0.000180
F	{100}	*0.000180	*0.000158	*0.000150	*0.000196	*0.000176		*0.000176
G	{500}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; MN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.3710000	.4210000	.4190000	.4050000	.3770000	.2020000	0.000000
A	{0}		*0.010085	*0.008991	*0.042202	0.640043	*0.000176	*0.000180
B	{0.01	*0.010085	0.875753	0.431434	*0.016357	*0.000158	*0.000174	
C	{0.1}	*0.008991	0.875753		0.283428	*0.012580	*0.000150	*0.000158
D	{1}	*0.042202	0.431434	0.283428		*0.042635	*0.000196	*0.000150
E	{10}	0.640043	*0.016357	*0.012580	*0.042635		*0.000180	*0.000196
F	{100}	*0.000176	*0.000158	*0.000150	*0.000196	*0.000180		*0.000176
G	{500}	*0.000180	*0.000174	*0.000158	*0.000150	*0.000196	*0.000176	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.3710000	.3720000	.3600000	.3410000	.2950000	.0560000	0.000000
A	{0}		0.935201	0.377361	0.063579	*0.000275	*0.000150	*0.000158
B	{0.01	0.935201		0.591937	0.091567	*0.000270	*0.000158	*0.000174
C	{0.1}	0.377361	0.591937		0.137641	*0.000415	*0.000196	*0.000150
D	{1}	0.063579	0.091567	0.137641		*0.002055	*0.000180	*0.000196
E	{10}	*0.000275	*0.000270	*0.000415	*0.002055823802948		*0.000176	*0.000180
F	{100}	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176		*0.000528
G	{500}	*0.000158	*0.000174	*0.000150	*0.000196	*0.000180	*0.000528	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.3710000	.3310000	.4000000	.3950000	.3510000	.1510000	0.000000
A	{0}		*0.007507	*0.049651	*0.047804	0.092175	*0.000196	*0.000150
B	{0.01	*0.007507	*0.000305	*0.000417	0.092175	*0.000176	*0.000180	
C	{0.1}	*0.049651	*0.000305		0.658234	*0.002953	*0.000158	*0.000174
D	{1}	*0.047804	*0.000417	0.658234		*0.003787	*0.000150	*0.000158
E	{10}	0.092175	0.092175	*0.002953	*0.003787		*0.000180	*0.000196
F	{100}	*0.000196	*0.000176	*0.000158	*0.000150	*0.000180		*0.000176
G	{500}	*0.000150	*0.000180	*0.000174	*0.000158	*0.000196	*0.000176	

APPENDIX 40: ANOVA: *Ventricaria ventricosa* : Growth (Growth rate)

* = Significant difference : p<0.05

ANOVA-GR-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .9120000	{0.01 mg/L .4450000	{0.1 mg/L} .3710000	{1 mg/L} .3410000	{10 mg/L} .0800000	{100 mg/L} 0.000000	{500 mg/L} 0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000174	*0.000158
B	{0.01	*0.000176668167114	*0.000179	*0.000180	*0.000196	*0.000158		*0.000150
C	{0.1}	*0.000180	*0.000179588794708	*0.013189	*0.000180	*0.000150	*0.000196	
D	{1}	*0.000196	*0.000180	*0.013189911842346	*0.000176	*0.000196	*0.000180	
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176668167114	*0.000183	*0.000177	
F	{100}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000183		1
G	{500}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000177	1	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .9120000	{0.01 mg/L .9660000	{0.1 mg/L} .8960000	{1 mg/L} .8990000	{10 mg/L} .8130000	{100 mg/L} .1200000	{500 mg/L} 0.000000
A	{0}		*0.029870	0.757825	0.569648	*0.002928	*0.000150	*0.000158
B	{0.01	*0.029870927333831	*0.032767	*0.024319	*0.000204	*0.000158		*0.000174
C	{0.1}	0.757825	*0.032767891883850	0.895118	*0.002444	*0.000180	*0.000196	
D	{1}	0.569648	*0.024319	0.895118		*0.004799	*0.000196	*0.000150
E	{10}	*0.002928	*0.000204	*0.002444	*0.004799365997314	*0.000176	*0.000180	
F	{100}	*0.000150	*0.000158	*0.000180	*0.000196	*0.000176668167114		*0.000258
G	{500}	*0.000158	*0.000174	*0.000196	*0.000150	*0.000180	*0.000258	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .9120000	{0.01 mg/L .8650000	{0.1 mg/L} .9620000	{1 mg/L} .9750000	{10 mg/L} .7700000	{100 mg/L} .3750000	{500 mg/L} 0.000000
A	{0}		0.07296	0.058241	0.051744	*0.000278	*0.000196	*0.000150
B	{0.01	0.07296		*0.003619	*0.002438	*0.001678	*0.000180	*0.000196
C	{0.1}	0.058241	*0.003619730472564	7	0.600152	*0.000199	*0.000150	*0.000158
D	{1}	0.051744	*0.002438	0.600152		*0.000153	*0.000158	*0.000174
E	{10}	*0.000278	*0.001678	*0.000199	*0.000153303146362	3	*0.000176	*0.000180
F	{100}	*0.000196	*0.000180	*0.000150	*0.000158	*0.000176668167114	2	*0.000176
G	{500}	*0.000150	*0.000196	*0.000158	*0.000174	*0.000180	*0.000176	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .9120000	{0.01 mg/L .8460000	{0.1 mg/L} .9020000	{1 mg/L} .9140000	{10 mg/L} .8200000	{100 mg/L} .7390000	{500 mg/L} .3850000
A	{0}		0.102666	0.741906	0.947451	*0.035568	*0.000491	*0.000158
B	{0.01	0.102666		0.080881	0.148793	0.397104	*0.007809	*0.000196
C	{0.1}	0.741906	0.080881		0.914845	*0.038704	*0.000587	*0.000150
D	{1}	0.947451	0.148793	0.914845		*0.046287	*0.000579	*0.000174
E	{10}	*0.035568	0.397104	*0.038704	*0.046287953853607	2	*0.016670	*0.000180
F	{100}	*0.000491	*0.007809	*0.000587	*0.000579	*0.016670644283294	7	*0.000176
G	{500}	*0.000158	*0.000196	*0.000150	*0.000174	*0.000180	*0.000176	

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .3710000	{0.01 mg/L .4150000	{0.1 mg/L} .4300000	{1 mg/L} .4100000	{10 mg/L} .3520000	{100 mg/L} .0700000	{500 mg/L} 0.000000
A	{0}		*0.003539	*0.000659	*0.003291	0.105018	*0.000180	*0.000196
B	{0.01	*0.003539979457855	2	0.192728	0.655323	*0.000440	*0.000150	*0.000158
C	{0.1}	*0.000659	0.192728		0.197618	*0.000186	*0.000158	*0.000174
D	{1}	*0.003291	0.655323	0.197618		*0.000463	*0.000196	*0.000150
E	{10}	0.105018	*0.000440	*0.000186	*0.000463187694595		*0.000176	*0.000180
F	{100}	*0.000180	*0.000150	*0.000158	*0.000196	*0.000176668167114	2	*0.000187
G	{500}	*0.000196	*0.000158	*0.000174	*0.000150	*0.000180	*0.000187	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .3710000	{0.01 mg/L .3840000	{0.1 mg/L} .4010000	{1 mg/L} .3810000	{10 mg/L} .3350000	{100 mg/L} .1290000	{500 mg/L} 0.000000
A	{0}		0.653022	0.21297	0.503219	*0.026882	*0.000180	*0.000196
B	{0.01	0.653022		0.262206	0.839697	*0.021232	*0.000150	*0.000158
C	{0.1}	0.21297	0.262206		0.379982	*0.003673	*0.000158	*0.000174
D	{1}	0.503219	0.839697	0.379982		*0.017925	*0.000196	*0.000150
E	{10}	*0.026882	*0.021232	*0.003673	*0.017925560474395	8	*0.000176	*0.000180
F	{100}	*0.000180	*0.000150	*0.000158	*0.000196	*0.000176668167114	2	*0.000176
G	{500}	*0.000196	*0.000158	*0.000174	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .3710000	{0.01 mg/L .3500000	{0.1 mg/L} .3860000	{1 mg/L} .3890000	{10 mg/L} .3420000	{100 mg/L} .3270000	{500 mg/L} .1510000
A	{0}		0.543774	0.663517	0.856385	0.673507	0.575316	*0.000245
B	{0.01	0.543774		0.548977	0.66272	0.81611	0.777789	*0.000378
C	{0.1}	0.663517	0.548977		0.930506	0.575316	0.438174	*0.000220
D	{1}	0.856385	0.66272	0.930506		0.641525	0.474852	*0.000239
E	{10}	0.673507	0.81611	0.575316	0.641525		0.663517	*0.000321
F	{100}	0.575316	0.777789	0.438174	0.474852	0.663517		*0.000289
G	{500}	*0.000245	*0.000378	*0.000220	*0.000239	*0.000321	*0.000289320945739746	

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} .3710000	{0.01 mg/L .4040000	{0.1 mg/L} .4060000	{1 mg/L} .3750000	{10 mg/L} .3650000	{100 mg/L} .3480000	{500 mg/L} .1190000
A	{0}		0.770795	0.880977	0.934245	0.901485	0.880093	*0.000735
B	{0.01	0.770795		0.967156	0.551768	0.843971	0.763397	*0.000503
C	{0.1}	0.880977	0.967156		0.794331	0.905904	0.820387	*0.000602
D	{1}	0.934245	0.551768	0.794331		0.976023	0.940048	*0.000894
E	{10}	0.901485	0.843971	0.905904	0.976023		0.726105	*0.000534
F	{100}	0.880093	0.763397	0.820387	0.940048	0.726105		*0.000426
G	{500}	*0.000735	*0.000503	*0.000602	*0.000894	*0.000534	*0.000426	

APPENDIX 40: ANOVA: *Ventricaria ventricosa* : Growth (Carotenoid)

* = Significant difference : p<0.05

ANOVA-CAR-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0349700	.0252100	.0217700	.0119600	.0031680	.0006640
A	{0}		*0.000196(	*0.000180	*0.000177(	*0.000765	*0.000180	*0.00019603967666626
B	{0.01	*0.000196039676666		*0.000176	*0.000180	*0.000150	*0.000158	*0.000174045562744141
C	{0.1}	*0.000180	*0.000176668167114		*0.000835	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000177(	*0.000180	*0.000835776329040		*0.000180	*0.000196	*0.000150978565216064
E	{10}	*0.000765	*0.000150	*0.000196	*0.000180900096893		*0.000176	*0.000180900096893311
F	{100}	*0.000180	*0.000158	*0.000150	*0.000196	*0.000176668167114		*0.00722813606262207
G	{500}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	*0.00722813606262207	

Newman-Keuls test; CO_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0285400	.0269200	.0231900	.0166600	.0024090	.0016600
A	{0}		*0.000388	*0.000769	*0.008446	0.585979	*0.000201	*0.000217616558074951
B	{0.01	*0.000388205051422		0.469569	0.067331	*0.000605	*0.000158	*0.000174045562744141
C	{0.1}	*0.000769	0.469569		0.109047	*0.001046	*0.000150	*0.000158190727233887
D	{1}	*0.008446	0.067331	0.109047		*0.009760	*0.000196	*0.000151038169860 84
E	{10}	0.585979	*0.000605	*0.001046	*0.009760856628417		*0.000205	*0.000222146511077881
F	{100}	*0.000201	*0.000158	*0.000150	*0.000196	*0.000205039978027		0.736235
G	{500}	*0.000217	*0.000174	*0.000158	*0.000151	*0.000222	0.736235	

Newman-Keuls test; CR_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0360500	.0384300	.0435400	.0285700	.0123700	.0002916
A	{0}		*0.000180	*0.000196	*0.000150	*0.000179	0.11916	*0.000181734561920166
B	{0.01	*0.000180900096893		0.219664	*0.003352	*0.001362	*0.000196	*0.000150978565216064
C	{0.1}	*0.000196	0.219664		*0.015504	*0.000446	*0.000150	*0.000158190727233887
D	{1}	*0.000150	*0.003352	*0.015504777431488		*0.000198	*0.000158	*0.000174045562744141
E	{10}	*0.000179	*0.001362	*0.000446	*0.000198543071746		*0.000181	*0.00019603967666626
F	{100}	0.11916	*0.000196	*0.000150	*0.000158	*0.000181138515474		*0.000185012817382812
G	{500}	*0.000181	*0.000150	*0.000158	*0.000174	*0.000196	*0.000185012817382812	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0192500	.0216860	.0238500	.0113200	.0016980	0.000000
A	{0}		0.059421	*0.012123	*0.002463	*0.043142	*0.000185	*0.00019758939743042
B	{0.01	0.059421		0.21005	0.064271	*0.002198	*0.000196	*0.000150978565216064
C	{0.1}	*0.012123	0.21005		0.262682	*0.000516	*0.000150	*0.000158190727233887
D	{1}	*0.002463	0.064271	0.262682		*0.000213	*0.000158	*0.000174045562744141
E	{10}	*0.043142	*0.002198	*0.000516	*0.000213861465454 1		*0.000295	*0.000243961811065674
F	{100}	*0.000185	*0.000196	*0.000150	*0.000158	*0.000295519828796		0.375368
G	{500}	*0.000197	*0.000150	*0.000158	*0.000174	*0.000243	0.375368	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0349300	.0331700	.0292600	.0286700	.0238000	.0002080
A	{0}		*0.000618	*0.001064	*0.006269	*0.005012	*0.039483	*0.000277340412139893
B	{0.01	*0.000618338584899		0.582498	0.201228	0.233463	*0.022165	*0.000174105167388916
C	{0.1}	*0.001064	0.582498		0.231664	0.348611	*0.042410	*0.000158190727233887
D	{1}	*0.006269	0.201228	0.231664		0.853135	0.223382	*0.000151515007019043
E	{10}	*0.005012	0.233463	0.348611	0.853135		0.141751	*0.000196456909179687
F	{100}	*0.039483	*0.022165	*0.042410	0.223382	0.141751		*0.000184118747711182
G	{500}	*0.000277	*0.000174	*0.000158	*0.000151	*0.000196	*0.000184118747711182	

Newman-Keuls test; CO_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0428500	.0376000	.0340300	.0216200	.0049380	0.000000
A	{0}		*0.000150	*0.000196	*0.000183	*0.048452	*0.000301	*0.000185847282409668
B	{0.01	*0.000150978565216		*0.037037	*0.004612	*0.000196	*0.000158	*0.000174045562744141
C	{0.1}	*0.000196	*0.037037789821624		0.139291	*0.000190	*0.000150	*0.000158190727233887
D	{1}	*0.000183	*0.004612	0.139291		*0.000247	*0.000196	*0.000150978565216064
E	{10}	*0.048452	*0.000196	*0.000190	*0.000247418880462		*0.000185	*0.000196158885955811
F	{100}	*0.000301	*0.000158	*0.000150	*0.000196	*0.000185847282409		*0.0479010939598083
G	{500}	*0.000185	*0.000174	*0.000158	*0.000150	*0.000196	*0.0479010939598083	

Newman-Keuls test; CR_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0316800	.0328500	.0357400	.0304900	.0032660	0.000000
A	{0}		*0.000182	*0.000197	*0.000151	*0.000178	*0.000180	*0.000181257724761963
B	{0.01	*0.000182926654815		0.554018	0.124842	0.547353	*0.000196	*0.000150978565216064
C	{0.1}	*0.000197	0.554018		0.156431	0.459533	*0.000150	*0.000158190727233887
D	{1}	*0.000151	0.124842	0.156431		0.070023	*0.000158	*0.000174045562744141
E	{10}	*0.000178	0.547353	0.459533	0.070023		*0.000180	*0.00019603967666626
F	{100}	*0.000180	*0.000196	*0.000150	*0.000158	*0.000180900096893		0.112691
G	{500}	*0.000181	*0.000150	*0.000158	*0.000174	*0.000196	0.112691	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0206600	.0365900	.0305200	.0282300	.0019940	0.000000
A	{0}		0.145579	*0.000167	*0.000666	*0.001517	*0.000218	*0.000207424163818359
B	{0.01	0.145579		*0.000293	*0.004967	*0.010824	*0.000186	*0.000198781490325928
C	{0.1}	*0.000167	*0.000293612480163		*0.033400	*0.015110	*0.000158	*0.000174045562744141
D	{1}	*0.000666	*0.004967	*0.033400952816009		0.388344	*0.000150	*0.000158190727233887
E	{10}	*0.001517	*0.010824	*0.015110	0.388344		*0.000196	*0.000150978565216064
F	{100}	*0.000218	*0.000186	*0.000158	*0.000150	*0.000196099281311		0.451087
G	{500}	*0.000207	*0.000198	*0.000174	*0.000158	*0.000150	0.451087	

APPENDIX 40: ANOVA: *Ventricaria ventricosa* : Growth (Carotenoid)

* = Significant difference : p<0.05

ANOVA-CAR-VEN

Newman-Keuls test; FE_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0423600	.0374400	.0365200	.0345100	.0047010	0.000000
A	{0}		*0.000150	*0.000196	*0.000180	*0.000176	*0.000177	*0.000180900096893311
B	{0.01	*0.000150978565216		*0.003261	*0.002393	*0.000467	*0.000158	*0.000174045562744141
C	{0.1}	*0.000196	*0.003261744976043		0.516131	0.121132	*0.000150	*0.000158190727233887
D	{1}	*0.000180	*0.002393	0.516131		0.167588	*0.000196	*0.000150978565216064
E	{10}	*0.000176	*0.000467	0.121132	0.167588		*0.000180	*0.00019603967666626
F	{100}	*0.000177	*0.000158	*0.000150	*0.000196	*0.000180900096893		*0.00442206859588623
G	{500}	*0.000180	*0.000174	*0.000158	*0.000150	*0.000196	*0.00442206859588623	

Newman-Keuls test; FE_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0388300	.0360100	.0343300	.0233900	.0037360	0.000000
A	{0}		*0.000151	*0.000197	*0.000182	*0.010816	*0.000219	*0.000185728073120117
B	{0.01	*0.00015115737910		0.235292	0.153981	*0.000227	*0.000158	*0.000174045562744141
C	{0.1}	*0.000197	0.235292		0.472274	*0.000354	*0.000150	*0.000158190727233887
D	{1}	*0.000182	0.153981	0.472274		*0.000428	*0.000196	*0.000150978565216064
E	{10}	*0.010816	*0.000227	*0.000354	*0.000428199768066		*0.000181	*0.000196099281311035
F	{100}	*0.000219	*0.000158	*0.000150	*0.000196	*0.000181257724761		0.122698
G	{500}	*0.000185	*0.000174	*0.000158	*0.000150	*0.000196	0.122698	

Newman-Keuls test; MN_V_C4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0245500	.0224700	.0190400	.0163700	.0020230	0.000000
A	{0}		*0.040495	0.104503	0.430736	0.747572	*0.000452	*0.000377476215362549
B	{0.01	*0.040495812892913		0.472551	0.15994	0.050271	*0.000166	*0.000176608562469482
C	{0.1}	0.104503	0.472551		0.243502	0.112424	*0.000179	*0.000166416168212891
D	{1}	0.430736	0.15994	0.243502		0.359362	*0.000338	*0.000213682651519775
E	{10}	0.747572	0.050271	0.112424	0.359362		*0.000593	*0.000408172607421875
F	{100}	*0.000452	*0.000166	*0.000179	*0.000338	*0.000593483448028		0.484553
G	{500}	*0.000377	*0.000176	*0.000166	*0.000213	*0.000408	0.484553	

Newman-Keuls test; MN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0425100	.0411200	.0380600	.0254700	.0048640	.0001397
A	{0}		*0.000150	*0.000196	*0.000180	*0.001880	*0.000292	*0.000186383724212646
B	{0.01	*0.00015097856521		0.550848	0.159658	*0.000203	*0.000158	*0.000174045562744141
C	{0.1}	*0.000196	0.550848		0.199791	*0.000192	*0.000150	*0.000158190727233887
D	{1}	*0.000180	0.159658	0.199791		*0.000236	*0.000196	*0.000150978565216064
E	{10}	*0.001880	*0.000203	*0.000192	*0.000236213207244		*0.000180	*0.00019603967666626
F	{100}	*0.000292	*0.000158	*0.000150	*0.000196	*0.000180		0.056697
G	{500}	*0.000186	*0.000174	*0.000158	*0.000150	*0.000196	0.056697	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0208400	.0216600	.0241000	.0297700	.0029030	.0001390
A	{0}		*0.010300	*0.010916	*0.001658	*0.000157	*0.000180	*0.000181376934051514
B	{0.01	*0.010300219058990		0.658765	0.207345	*0.001296	*0.000180	*0.00019603967666626
C	{0.1}	*0.010916	0.658765		0.200712	*0.001574	*0.000196	*0.000150978565216064
D	{1}	*0.001658	0.207345	0.200712		*0.007662	*0.000150	*0.000158190727233887
E	{10}	*0.000157	*0.001296	*0.001574	*0.007662773132324		*0.000158	*0.000174045562744141
F	{100}	*0.000180	*0.000180	*0.000196	*0.000150	*0.000158190727233		0.150537
G	{500}	*0.000181	*0.000196	*0.000150	*0.000158	*0.000174	0.150537	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0283100	.0250900	.0206900	.0181800	.0014180	.0000811
A	{0}		*0.000395	*0.003521	0.134299	0.45658	*0.000177	*0.000181317329406738
B	{0.01	*0.00039511919021		0.118874	*0.004123	*0.000811	*0.000158	*0.000174045562744141
C	{0.1}	*0.003521	0.118874		*0.039576	*0.008264	*0.000150	*0.000158190727233887
D	{1}	0.134299	*0.004123	*0.039576232433319		0.216195	*0.000196	*0.000150978565216064
E	{10}	0.45658	*0.000811	*0.008264	0.216195		*0.000181	*0.000196218490600586
F	{100}	*0.000177	*0.000158	*0.000150	*0.000196	*0.000181257724761		0.501606
G	{500}	*0.000181	*0.000174	*0.000158	*0.000150	*0.000196	0.501606	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0138200	.0240100	.0174700	.0105600	.0019350	0.000000
A	{0}		0.391874	*0.001136	0.289407	*0.046574	*0.000206	*0.000153541564941406
B	{0.01	0.391874		*0.000545	0.152518	0.098076	*0.000209	*0.00020366907119751
C	{0.1}	*0.001136	*0.000545620918273		*0.003303	*0.000177	*0.000158	*0.000174045562744141
D	{1}	0.289407	0.152518	*0.003303050994873		*0.010192	*0.000153	*0.000158488750457764
E	{10}	*0.046574	0.098076	*0.000177	*0.010192990303039		*0.000496	*0.000302135944366455
F	{100}	*0.000206	*0.000209	*0.000158	*0.000153	*0.000496864318847		0.310534
G	{500}	*0.000153	*0.000203	*0.000174	*0.000158	*0.000302	0.310534	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0213800	.0356100	.0341200	.0205800	.0030060	0.000000
A	{0}		0.36718	*0.000601	*0.000832	0.264997	*0.001232	*0.000682175159454346
B	{0.01	0.36718		*0.002288	*0.002071	0.814518	*0.000576	*0.000268518924713135
C	{0.1}	*0.000601	*0.002288639545440		0.662856	*0.002639	*0.000158	*0.000174105167388916
D	{1}	*0.000832	*0.002071	0.662856		*0.003326	*0.000151	*0.000158309936523437
E	{10}	0.264997	0.814518	*0.002639	*0.003326475620269		*0.000484	*0.000301480293273926
F	{100}	*0.001232	*0.000576	*0.000158	*0.000151	*0.000484049320220		0.384077
G	{500}	*0.000682	*0.000268	*0.000174	*0.000158	*0.000301	0.384077	

APPENDIX 40: ANOVA: *Ventricaria ventricosa* : Growth (Carotenoid)

* = Significant difference : p<0.05

ANOVA-CAR-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0104000	.0084700	.0070600	.0014330	.0002059	0.000000
A	{0}		*0.001714	*0.000409	*0.000251	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.001714289188385		0.157131	0.052908	*0.000219	*0.000157	*0.000165104866027832
C	{0.1}	*0.000409	0.157131		0.293165	*0.000389	*0.000261	*0.000238716602325439
D	{1}	*0.000251	0.052908	0.293165		*0.000796	*0.000453	*0.00059354305267334
E	{10}	*0.000150	*0.000219	*0.000389	*0.000796496868133		0.358002	0.523659
F	{100}	*0.000158	*0.000157	*0.000261	*0.000453	0.358002		0.875642
G	{500}	*0.000174	*0.000165	*0.000238	*0.000593	0.523659	0.875642	

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0444700	.0459300	.0394000	.0241100	.0017320	0.000000
A	{0}		*0.000196	*0.000150	*0.000180	*0.006031	*0.000184	*0.00018620491027832
B	{0.01	*0.000196039676666		0.53343	*0.043712	*0.000181	*0.000150	*0.000158190727233887
C	{0.1}	*0.000150	0.53343		*0.032012	*0.000196	*0.000158	*0.000174045562744141
D	{1}	*0.000180	*0.043712	*0.032012462615966		*0.000182	*0.000196	*0.000150978565216064
E	{10}	*0.006031	*0.000181	*0.000196	*0.000182449817657		*0.000180	*0.00019603967666626
F	{100}	*0.000184	*0.000150	*0.000158	*0.000196	*0.000180959701538		0.461313
G	{500}	*0.000186	*0.000158	*0.000174	*0.000150	*0.000196	0.461313	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0284900	.0248100	.0249600	.0154200	.0020380	0.000000
A	{0}		*0.000196	*0.000181	*0.000193	0.986003	*0.000180	*0.00019603967666626
B	{0.01	*0.000196218490600		*0.047255	*0.023659	*0.000151	*0.000158	*0.000174045562744141
C	{0.1}	*0.000181	*0.0472555160522461		0.915669	*0.000196	*0.000196	*0.000150978565216064
D	{1}	*0.000193	*0.023659	0.915669		*0.000223	*0.000150	*0.000158190727233887
E	{10}	0.986003	*0.000151	*0.000196	*0.000223100185394		*0.000176	*0.000180900096893311
F	{100}	*0.000180	*0.000158	*0.000196	*0.000150	*0.000176727771759		0.164578
G	{500}	*0.000196	*0.000174	*0.000150	*0.000158	*0.000180	0.164578	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0336100	.0372100	.0324200	.0154300	.0036520	0.000000
A	{0}		*0.000402	*0.000243	*0.000337	0.691257	*0.002621	*0.000689685344696045
B	{0.01	*0.000402867794036		0.26813	0.708825	*0.000403	*0.000151	*0.000158190727233887
C	{0.1}	*0.000243	0.26813		0.305427	*0.000195	*0.000158	*0.000174045562744141
D	{1}	*0.000337	0.708825	0.305427		*0.000392	*0.000196	*0.000150978565216064
E	{10}	0.691257	*0.000403	*0.000195	*0.000392794609069		*0.002210	*0.000731170177459717
F	{100}	*0.002621	*0.000151	*0.000158	*0.000196	*0.002210259437561		0.261576
G	{500}	*0.000689	*0.000158	*0.000174	*0.000150	*0.000731	0.261576	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0254500	.0369000	.0387800	.0170900	.0033040	.0001540
A	{0}		*0.001420	*0.000196	*0.000150	0.469392	*0.000242	*0.000192403793334961
B	{0.01	*0.001420259475708		*0.000298	*0.000253	*0.002183	*0.000196	*0.000150978565216064
C	{0.1}	*0.000196	*0.000298321247100		0.409694	*0.000181	*0.000150	*0.000158190727233887
D	{1}	*0.000150	*0.000253	0.409694		*0.000196	*0.000158	*0.000174045562744141
E	{10}	0.469392	*0.002183	*0.000181	*0.000196099281311		*0.000231	*0.000201880931854248
F	{100}	*0.000242	*0.000196	*0.000150	*0.000158	*0.000231564044952		0.176327
G	{500}	*0.000192	*0.000150	*0.000158	*0.000174	*0.000201	0.176327	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0289500	.0371600	.0394500	.0204400	.0106100	.0044000
A	{0}		0.065446	*0.005134	*0.003347	0.462857	0.239994	0.064448
B	{0.01	0.065446		0.12013	0.122104	0.108261	*0.011396	*0.00177470111848692
C	{0.1}	*0.005134	0.12013		0.651415	*0.011996	*0.000935	*0.000273227691650391
D	{1}	*0.003347	0.122104	0.651415		*0.008848	*0.000631	*0.00023806095123291
E	{10}	0.462857	0.108261	*0.011996	*0.008848487261962		0.153199	*0.027249157428741
F	{100}	0.239994	*0.011396	*0.000935	*0.000631	0.153199		0.231025
G	{500}	0.064448	*0.001777	*0.000273	*0.000238	*0.027249	0.231025	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0154450	.0219200	.0288000	.0294100	.0199900	.0160300	.0037800
A	{0}		0.2213	*0.006741	*0.006554	0.353023	0.856719	*0.00267326831817627
B	{0.01	0.2213		*0.048293	0.080449	0.553534	0.188995	*0.000561833381652832
C	{0.1}	*0.006741	*0.048293232917785		0.850665	*0.037565	*0.006278	*0.000168323516845703
D	{1}	*0.006554	0.080449	0.850665		*0.045075	*0.006644	*0.000183641910552979
E	{10}	0.353023	0.553534	*0.037565	*0.045075476169586		0.233317	*0.000975906848907471
F	{100}	0.856719	0.188995	*0.006278	*0.006644	0.233317		*0.00478881597518921
G	{500}	*0.002673	*0.000561	*0.000168	*0.000183	*0.000975	*0.00478881597518921	

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		.0166960	.0408400	.0418400	.0314200	.0275300	.0229200	.0030900
A	{0}		*0.000152	*0.000158	*0.000666	*0.003952	*0.039461	*0.000361382961273193
B	{0.01	*0.000152051448822		0.720586	*0.004141	*0.000828	*0.000246	*0.000158190727233887
C	{0.1}	*0.000158	0.720586		*0.005256	*0.000815	*0.000200	*0.000174045562744141
D	{1}	*0.000666	*0.004141	*0.005256831645965		0.177507	*0.020037	*0.000150978565216064
E	{10}	*0.003952	*0.000828	*0.000815	0.177507		0.114602	*0.000196456909179687
F	{100}	*0.039461	*0.000246	*0.000200	*0.020037	0.114602		*0.00018697977066604
G	{500}	*0.000361	*0.000158	*0.000174	*0.000150	*0.000196	*0.00018697977066604	

APPENDIX 41: ANOVA: *Ventricaria ventricosa* : Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.521200	3.222800	3.258700	3.270700	2.299000	.3308800	.0269600
A	{1}		*0.003069	*0.005510	*0.008722	0.273965	*0.000180	*0.000196
B	{2}	*0.0030693411827087		0.856761	0.967481	*0.001004	*0.000196	*0.000150
C	{3}	*0.005510	0.856761		0.951923	*0.001289	*0.000150	*0.000158
D	{4}	*0.008722	0.967481	0.951923		*0.001692	*0.000158	*0.000174
E	{5}	0.273965	*0.001004	*0.001289	*0.0016924142837524		*0.000176	*0.000180
F	{6}	*0.000180	*0.000196	*0.000150	*0.000158	*0.000176727771759		0.141732
G	{7}	*0.000196	*0.000150	*0.000158	*0.000174	*0.000180	0.141732	

Newman-Keuls test; CO_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.521200	4.865000	3.951000	3.069000	2.355000	.3755000	.0999200
A	{1}		*0.000196	*0.000222	*0.030960	0.478561	*0.000180	*0.000196
B	{2}	*0.0001960992813110		*0.001445	*0.000182	*0.000150	*0.000158	*0.000174
C	{3}	*0.000222	*0.0014454126358032		*0.001860	*0.000217	*0.000150	*0.000158
D	{4}	*0.030960	*0.000182	*0.0018609166145324		*0.019090	*0.000196	*0.000150
E	{5}	0.478561	*0.000150	*0.000217	*0.0190902948379517		*0.000176	*0.000180
F	{6}	*0.000180	*0.000158	*0.000150	*0.000196	*0.0001768469810485		0.247269
G	{7}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	0.247269	

Newman-Keuls test; CR_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.521200	3.265000	2.841000	2.514000	1.775000	.5975000	.0277500
A	{0}		*0.014783	0.182559	0.975379	*0.014489	*0.000197	*0.000150
B	{0.01	*0.0147839784622192		0.084151	*0.024382	*0.000242	*0.000158	*0.000174
C	{0.1}	0.182559	0.084151		0.350936	*0.001923	*0.000151	*0.000158
D	{1}	0.975379	*0.024382	0.350936		*0.006053	*0.000181	*0.000196
E	{10}	*0.014489	*0.000242	*0.001923	*0.006053626537323		*0.000301	*0.000183
F	{100}	*0.000197	*0.000158	*0.000151	*0.000181	*0.000301539897918		*0.025638
G	{500}	*0.000150	*0.000174	*0.000158	*0.000196	*0.000183	*0.025638	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.521200	2.351000	3.135000	1.926000	1.037000	.3331000	0.000000
A	{0}		0.467798	*0.017663	0.050703	*0.000249	*0.000151	*0.000158
B	{0.01	0.467798		*0.010545	0.083495	*0.000297	*0.000196	*0.000150
C	{0.1}	*0.017663	*0.0105452537536621		*0.000732	*0.000151	*0.000158	*0.000174
D	{1}	0.050703	0.083495	*0.000732898712158		*0.001742	*0.000190	*0.000197
E	{10}	*0.000249	*0.000297	*0.000151	*0.0017426609992981		*0.008189	*0.001362
F	{100}	*0.000151	*0.000196	*0.000158	*0.000190	*0.008189678192138		0.166158
G	{500}	*0.000158	*0.000150	*0.000174	*0.000197	*0.001362	0.166158	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.361600	3.325100	3.337100	3.342500	3.159700	2.348000	.0299420
A	{1}		0.994046	0.984191	0.896234	0.634213	*0.000205	*0.000174
B	{2}	0.994046		0.934708	0.992022	0.268924	*0.000195	*0.000196
C	{3}	0.984191	0.934708		0.970678	0.453113	*0.000222	*0.000150
D	{4}	0.896234	0.992022	0.970678		0.593921	*0.000199	*0.000158
E	{5}	0.634213	0.268924	0.453113	0.593921		*0.000224	*0.000180
F	{6}	*0.000205	*0.000195	*0.000222	*0.000199	*0.000224292278289	70	*.000176
G	{7}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000180	*0.000176	

Newman-Keuls test; CO_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.361600	4.996000	4.816000	3.592000	2.853000	1.984000	.3820000
A	{1}		*0.000219	*0.000236	0.344395	*0.048653	*0.000280	*0.000196
B	{2}	*0.000219345092773		0.457293	*0.000262	*0.000151	*0.000158	*0.000174
C	{3}	*0.000236	0.457293		*0.000293	*0.000197	*0.000150	*0.000158
D	{4}	0.344395	*0.000262	*0.0002931952476501		*0.018708	*0.000225	*0.000150
E	{5}	*0.048653	*0.000151	*0.000197	*0.0187085866928101		*0.002568	*0.000180
F	{6}	*0.000280	*0.000158	*0.000150	*0.000225	*0.002568781375850		*0.000181
G	{7}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	*0.000181	

Newman-Keuls test; CR_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.361600	3.494000	3.490000	2.939000	2.841000	1.069000	.3135000
A	{0}		0.841906	0.594119	0.094325	0.103842	*0.000196	*0.000150
B	{0.01	0.841906		0.986795	0.131881	0.091911	*0.000158	*0.000174
C	{0.1}	0.594119	0.986795		0.082743	0.065659	*0.000150	*0.000158
D	{1}	0.094325	0.131881	0.082743		0.683591	*0.000182	*0.000196
E	{10}	0.103842	0.091911	0.065659	0.683591		*0.000177	*0.000180
F	{100}	*0.000196	*0.000158	*0.000150	*0.000182	*0.000177681446075	4	*0.006441
G	{500}	*0.000150	*0.000174	*0.000158	*0.000196	*0.000180	*0.006441	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.361600	3.343600	3.356700	3.333800	2.340300	2.554500	.2354200
A	{0}		0.980273	0.95945	0.990787	*0.000158	*0.000152	*0.000174
B	{0.01	0.980273		0.891735	0.918907	*0.000196	*0.000181	*0.000150
C	{0.1}	0.95945	0.891735		0.968236	*0.000150	*0.000197	*0.000158
D	{1}	0.990787	0.918907	0.968236		*0.000180	*0.000176	*0.000196
E	{10}	*0.000158	*0.000196	*0.000150	*0.000180900096893	3	*0.039713	*0.000176
F	{100}	*0.000152	*0.000181	*0.000197	*0.000176	*0.039713382720947	3	*0.000180
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000176	*0.000180	

APPENDIX 41: ANOVA: *Ventricaria ventricosa* : Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-VEN

Newman-Keuls test; FE_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.521200	2.743000	3.265000	2.841000	2.375000	.6367000	0.000000
A	{0}		0.347198	*0.025866(	0.366104	0.531803	*0.0001815	*0.000196
B	{0.01	0.347198		0.090565	0.673937	0.272375	*0.000196:	*0.000150
C	{0.1}	*0.025866(	0.090565		0.084148	*0.011703(	*0.000158:	*0.000174
D	{1}	0.366104	0.673937	0.084148		0.218962	*0.000151:	*0.000158
E	{10}	0.531803	0.272375	*0.011703(	0.218962		*0.0001774	*0.000180
F	{100}	*0.000181:	*0.000196:	*0.000158:	*0.000151:	*0.0001774430274963		*0.014528
G	{500}	*0.000196(	*0.000150:	*0.000174(	*0.000158:	*0.000180:	*0.014528	

Newman-Keuls test; MN_V_C4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.521200	3.461000	3.233000	2.775000	1.004000	.2808000	.0261200
A	{0}		*0.0051781	*0.019330(	0.284424	*0.000182(	*0.000180:	*0.000196
B	{0.01	*0.0051781535148620		0.334331	*0.023981(	*0.000150:	*0.000158:	*0.000174
C	{0.1}	*0.019330(	0.334331		0.064335	*0.000196(	*0.000150:	*0.000158
D	{1}	0.284424	*0.023981(	0.064335		*0.000182:	*0.000196(	*0.000150
E	{10}	*0.000182(	*0.000150:	*0.000196(	*0.000182926654815(		*0.006936:	*0.002152
F	{100}	*0.000180:	*0.000158:	*0.000150:	*0.000196(	*0.006936907768249:		0.282826
G	{500}	*0.000196(	*0.000174(	*0.000158:	*0.000150:	*0.002152:	0.282826	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.521200	1.861000	1.929000	2.220000	2.122000	.2024000	.1829000
A	{0}		0.074605	0.087642	0.208125	0.22235	*0.000158:	*0.000174
B	{0.01	0.074605		0.770205	0.423879	0.504329	*0.000178:	*0.000185
C	{0.1}	0.087642	0.770205		0.431365	0.412054	*0.000184(	*0.000201
D	{1}	0.208125	0.423879	0.431365		0.674242	*0.000152(	*0.000159
E	{10}	0.22235	0.504329	0.412054	0.674242		*0.000197:	*0.000153
F	{100}	*0.000158:	*0.000178:	*0.000184(	*0.000152(	*0.0001973509788513		0.933217
G	{500}	*0.000174:	*0.000185:	*0.000201:	*0.000159(	*0.000153(	0.933217	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.521200	2.351000	2.449000	2.384000	1.677000	.2155000	0.000000
A	{0}		0.876653	0.75625	0.821467	*0.016997:	*0.000158:	*0.000174
B	{0.01	0.876653		0.903893	0.887083	*0.010564(	*0.000180:	*0.000196
C	{0.1}	0.75625	0.903893		0.779834	*0.020506:	*0.000151:	*0.000158
D	{1}	0.821467	0.887083	0.779834		*0.020121:	*0.000196:	*0.000150
E	{10}	*0.016997:	*0.010564(	*0.020506:	*0.020121932029724:		*0.000187(	*0.000185
F	{100}	*0.000158:	*0.000180:	*0.000151:	*0.000196:	*0.0001870393753051		0.360697
G	{500}	*0.000174:	*0.000196(	*0.000158:	*0.000150:	*0.000185:	0.360697	

Newman-Keuls test; FE_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.361600	6.494000	5.971000	5.126000	3.592000	.1630000	.0650000
A	{0}		*0.000150:	*0.000196(	*0.000181:	0.296052	*0.000176(	*0.000180
B	{0.01	*0.0001509785652160		*0.027404:	*0.000209:	*0.000196(	*0.000158:	*0.000174
C	{0.1}	*0.000196(	*0.027404844760894:		*0.001506:	*0.000180:	*0.000150:	*0.000158
D	{1}	*0.000181:	*0.000209:	*0.001506388187408:		*0.000178:	*0.000196(	*0.000150
E	{10}	0.296052	*0.000196(	*0.000180:	*0.000178456306457:		*0.000180:	*0.000196
F	{100}	*0.000176(	*0.000158:	*0.000150:	*0.000196(	*0.000180900096893:		0.651447
G	{500}	*0.000180:	*0.000174(	*0.000158:	*0.000150:	*0.000196(	0.651447	

Newman-Keuls test; MN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.361600	2.318000	2.612000	2.971000	2.690000	.2939000	.0284400
A	{0}		*0.001881(	*0.015584:	0.087084	*0.017829:	*0.000158:	*0.000174
B	{0.01	*0.0018810033798217		0.187712	*0.036571:	0.22114	*0.000176:	*0.000180
C	{0.1}	*0.015584:	0.187712		0.242782	0.718814	*0.000180:	*0.000196
D	{1}	0.087084	*0.036571:	0.242782		0.206765	*0.000150:	*0.000158
E	{10}	*0.017829:	0.22114	0.718814	0.206765		*0.000196(	*0.000150
F	{100}	*0.000158:	*0.000176:	*0.000180:	*0.000150:	*0.0001960396766662		0.231561
G	{500}	*0.000174(	*0.000180:	*0.000196(	*0.000158:	*0.000150:	0.231561	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.361600	1.926000	2.090000	2.318000	2.514000	2.286000	.2057000
A	{0}		*0.000445:	*0.000871:	*0.001669:	*0.003041:	*0.002314:	*0.000174
B	{0.01	*0.0004453659057617		0.497524	0.376761	0.146851	0.30776	*0.000178
C	{0.1}	*0.000871:	0.497524		0.607753	0.313253	0.419158	*0.000182
D	{1}	*0.001669:	0.376761	0.607753		0.419158	0.893927	*0.000151
E	{10}	*0.003041:	0.146851	0.313253	0.419158		0.607753	*0.000158
F	{100}	*0.002314:	0.30776	0.419158	0.893927	0.607753		*0.000196
G	{500}	*0.000174(	*0.000178:	*0.000182(	*0.000151:	*0.000158:	*0.000196	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.361600	2.318000	3.592000	3.265000	2.024000	.2188000	0.000000
A	{0}		*0.000759(	0.296047	0.656066	*0.000275:	*0.000150:	*0.000158
B	{0.01	*0.0007596611976623:		*0.000348:	*0.000681(	0.187707	*0.000180:	*0.000196
C	{0.1}	0.296047	*0.000348389148712:		0.302784	*0.000175:	*0.000158:	*0.000174
D	{1}	0.656066	*0.000681(	0.302784		*0.000281(	*0.000196(	*0.000150
E	{10}	*0.000275:	0.187707	*0.000175:	*0.000281095504760:		*0.000176:	*0.000180
F	{100}	*0.000150:	*0.000180:	*0.000158:	*0.000196(	*0.0001768469810485		0.320104
G	{500}	*0.000158:	*0.000196(	*0.000174(	*0.000150:	*0.000180:	0.320104	

APPENDIX 41: ANOVA: *Ventricaria ventricosa* : Biochemical composition (Carbohydrate)

* = Significant difference : p<0.05

ANOVA-CHO-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 2.521200	{0.01 mg/L} 2.220000	{0.1 mg/L} 2.355000	{1 mg/L} 2.547000	{10 mg/L} 1.743000	{100 mg/L} .2188000	{500 mg/L} 0.000000
A	{0}		0.407158	0.478131	0.911615	0.019485	*0.000151(	*0.000158
B	{0.01	0.407158		0.563285	0.499931	0.055219	*0.000181:	*0.000196
C	{0.1}	0.478131	0.563285		0.683927	*0.044237:	*0.000196:	*0.000150
D	{1}	0.911615	0.499931	0.683927		*0.023564	*0.000158:	*0.000174
E	{10}	0.019485	0.055219	*0.044237:	*0.023564457893371(		*0.000182:	*0.000183
F	{100}	*0.000151(	*0.000181:	*0.000196:	*0.000158:	*0.000182569026947(		*0.353559
G	{500}	*0.000158:	*0.000196(	*0.000150:	*0.000174:	*0.000183:	*0.353559	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 2.521200	{0.01 mg/L} 3.396000	{0.1 mg/L} 3.592000	{1 mg/L} 3.363000	{10 mg/L} 2.612000	{100 mg/L} .2220000	{500 mg/L} 0.000000
A	{0}		*0.008790:	*0.002756:	0.006498	*0.696531:	*0.000176:	*0.000180
B	{0.01	*0.008790552616119:		0.40455	0.887083	*0.010545:	*0.000150:	*0.000158
C	{0.1}	*0.002756:	0.40455		0.586174	*0.003741(	*0.000158:	*0.000174
D	{1}	*0.006498:	0.887083	0.586174		*0.005468	*0.000196(	*0.000150
E	{10}	0.696532	*0.010545:	*0.003741(	*0.005468428134918:		*0.000180:	*0.000196
F	{100}	*0.000176:	*0.000150:	*0.000158:	*0.000196(	*0.000180900096893:		0.346777
G	{500}	*0.000180:	*0.000158:	*0.000174(	*0.000150:	*0.000196(	0.346777	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 2.521200	{0.01 mg/L} 3.382800	{0.1 mg/L} 3.386100	{1 mg/L} 3.382800	{10 mg/L} 2.386100	{100 mg/L} .3383900	{500 mg/L} .0240000
A	{0}		*0.000178(	*0.000209(	*0.000187:	0.278003	*0.000180:	*0.000196
B	{0.01	*0.000178635120391:		0.99963	1	*0.000181	*0.000196(	*0.000150
C	{0.1}	*0.000209(	0.99963		0.978502	*0.000153(	*0.000158:	*0.000174
D	{1}	*0.000187:	1	0.978502		*0.000197:	*0.000150:	*0.000158
E	{10}	0.278003	*0.000181	*0.000153(	*0.000197589397430		*0.000176(	*0.000180
F	{100}	*0.000180:	*0.000196(	*0.000158:	*0.000150:	*0.000176668167114		*0.020043
G	{500}	*0.000196(	*0.000150:	*0.000174(	*0.000158:	*0.000180:	*0.020043	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 2.521200	{0.01 mg/L} 3.400200	{0.1 mg/L} 3.399100	{1 mg/L} 3.399100	{10 mg/L} 2.395900	{100 mg/L} .3398000	{500 mg/L} .0194060
A	{0}		*0.000227:	*0.000181	*0.000195:	0.349581	*0.000180:	*0.000196
B	{0.01	*0.000227570533752		0.999965	0.993444	*0.000159(	*0.000158:	*0.000174
C	{0.1}	*0.000181	0.999965		1	*0.000182:	*0.000196(	*0.000150
D	{1}	*0.000195:	0.993444	1		*0.000200	*0.000150:	*0.000158
E	{10}	0.349581	*0.000159(	*0.000182:	*0.000200808048248		*0.000176(	*0.000180
F	{100}	*0.000180:	*0.000158:	*0.000196(	*0.000150:	*0.000176668167114		*0.026846

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 3.361600	{0.01 mg/L} 3.368600	{0.1 mg/L} 3.369700	{1 mg/L} 3.369700	{10 mg/L} 2.373000	{100 mg/L} .3368600	{500 mg/L} .0258610
A	{0}		0.94463	0.996388	0.99981	*0.000176	*0.000180:	*0.000196
B	{0.01	0.94463		0.991395	0.99994	*0.000180:	*0.000196(	*0.000150
C	{0.1}	0.996388	0.991395		1	*0.000196(	*0.000150:	*0.000158
D	{1}	0.99981	0.99994	1		*0.000151(	*0.000158:	*0.000174
E	{10}	*0.000176:	*0.000180:	*0.000196(	*0.000151038169860		*0.000176(	*0.000180
F	{100}	*0.000180:	*0.000196(	*0.000150:	*0.000158:	*0.000176668167114		*0.007293
G	{500}	*0.000196(	*0.000150:	*0.000158:	*0.000174(	*0.000180:	*0.007293	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 3.361600	{0.01 mg/L} 3.359900	{0.1 mg/L} 3.378400	{1 mg/L} 3.376300	{10 mg/L} 2.378400	{100 mg/L} .2152900	{500 mg/L} .0196790
A	{0}		0.988168	0.987702	0.897248	*0.000181(	*0.000196(	*0.000150
B	{0.01	0.988168		0.998364	0.988277	*0.000176	*0.000180:	*0.000196
C	{0.1}	0.987702	0.998364		0.98536	*0.000151	*0.000158:	*0.000174
D	{1}	0.897248	0.988277	0.98536		*0.000196	*0.000150:	*0.000158
E	{10}	*0.000181(	*0.000176	*0.000151	*0.000196456909179(		*0.000176(	*0.000180
F	{100}	*0.000196(	*0.000180:	*0.000158:	*0.000150:	*0.000176668167114		0.101743
G	{500}	*0.000150:	*0.000196(	*0.000174(	*0.000158:	*0.000180:	0.101743	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 3.361600	{0.01 mg/L} 3.392600	{0.1 mg/L} 3.394800	{1 mg/L} 3.392600	{10 mg/L} 2.395900	{100 mg/L} .3392600	{500 mg/L} .0277650
A	{0}		0.759345	0.986581	0.947856	*0.000176	*0.000180:	*0.000196
B	{0.01	0.759345		0.999761	1	*0.000180:	*0.000196(	*0.000150
C	{0.1}	0.986581	0.999761		0.982726	*0.000151(	*0.000158:	*0.000174
D	{1}	0.947856	1	0.982726		*0.000196(	*0.000150:	*0.000158
E	{10}	*0.000176:	*0.000180:	*0.000151(	*0.000196099281311(		*0.000176(	*0.000180
F	{100}	*0.000180:	*0.000196(	*0.000158:	*0.000150:	*0.000176668167114		*0.007375
G	{500}	*0.000196(	*0.000150:	*0.000174(	*0.000158:	*0.000180:	*0.007375	

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L} 3.361600	{0.01 mg/L} 3.081300	{0.1 mg/L} 3.400200	{1 mg/L} 3.402400	{10 mg/L} 2.406700	{100 mg/L} .3411100	{500 mg/L} .0234770
A	{0}		*0.037429:	0.75618	0.940395	*0.000182:	*0.000196(	*0.000150
B	{0.01	*0.037429511547088(		0.050104	0.081635	*0.000236	*0.000180:	*0.000196
C	{0.1}	0.75618	0.050104		0.985956	*0.000198	*0.000150:	*0.000158
D	{1}	0.940395	0.081635	0.985956		*0.000154	*0.000158:	*0.000174
E	{10}	*0.000182:	*0.000236	*0.000198	*0.000154852867126		*0.000176(	*0.000180
F	{100}	*0.000196(	*0.000180:	*0.000150:	*0.000158:	*0.000176668167114		*0.020838

G {500} *0.0001960 *0.0001740 *0.0001505 *0.0001583 *0.0001805 *0.026846

G {500} *0.0001505 *0.0001960 *0.0001583 *0.0001740 *0.0001805 *0.020838

APPENDIX 41: ANOVA: *Ventricaria ventricosa* : Biochemical composition (Protein)

* = Significant difference : p<0.05

ANOVA-PROTEIN-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	7.779000	7.759000	8.004000	7.820000	6.920000	.7769000
A	{1}		*0.000196(	*0.000180	*0.000158	*0.000150	*0.000176(	*0.000565111637115479
B	{2}	*0.0001960396766662		0.9588	0.82595	0.915547	0.094569	*0.0001509785652
C	{3}	*0.000180	0.9588		0.91524	0.985939	*0.044175	*0.00019603967666626
D	{4}	*0.000158	0.82595	0.91524		0.63516	0.079299	*0.000174045562744141
E	{5}	*0.000150	0.915547	0.985939	0.63516		0.12848	*0.000158190727233887
F	{6}	*0.000176(	0.094569	*0.044175	0.079299	0.12848		*0.000180900096893311
G	{7}	*0.000565	*0.000150	*0.000196(	*0.000174(	*0.000158	*0.000180900096893311	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	8.107000	8.075000	8.346000	8.446000	7.295000	.7727000
A	{1}		*0.000196(	*0.000180	*0.000150	*0.000158	*0.000176	*0.000177323818206787
B	{2}	*0.0001960396766662		0.935736	0.549119	0.666595	0.128748	*0.0001509785652
C	{3}	*0.000180	0.935736		0.769542	0.777264	0.064905	*0.00019603967666626
D	{4}	*0.000150	0.549119	0.769542		0.801069	0.07269	*0.000158190727233887
E	{5}	*0.000158	0.666595	0.777264	0.801069		0.066559	*0.000174045562744141
F	{6}	*0.000176	0.128748	0.064905	0.07269	0.066559		*0.000180900096893311
G	{7}	*0.000177	*0.000150	*0.000196(	*0.000158	*0.000174(	*0.000180900096893311	

Newman-Keuls test; CO_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	5.563000	5.824000	5.650000	5.631000	.5631000	.0545700
A	{1}		*0.000176(	*0.000150	*0.000196(	*0.000180	*0.000176	*0.000180900096893311
B	{2}	*0.0001766681671142		0.600815	0.907915	0.747501	*0.000180	*0.00019603967666626
C	{3}	*0.000150	0.600815		0.414836	0.629586	*0.000158	*0.000174045562744141
D	{4}	*0.000196(	0.907915	0.414836		0.928269	*0.000150	*0.000158190727233887
E	{5}	*0.000180	0.747501	0.629586	0.928269		*0.000196(	*0.000150978565216064
F	{6}	*0.000176	*0.000180	*0.000158	*0.000150	*0.0001960396766662	*0.0278107523918152	
G	{7}	*0.000180	*0.000196(	*0.000174(	*0.000158	*0.000150	*0.0278107523918152	

Newman-Keuls test; CO_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	4.402000	4.180000	4.112000	3.141000	.4209000	.3899000
A	{1}		0.063645	0.209894	0.160085	*0.013342	*0.000180	*0.00019603967666626
B	{2}	0.063645		0.340099	0.423278	*0.000638	*0.000158	*0.000174045562744141
C	{3}	0.209894	0.340099		0.766774	*0.002104	*0.000150	*0.000158190727233887
D	{4}	0.160085	0.423278	0.766774		*0.002032	*0.000196(	*0.0001509785652
E	{5}	*0.013342	*0.000638	*0.002104	*0.002032101154327		*0.000176(	*0.000180900096893311
F	{6}	*0.000180	*0.000158	*0.000150	*0.000196(	*0.0001766681671142	0.892379	
G	{7}	*0.000196(	*0.000174(	*0.000158	*0.000150	*0.000180	0.892379	

Newman-Keuls test; CR_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	5.515000	5.566000	5.679000	5.640000	.5418000	.0548600
A	{0}		*0.000176(	*0.000180	*0.000150	*0.000196(	*0.000176	*0.000180900096893311
B	{0.01}	*0.0001766681671142		0.809077	0.856798	0.82038	*0.000180	*0.00019603967666626
C	{0.1}	*0.000180	0.809077		0.850247	0.726158	*0.000196(	*0.000150978565216064
D	{1}	*0.000150	0.856798	0.850247		0.853362	*0.000158	*0.000174045562744141
E	{10}	*0.000196(	0.82038	0.726158	0.853362		*0.000150	*0.000158190727233887
F	{100}	*0.000176	*0.000180	*0.000196(	*0.000150	*0.000150978565216(	*0.0339251160621643	
G	{500}	*0.000180	*0.000196(	*0.000150	*0.000174(	*0.000158	*0.0339251160621643	

Newman-Keuls test; CR_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	4.160000	4.131000	3.938000	2.063000	.4354000	.3909000
A	{0}		0.361025	0.291054	0.48966	*0.000177	*0.000180	*0.00019603967666626
B	{0.01	0.361025		0.899279	0.596174	*0.000151	*0.000158	*0.000174045562744141
C	{0.1}	0.291054	0.899279		0.405062	*0.000196	*0.000150	*0.000158190727233887
D	{1}	0.48966	0.596174	0.405062		*0.000181	*0.000196(	*0.000150978565216064
E	{10}	*0.000177	*0.000151	*0.000196	*0.000181496143341(		*0.000178	*0.000184834003448486
F	{100}	*0.000180	*0.000158	*0.000150	*0.000196(	*0.000178456306457	0.845991	
G	{500}	*0.000196(	*0.000174(	*0.000158	*0.000150	*0.000184	0.845991	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	5.418000	5.486000	5.340000	5.311000	.5399000	0.000000
A	{0}		*0.000196(	*0.000150	*0.000180	*0.000176	*0.000176	*0.000180900096893311
B	{0.01}	*0.0001960396766662		0.747474	0.71203	0.864439	*0.000150	*0.000158190727233887
C	{0.1}	*0.000150	0.747474		0.764364	0.832036	*0.000158	*0.000174045562744141
D	{1}	*0.000180	0.71203	0.764364		0.890693	*0.000196(	*0.000150978565216064
E	{10}	*0.000176	0.864439	0.832036	0.890693		*0.000180	*0.00019603967666626
F	{100}	*0.000176	*0.000150	*0.000158	*0.000196(	*0.0001809000968	*0.0207716822624207	
G	{500}	*0.000180	*0.000158	*0.000174(	*0.000150	*0.000196(	*0.0207716822624207	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	8.411000	8.443000	8.585000	7.827000	.7904000	.0795600
A	{0}		*0.000180	*0.000196(	*0.000150	*0.000176	*0.000177	*0.000180959701538086
B	{0.01	*0.0001809000968933		0.935534	0.895908	0.154585	*0.000196(	*0.000150978565216064
C	{0.1}	*0.000196(	0.935534		0.719931	0.28309	*0.000150	*0.000158190727233887
D	{1}	*0.000150	0.895908	0.719931		0.251141	*0.000158	*0.000174045562744141
E	{10}	*0.000176	0.154585	0.28309	0.251141		*0.000180	*0.00019603967666626
F	{100}	*0.000177	*0.000196(	*0.000150	*0.000158	*0.0001809000968933	0.088377	
G	{500}	*0.000180	*0.000150	*0.000158	*0.000174(	*0.000196(	0.088377	

APPENDIX 41: ANOVA: *Ventricaria ventricosa* : Biochemical composition (Protein)

* = Significant difference : p<0.05

ANOVA-PROTEIN-VEN

Newman-Keuls test; FE_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	5.486000	5.437000	5.592000	5.650000	.5844000	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000176	*0.000180900096893311
B	{0.01	*0.000180900096893		0.816393	0.616643	0.713649	*0.000196	*0.000150978565216064
C	{0.1}	*0.000176	0.816393		0.73924	0.73571	*0.000180	*0.00019603967666626
D	{1}	*0.000196	0.616643	0.73924		0.783505	*0.000150	*0.000158190727233887
E	{10}	*0.000150	0.713649	0.73571	0.783505		*0.000158	*0.000174045562744141
F	{100}	*0.000176	*0.000196	*0.000180	*0.000150	*0.000158190727233		*0.0136872529983521
G	{500}	*0.000180	*0.000150	*0.000196	*0.000158	*0.000174	*0.0136872529983521	

Newman-Keuls test; FE_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	4.112000	3.996000	4.005000	3.841000	.3967000	0.000000
A	{0}		0.58808	0.608168	0.747507	0.785083	*0.000176	*0.000180900096893311
B	{0.01	0.58808		0.864807	0.641424	0.633264	*0.000158	*0.000174045562744141
C	{0.1}	0.608168	0.864807		0.968762	0.501743	*0.000196	*0.000150978565216064
D	{1}	0.747507	0.641424	0.968762		0.750332	*0.000150	*0.000158190727233887
E	{10}	0.785083	0.633264	0.501743	0.750332		*0.000180	*0.00019603967666626
F	{100}	*0.000176	*0.000158	*0.000196	*0.000150	*0.000180900096893		0.099398
G	{500}	*0.000180	*0.000174	*0.000150	*0.000158	*0.000196	0.099398	

Newman-Keuls test; MN_V_C4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	5.544000	5.540000	5.534000	5.379000	.5505000	0.000000
A	{0}		*0.000150	*0.000196	*0.000180	*0.000176	*0.000176	*0.000180900096893311
B	{0.01	*0.000150978565216		0.984957	0.998787	0.854577	*0.000158	*0.000174045562744141
C	{0.1}	*0.000196	0.984957		0.9774	0.722216	*0.000150	*0.000158190727233887
D	{1}	*0.000180	0.998787	0.9774		0.466447	*0.000196	*0.000150978565216064
E	{10}	*0.000176	0.854577	0.722216	0.466447		*0.000180	*0.00019603967666626
F	{100}	*0.000176	*0.000158	*0.000150	*0.000196	*0.000180900096893		*0.0188148021697998
G	{500}	*0.000180	*0.000174	*0.000158	*0.000150	*0.000196	*0.0188148021697998	

Newman-Keuls test; MN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	4.150000	4.121000	4.296000	2.947000	.4005000	.0368600
A	{0}		0.257153	0.149845	0.14453	*0.002528	*0.000180	*0.00019603967666626
B	{0.01	0.257153		0.89927	0.526514	*0.000686	*0.000150	*0.000158190727233887
C	{0.1}	0.149845	0.89927		0.721694	*0.000502	*0.000196	*0.000150978565216064
D	{1}	0.14453	0.526514	0.721694		*0.000391	*0.000158	*0.000174045562744141
E	{10}	*0.002528	*0.000686	*0.000502	*0.000391304492950		*0.000176	*0.000180900096893311
F	{100}	*0.000180	*0.000150	*0.000196	*0.000158	*0.000176668167114		0.12803
G	{500}	*0.000196	*0.000158	*0.000150	*0.000174	*0.000180	0.12803	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	5.205000	5.340000	5.302000	5.292000	2.205000	.5292000
A	{0}		*0.000176	*0.000150	*0.000196	*0.000180	0.155797	*0.000180959701538086
B	{0.01	*0.000176668167114		0.915561	0.889265	0.684001	*0.000180	*0.00019603967666626
C	{0.1}	*0.000150	0.915561		0.858615	0.971544	*0.000158	*0.000174045562744141
D	{1}	*0.000196	0.889265	0.858615		0.962674	*0.000150	*0.000158190727233887
E	{10}	*0.000180	0.684001	0.971544	0.962674		*0.000196	*0.000150978565216064
F	{100}	0.155797	*0.000180	*0.000158	*0.000150	*0.000196603967666		*0.000176966190338135
G	{500}	*0.000180	*0.000196	*0.000174	*0.000158	*0.000150	*0.000176966190338135	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	3.860000	3.889000	3.947000	3.870000	2.831000	.3889000
A	{0}		0.724831	0.960734	0.942549	0.914807	*0.001069	*0.000180900096893311
B	{0.01	0.724831		0.991117	0.980106	0.965571	*0.001390	*0.00019603967666626
C	{0.1}	0.960734	0.991117		0.802004	0.934532	*0.002930	*0.000158190727233887
D	{1}	0.942549	0.980106	0.802004		0.9388	*0.002579	*0.000174045562744141
E	{10}	0.914807	0.965571	0.934532	0.9388		*0.002270	*0.000150978565216064
F	{100}	*0.001069	*0.001390	*0.002930	*0.002579	*0.002270428962707		*0.000176668167114258
G	{500}	*0.000180	*0.000196	*0.000158	*0.000174	*0.000150	*0.000176668167114258	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	5.389000	5.466000	5.495000	4.466000	.4034000	0.000000
A	{0}		*0.000180	*0.000196	*0.000150	*0.000176	*0.000176	*0.000180900096893311
B	{0.01	*0.000180900096893		0.715543	0.86676	*0.000684	*0.000196	*0.000150978565216064
C	{0.1}	*0.000196	0.715543		0.890693	*0.000864	*0.000150	*0.000158190727233887
D	{1}	*0.000150	0.86676	0.890693		*0.001186	*0.000158	*0.000174045562744141
E	{10}	*0.000176	*0.000684	*0.000864	*0.001186311244964		*0.000180	*0.00019603967666626
F	{100}	*0.000176	*0.000196	*0.000150	*0.000158	*0.000180900096893		0.071724
G	{500}	*0.000180	*0.000150	*0.000158	*0.000174	*0.000196	0.071724	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	3.996000	4.238000	3.986000	2.044000	.4015000	0.000000
A	{0}		0.608168	0.218718	0.371592	*0.000177	*0.000180	*0.00019603967666626
B	{0.01	0.608168		0.299801	0.965248	*0.000196	*0.000150	*0.00015819072
C	{0.1}	0.218718	0.299801		0.51718	*0.000151	*0.000158	*0.000174045562744141
D	{1}	0.371592	0.965248	0.51718		*0.000181	*0.000196	*0.000150978565216064
E	{10}	*0.000177	*0.000196	*0.000151	*0.0001812577247		*0.000178	*0.000180959701538086
F	{100}	*0.000180	*0.000150	*0.000158	*0.000196	*0.000178217887878		0.095753
G	{500}	*0.000196	*0.000158	*0.000174	*0.000150	*0.000180	0.095753	

APPENDIX 41: ANOVA: *Ventricaria ventricosa* : Biochemical composition (Protein)

* = Significant difference : p<0.05

ANOVA-PROTEIN-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	3.870000	3.851000	3.841000	2.851000	.3889000	0.000000
A	{0}		*0.000244(	*0.000257(	*0.000213(	0.131126	*0.000176(	*0.000180900096893311
B	{0.01	*0.000244021415710(		0.928261	0.989326	*0.001279(	*0.000158(	*0.000174045562744141
C	{0.1}	*0.000257(	0.928261		0.962262	*0.000864(	*0.000150(	*0.000158190727233887
D	{1}	*0.000213(	0.989326	0.962262		*0.000443(	*0.000196(	*0.000150978565216064
E	{10}	0.131126	*0.001279(	*0.000864(	*0.000443160533905(		*0.000180(	*0.00019603967666626
F	{100}	*0.000176(	*0.000158(	*0.000150(	*0.000196(	*0.000180900096893(		0.081325
G	{500}	*0.000180(	*0.000174(	*0.000158(	*0.000150(	*0.000196(	0.081325	

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	8.014000	8.153000	8.394000	4.333000	.8298000	.0849400
A	{0}		*0.000180(	*0.000196(	*0.000150(	0.175232	*0.000177(	*0.000180959701538086
B	{0.01	*0.000180900096893(		0.725761	0.601602	*0.000176(	*0.000196(	*0.000150978565216064
C	{0.1}	*0.000196(	0.725761		0.544838	*0.000180(	*0.000150(	*0.000158190727233887
D	{1}	*0.000150(	0.601602	0.544838		*0.000196(	*0.000158(	*0.000174045562744141
E	{10}	0.175232	*0.000176(	*0.000180(	*0.000196039676666(		*0.000180(	*0.00019603967666626
F	{100}	*0.000177(	*0.000196(	*0.000150(	*0.000158(	*0.000180959701538(		0.07576
G	{500}	*0.000180(	*0.000150(	*0.000158(	*0.000174(	*0.000196(	0.07576	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	4.508000	4.354000	4.131000	3.847000	.4354000	0.000000
A	{0}		*0.000151(	*0.000196(	*0.000182(	*0.000186(	*0.000176(	*0.000180900096893311
B	{0.01	*0.000151157379150(		0.469278	0.19871	*0.029425(	*0.000158(	*0.000174045562744141
C	{0.1}	*0.000196(	0.469278		0.29958	0.068065	*0.000150(	*0.000158190727233887
D	{1}	*0.000182(	0.19871	0.29958		0.191685	*0.000196(	*0.000150978565216064
E	{10}	*0.000186(	*0.029425(	0.068065	0.191685		*0.000180(	*0.00019603967666626
F	{100}	*0.000176(	*0.000158(	*0.000150(	*0.000196(	*0.000180900096893(		0.054068
G	{500}	*0.000180(	*0.000174(	*0.000158(	*0.000150(	*0.000196(	0.054068	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	8.362000	8.275000	8.220000	4.462000	.8056000	.0844300
A	{0}		*0.000150(	*0.000196(	*0.000180(	0.133688	*0.000180(	*0.000181257724761963
B	{0.01	*0.000150978565216(		0.842387	0.941767	*0.000196(	*0.000158(	*0.000174045562744141
C	{0.1}	*0.000196(	0.842387		0.899969	*0.000181(	*0.000150(	*0.000158190727233887
D	{1}	*0.000180(	0.941767	0.899969		*0.000176(	*0.000196(	*0.000150978565216064
E	{10}	0.133688	*0.000196(	*0.000181(	*0.000176846981048(		*0.000181(	*0.000196099281311035
F	{100}	*0.000180(	*0.000158(	*0.000150(	*0.000196(	*0.000181317329406(		0.115156
G	{500}	*0.000181(	*0.000174(	*0.000158(	*0.000150(	*0.000196(	0.115156	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	8.275000	8.085000	8.295000	7.256000	.8117000	.0814300
A	{0}		*0.000196(	*0.000180(	*0.000150(	*0.000176(	*0.001289(	*0.000288784503936768
B	{0.01	*0.000196039676666(		0.65786	0.962785	0.070836	*0.000150(	*0.000158190727233887
C	{0.1}	*0.000180(	0.65786		0.872396	0.068441	*0.000196(	*0.000150978565216064
D	{1}	*0.000150(	0.962785	0.872396		0.107977	*0.000158(	*0.000174045562744141
E	{10}	*0.000176(	0.070836	0.068441	0.107977		*0.000180(	*0.00019603967666626
F	{100}	*0.001289(	*0.000150(	*0.000196(	*0.000158(	*0.000180900096893(		0.103935
G	{500}	*0.000288(	*0.000158(	*0.000150(	*0.000174(	*0.000196(	0.103935	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	8.346000	8.536000	8.514000	4.456000	.8456000	.0806600
A	{0}		*0.000180(	*0.000150(	*0.000196(	0.067917	*0.000176(	*0.000180900096893311
B	{0.01	*0.000180900096893(		0.845663	0.631295	*0.000176(	*0.000196(	*0.000150978565216064
C	{0.1}	*0.000150(	0.845663		0.949753	*0.000196(	*0.000158(	*0.000174045562744141
D	{1}	*0.000196(	0.631295	0.949753		*0.000180(	*0.000150(	*0.000158190727233887
E	{10}	0.067917	*0.000176(	*0.000196(	*0.000180900096893(		*0.000180(	*0.00019603967666626
F	{100}	*0.000176(	*0.000196(	*0.000158(	*0.000150(	*0.0001809000968		0.042377
G	{500}	*0.000180(	*0.000150(	*0.000174(	*0.000158(	*0.000196(	0.042377	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		2.519000	8.198000	8.233000	8.256000	8.246000	7.008000	.8124000
A	{0}		*0.000180(	*0.000196(	*0.000158(	*0.000150(	*0.000176(	*0.00133544206619263
B	{0.01	*0.000180900096893(		0.935068	0.999076	0.992938	*0.013658(	*0.00019603967666626
C	{0.1}	*0.000196(	0.935068		0.998429	0.975944	*0.029075(	*0.000150978565216064
D	{1}	*0.000158(	0.999076	0.998429		0.981503	0.065976	*0.000174045562744141
E	{10}	*0.000150(	0.992938	0.975944	0.981503		*0.047206(	*0.000158190727233887
F	{100}	*0.000176(	*0.013658(	*0.029075(	0.065976	*0.047206580638885(		*0.000180900096893311

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.778500	8.172000	8.427000	8.507000	8.591000	4.485000	.8146000
A	{0}		*0.000180(	*0.000196(	*0.000150(	*0.000158(	0.05908	*0.000176846981048584
B	{0.01	*0.000180900096893(		0.470529	0.604082	0.625524	*0.000176(	*0.00019603967666626
C	{0.1}	*0.000196(	0.470529		0.819424	0.883119	*0.000180(	*0.000150978565216064
D	{1}	*0.000150(	0.604082	0.819424		0.810581	*0.000196(	*0.000158190727233887
E	{10}	*0.000158(	0.625524	0.883119	0.810581		*0.000150(	*0.000174045562744141
F	{100}	0.05908	*0.000176(	*0.000180(	*0.000196(	*0.000150978565216(		*0.000180900096893311

G {500} *0.001335 *0.000196(*0.000150 *0.0001740 *0.000158 *0.000180900096893311

G {500} *0.000176 *0.000196(*0.000150 *0.000158 *0.000174(*0.000180900096893311

APPENDIX 41: ANOVA: *Ventricaria ventricosa* : Biochemical composition (Lipid)

* = Significant difference : p<0.05

ANOVA-LIPID-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	4.009720	4.637690	6.426000	3.740661	3.580011	.2557338
A	{0}		*0.003502	*0.000177	*0.000158	*0.021791	*0.037629	*0.000176668167114258
B	{0.01}	*0.003502607345581		*0.010138	*0.000180	0.222866	0.139772	*0.000150978565216064
C	{0.1}	*0.000177	*0.010138750076293		*0.000176	*0.002294	*0.001109	*0.000158190727233887
D	{1}	*0.000158	*0.000180	*0.000176846981048		*0.000196	*0.000150	*0.000174045562744141
E	{10}	*0.021791	0.222866	*0.002294	*0.000196039676666		0.458976	*0.000196039676666626
F	{100}	*0.037629	0.139772	*0.001109	*0.000150	0.458976		*0.000180900096893311
G	{500}	*0.000176	*0.000150	*0.000158	*0.000174	*0.000196	*0.000180900096893311	

Newman-Keuls test; CO_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	3.983600	3.306100	3.278300	2.692230	.1737110	0.000000
A	{0}		*0.001890	0.522951	0.351122	0.051732	*0.000180	*0.000196039676666626
B	{0.01}	*0.001890003681182		*0.003194	*0.006152	*0.000208	*0.000158	*0.000174045562744141
C	{0.1}	0.522951	*0.003194034099578		0.885589	*0.027035	*0.000150	*0.000158190727233887
D	{1}	0.351122	*0.006152	0.885589		*0.020466	*0.000196	*0.000150978565216064
E	{10}	0.051732	*0.000208	*0.027035	*0.020466268062591		*0.000176	*0.000180900096893311
F	{100}	*0.000180	*0.000158	*0.000150	*0.000196	*0.000176668167114		0.375035
G	{500}	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	0.375035	

Newman-Keuls test; CR_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	3.722380	4.466850	4.666110	3.056270	.3774620	0.000000
A	{0}		*0.005326	*0.000187	*0.000197	0.839295	*0.000180	*0.000196039676666626
B	{0.01}	*0.005326151847839		*0.001656	*0.000694	*0.009119	*0.000196	*0.000150978565216064
C	{0.1}	*0.000187	*0.001656055450439		0.310983	*0.000204	*0.000150	*0.000158190727233887
D	{1}	*0.000197	*0.000694	0.310983		*0.000153	*0.000158	*0.000174045562744141
E	{10}	0.839295	*0.009119	*0.000204	*0.000153064727783		*0.000176	*0.000180900096893311
F	{100}	*0.000180	*0.000196	*0.000150	*0.000158	*0.000176668167114		0.066396
G	{500}	*0.000196	*0.000150	*0.000158	*0.000174	*0.000180	0.066396	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	3.134630	3.670130	3.117550	1.031820	.0186772	0.000000
A	{0}		0.976834	*0.039734	0.908935	*0.000176	*0.000180	*0.000196039676666626
B	{0.01}	0.976834		*0.013635	0.929569	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.039734	*0.013635373527527		*0.028644	*0.000150	*0.000158	*0.000174045562744141
D	{1}	0.908935	0.929569	*0.028644561767578		*0.000180	*0.000196	*0.000150978565216064
E	{10}	*0.000176	*0.000196	*0.000150	*0.000180900096893		*0.000263	*0.000392556190490723
F	{100}	*0.000180	*0.000150	*0.000158	*0.000196	*0.000263988971710		0.923005
G	{500}	*0.000196	*0.000158	*0.000174	*0.000150	*0.000392	0.923005	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	3.248160	3.626930	3.150306	2.498276	.3115041	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000180900096893		0.05242	0.592556	*0.002534	*0.000196	*0.000150978565216064
C	{0.1}	*0.000176	0.05242		*0.045600	*0.000273	*0.000150	*0.000158190727233887
D	{1}	*0.000196	0.592556	*0.045600354671478		*0.002773	*0.000180	*0.000196039676666626
E	{10}	*0.000150	*0.002534	*0.000273	*0.002773940563201		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176668167114		0.10319
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	0.10319	

Newman-Keuls test; CO_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	2.324850	1.146753	.7314100	.5224400	.0496320	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176668167114		*0.000181	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	*0.000181674957275		*0.032100	*0.008056	*0.000277	*0.000236900444030076
D	{1}	*0.000196	*0.000180	*0.032100379467010		0.250805	*0.004315	*0.00452804565429687
E	{10}	*0.000150	*0.000196	*0.008056	0.250805		*0.017038	*0.0246045589447021
F	{100}	*0.000158	*0.000150	*0.000277	*0.004315	*0.017038166522979		0.780237
G	{500}	*0.000174	*0.000158	*0.000236	*0.004528	*0.024604	0.780237	

Newman-Keuls test; CR_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	7.834230	8.114501	7.196590	6.261900	4.359000	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000180959701538		0.139753	*0.003265	*0.000181	*0.000196	*0.000150978565216064
C	{0.1}	*0.000176	0.139753		*0.000566	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000196	*0.003265	*0.000566363334655		*0.000287	*0.000180	*0.000196039676666626
E	{10}	*0.000150	*0.000181	*0.000196	*0.000287950038909		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176668167114		*0.000176668167114258
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	*0.000176668167114258	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	3.441156	3.600800	3.322710	2.671438	.2422810	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000180900096893		0.375761	0.508346	*0.001731	*0.000196	*0.000150978565216064
C	{0.1}	*0.000176	0.375761		0.280489	*0.000709	*0.000150	*0.000158190727233887
D	{1}	*0.000196	0.508346	0.280489		*0.002379	*0.000180	*0.000196039676666626
E	{10}	*0.000150	*0.001731	*0.000709	*0.002379357814788		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000196	*0.000150	*0.000180	*0.000176668167114		0.186668
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000180	0.186668	

APPENDIX 41: ANOVA: *Ventricaria ventricosa* : Biochemical composition (Lipid)

* = Significant difference : p<0.05

ANOVA-LIPID-VEN

Newman-Keuls test; FE_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	9.847970	9.064310	7.418630	4.601480	.4009720	0.000000
A	{0}		*0.000150	*0.000196	*0.000180	*0.000177	*0.000176	*0.000180900096893311
B	{0.01}	*0.000150		*0.001148	*0.000180	*0.000196	*0.000158	*0.000174045562744141
C	{0.1}	*0.000196	*0.001148		*0.000176	*0.000180	*0.000150	*0.000158190727233887
D	{1}	*0.000180	*0.000180	*0.000176		*0.000176	*0.000196	*0.000150978565216064
E	{10}	*0.000177	*0.000196	*0.000180	*0.000176		*0.000180	*0.00019603967666626
F	{100}	*0.000176	*0.000158	*0.000150	*0.000196	*0.000180		0.05288
G	{500}	*0.000180	*0.000174	*0.000158	*0.000150	*0.000196	0.05288	

Newman-Keuls test; FE_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	4.858680	1.018756	.9143000	.1306100	.0613870	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	*0.000176		0.558892	*0.000594	*0.000579	*0.00047612190246582
D	{1}	*0.000196	*0.000180	0.558892		*0.000651	*0.000790	*0.000796020030975342
E	{10}	*0.000150	*0.000196	*0.000594	*0.000651		0.697523	0.739235
F	{100}	*0.000158	*0.000150	*0.000579	*0.000790	0.697523		0.730203
G	{500}	*0.000174	*0.000158	*0.000476	*0.000796	0.739235	0.730203	

Newman-Keuls test; MN_V_C4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	3.474220	2.653050	2.571340	1.339590	.1959150	0.000000
A	{0}		0.065569	*0.035111	*0.038020	*0.000196	*0.000150	*0.000158190727233887
B	{0.01}	0.065569		*0.001988	*0.001658	*0.000150	*0.000158	*0.000174045562744141
C	{0.1}	*0.035111	*0.001988		0.673042	*0.000191	*0.000196	*0.000150978565216064
D	{1}	*0.038020	*0.001658	0.673042		*0.000185	*0.000180	*0.00019603967666626
E	{10}	*0.000196	*0.000150	*0.000191	*0.000185		*0.000198	*0.000189423561096191
F	{100}	*0.000150	*0.000158	*0.000196	*0.000180	*0.000198		0.318876
G	{500}	*0.000158	*0.000174	*0.000150	*0.000196	*0.000189	0.318876	

Newman-Keuls test; MN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	6.121570	5.563970	3.123240	2.808110	.6060290	.0522439
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000176		*0.000180	*0.006621	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	*0.006621		*0.000176	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.000176		0.092495	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000196	*0.000180	0.092495		*0.000176	*0.000180900096893311
F	{100}	*0.000150	*0.000150	*0.000196	*0.000180	*0.000176		*0.00690716505050659
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.00690716505050659	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	6.661090	3.448100	2.925660	1.304430	.2599130	.0637811
A	{0}		*0.000180	0.08402	0.385605	*0.000180	*0.000196	*0.000150978565216064
B	{0.01}	*0.000180		*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
C	{0.1}	0.08402	*0.000176		*0.038652	*0.000196	*0.000150	*0.000158190727233887
D	{1}	0.385605	*0.000196	*0.038652		*0.000176	*0.000180	*0.00019603967666626
E	{10}	*0.000180	*0.000150	*0.000196	*0.000176		*0.000239	*0.000204741954803467
F	{100}	*0.000196	*0.000158	*0.000150	*0.000180	*0.000239		0.31836
G	{500}	*0.000150	*0.000174	*0.000158	*0.000196	*0.000204	0.31836	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	8.835900	8.625250	8.189230	2.194240	.2677500	.1906900
A	{0}		*0.001297	*0.000474	*0.000200	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.001297		0.247424	*0.006335	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000474	0.247424		*0.025658	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000200	*0.006335	*0.025658		*0.000176	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176		*0.000176	*0.000180900096893311
F	{100}	*0.000150	*0.000150	*0.000196	*0.000180	*0.000176		0.665619
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	0.665619	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	3.722380	2.339590	1.371400	.7614540	.0927329	0.000000
A	{0}		*0.005326	*0.001489	*0.000180	*0.000196	*0.000150	*0.000158190727233887
B	{0.01}	*0.005326		*0.000186	*0.000196	*0.000150	*0.000158	*0.000174045562744141
C	{0.1}	*0.001489	*0.000186		*0.000316	*0.000181	*0.000196	*0.000150978565216064
D	{1}	*0.000180	*0.000196	*0.000316		*0.006332	*0.000197	*0.000209271907806396
E	{10}	*0.000196	*0.000150	*0.000181	*0.006332		*0.003492	*0.00352191925048828
F	{100}	*0.000150	*0.000158	*0.000196	*0.000197	*0.003492		0.632314
G	{500}	*0.000158	*0.000174	*0.000150	*0.000209	*0.003521	0.632314	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	2.481580	2.755860	2.481580	1.495011	.2364040	0.000000
A	{0}		*0.000196	*0.000176	*0.000180	*0.000150	*0.000158	*0.000174045562744141
B	{0.01}	*0.000196		0.289484	1	*0.000223	*0.000180	*0.00019603967666626
C	{0.1}	*0.000176	0.289484		0.138326	*0.000209	*0.000150	*0.000158190727233887
D	{1}	*0.000180	1	0.138326		*0.000323	*0.000196	*0.000150978565216064
E	{10}	*0.000150	*0.000223	*0.000209	*0.000323		*0.000178	*0.000181317329406738
F	{100}	*0.000158	*0.000180	*0.000150	*0.000196	*0.000178		0.196914
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000181	0.196914	

APPENDIX 41: ANOVA: *Ventricaria ventricosa* : Biochemical composition (Lipid)

* = Significant difference : p<0.05

ANOVA-LIPID-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	1.332220	1.305627	1.239016	.5537850	.3461160	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176		0.890538	0.876383	*0.005322	*0.001178	*0.000208497047424316
C	{0.1}	*0.000180	0.890538		0.730584	*0.003868	*0.001031	*0.000201821327209473
D	{1}	*0.000196	0.876383	0.730584		*0.002958	*0.001043	*0.000247061252593994
E	{10}	*0.000150	*0.005322	*0.003868	*0.002958	774566503	0.291764	*0.0283021926879883
F	{100}	*0.000158	*0.001178	*0.001031	*0.001043	0.291764		0.089321
G	{500}	*0.000174	*0.000208	*0.000201	*0.000247	*0.028302	0.089321	

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	6.269300	6.329550	2.207300	1.254166	.6530480	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000180		0.735064	*0.000176	*0.000180	*0.000196	*0.000150978565216064
C	{0.1}	*0.000176	0.735064		*0.000180	*0.000196	*0.000150	*0.000158190727233887
D	{1}	*0.000196	*0.000176	*0.000180		*0.000245	*0.000181	*0.00019603967666626
E	{10}	*0.000150	*0.000180	*0.000196	*0.000245		*0.004091	*0.000187516212463379
F	{100}	*0.000158	*0.000196	*0.000150	*0.000181	*0.004091		*0.00233536958694458
G	{500}	*0.000174	*0.000150	*0.000158	*0.000196	*0.000187	*0.00233536958694458	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	4.780310	2.324850	2.351340	1.442400	.1162430	0.000000
A	{0}		*0.000176	*0.003222	*0.001661	*0.000196	*0.000150	*0.000158190727233887
B	{0.01	*0.000176		*0.000196	*0.000180	*0.000150	*0.000158	*0.000174045562744141
C	{0.1}	*0.003222	*0.000196		0.890959	*0.000519	*0.000180	*0.00019603967666626
D	{1}	*0.001661	*0.000180	0.890959		*0.000912	*0.000196	*0.000150978565216064
E	{10}	*0.000196	*0.000150	*0.000519	*0.000912	785530090	*0.000179	*0.000183761119842529
F	{100}	*0.000150	*0.000158	*0.000180	*0.000196	*0.000179	708039978	0.549596
G	{500}	*0.000158	*0.000174	*0.000196	*0.000150	*0.000183	0.549596	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	4.284000	4.048900	3.042210	1.449770	.2576929	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176		0.199249	*0.000188	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	0.199249		*0.000214	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000188	*0.000214099884033		*0.000176	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000196	*0.000180	*0.000176727771759		*0.000181	*0.000181496143341064
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000181078910827		0.161868
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000181	0.161868	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	2.977900	3.142000	3.534510	2.362360	.9612870	0.000000
A	{0}		0.545177	0.809658	0.086357	*0.004665	*0.000196	*0.000150978565216064
B	{0.01	0.545177		0.669708	*0.047353	*0.005980	*0.000180	*0.00019603967666626
C	{0.1}	0.809658	0.669708		0.057427	*0.005261	*0.000150	*0.000158190727233887
D	{1}	0.086357	*0.047353	0.057427		*0.000320	*0.000158	*0.000174045562744141
E	{10}	*0.004665	*0.005980	*0.005261	*0.000320		*0.000178	*0.000180900096893311
F	{100}	*0.000196	*0.000180	*0.000150	*0.000158	*0.000178		*0.000326812267303467
G	{500}	*0.000150	*0.000196	*0.000158	*0.000174	*0.000180	*0.000326812267303467	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	6.282330	5.335000	3.560630	2.322240	.9273290	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
B	{0.01	*0.000176		*0.000250	*0.000180	*0.000196	*0.000150	*0.000158190727233887
C	{0.1}	*0.000180	*0.000250399112701		*0.000176	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000196	*0.000180	*0.000176668167114		*0.000179	*0.000180	*0.00019603967666626
E	{10}	*0.000150	*0.000196	*0.000180	*0.000179171562194		*0.000176	*0.000180900096893311
F	{100}	*0.000158	*0.000150	*0.000196	*0.000180	*0.000176	9661903381	*0.000269412994384766
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000269412994384766	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.095450	7.692910	4.690560	3.761560	3.300507	1.119325	.4414610
A	{0}		*0.000150	*0.000197	*0.009119	0.297634	*0.000176	*0.000180900096893311
B	{0.01	*0.000150		*0.000176	*0.000180	*0.000196	*0.000158	*0.000174045562744141
C	{0.1}	*0.000197	*0.000176668167114		*0.000387	*0.000185	*0.000150	*0.000158190727233887
D	{1}	*0.009119	*0.000180	*0.000387132167816		*0.029117	*0.000196	*0.000150978565216064
E	{10}	0.297634	*0.000196	*0.000185	*0.029117286205291		*0.000180	*0.00019603967666626
F	{100}	*0.000176	*0.000158	*0.000150	*0.000196	*0.000180900096893		*0.00318241119384766

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		9.545000	10.78836	3.369730	2.237344	1.280811	.9299410	.5485610
A	{0}		*0.000179	*0.000176	*0.000180	*0.000196	*0.000150	*0.000158190727233887
B	{0.01	*0.000179		*0.000180	*0.000196	*0.000150	*0.000158	*0.000174045562744141
C	{0.1}	*0.000176	*0.000180900096893		*0.000185	*0.000180	*0.000196	*0.000150978565216064
D	{1}	*0.000180	*0.000196	*0.000185608863830		*0.000243	*0.000184	*0.000196099281311035
E	{10}	*0.000196	*0.000150	*0.000180	*0.000243246555328		0.064125	*0.00254172086715698
F	{100}	*0.000150	*0.000158	*0.000196	*0.000184	0.064125		*0.0464401245117187

G {500} *0.0001809 *0.000174(*0.000158: *0.000150(*0.000196(*0.00318241119384766

G {500} *0.000158: *0.000174(*0.000150(*0.000196(*0.002541; *0.0464401245117187

APPENDIX 42: ANOVA: *Ventricaria ventricosa* : DNA Damage (RAPD-Appearance of new band)

* = Significant difference : p<0.05

ANOVA-APPEARANCE-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	25.00000	50.00000	25.00000	0.000000	0.000000	0.000000
A	{0}		*0.000150	*0.000174	*0.000158	1	1	1
B	{0.01	*0.000150	978565216	*0.000180	1	*0.000196	*0.000180	*0.000176
C	{0.1}	*0.000174	*0.000180	900096893	*0.000176	*0.000158	*0.000150	*0.000196
D	{1}	*0.000158	1	*0.000176	6681671142	*0.000150	*0.000196	*0.000180
E	{10}	1	*0.000196	*0.000158	*0.000150	978565216	1	1
F	{100}	1	*0.000180	*0.000150	*0.000196	1		1
G	{500}	1	*0.000176	*0.000196	*0.000180	1	1	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	42.78000	44.86000	35.14000	30.24000	2.080000	0.000000
A	{0}		*0.001029	*0.000897	*0.003918	*0.008226	0.961832	1
B	{0.01	*0.001029	7298431396	0.793929	0.34455	0.27568	*0.000829	*0.000775
C	{0.1}	*0.000897	0.793929		0.447734	0.283058	*0.000775	*0.000699
D	{1}	*0.003918	0.34455	0.447734		0.540519	*0.002376	*0.002610
E	{10}	*0.008226	0.27568	0.283058	0.540519		*0.003009	*0.004624
F	{100}	0.961832	*0.000829	*0.000775	*0.002376	*0.003009	7365379333	0.793929
G	{500}	1	*0.000775	*0.000699	*0.002610	*0.004624	0.793929	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	27.96000	20.00000	16.67000	12.22000	9.170000	8.890000
A	{0}		*0.000174	*0.000158	*0.000150	*0.000196	*0.000185	*0.000178
B	{0.01	*0.000174	0455627441	*0.000187	*0.000180	*0.000196	*0.000150	*0.000158
C	{0.1}	*0.000158	*0.000187	0393753051	*0.018018	*0.000222	*0.000196	*0.000151
D	{1}	*0.000150	*0.000180	*0.018018	3649063111	*0.003137	*0.000251	*0.000281
E	{10}	*0.000196	*0.000196	*0.000222	*0.003137	7673149108	*0.027816	*0.044434
F	{100}	*0.000185	*0.000150	*0.000196	*0.000251	*0.027816	7246093758	0.824936
G	{500}	*0.000178	*0.000158	*0.000151	*0.000281	*0.044434	0.824936	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	34.76000	36.38000	29.76000	28.81000	26.45000	0.000000
A	{0}		*0.000359	*0.000335	*0.000896	*0.000831	*0.000929	1
B	{0.01	*0.000359	7736358642	0.773898	0.381198	0.543517	0.461528	*0.000292
C	{0.1}	*0.000335	0.773898		0.47387	0.537246	0.413065	*0.000278
D	{1}	*0.000896	0.381198	0.47387		0.866127	0.823091	*0.000660
E	{10}	*0.000831	0.543517	0.537246	0.866127		0.676049	*0.000510
F	{100}	*0.000929	0.461528	0.413065	0.823091	0.676049		*0.000442
G	{500}	1	*0.000292	*0.000278	*0.000660	*0.000510	*0.000442	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	15.93000	15.93000	15.93000	11.11000	0.000000	0.000000
A	{0}		*0.000151	*0.000158	*0.000174	*0.000218	1	1
B	{0.01	*0.000151	0381698608	1	1	*0.009338	*0.000196	*0.000180
C	{0.1}	*0.000158	1		1	*0.023491	*0.000151	*0.000196
D	{1}	*0.000174	1	1		*0.040646	*0.000158	*0.000151
E	{10}	*0.000218	*0.009338	*0.023491	*0.040646255016326		*0.000191	*0.000180
F	{100}	1	*0.000196	*0.000151	*0.000158	*0.000191		1
G	{500}	1	*0.000180	*0.000196	*0.000151	*0.000180	1	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	52.56000	43.47000	31.43000	28.39000	18.60000	16.67000
A	{0}		*0.000174	*0.000158	*0.000207	*0.000299	*0.003385	*0.002934
B	{0.01	*0.000174	0455627441	0.068556	*0.001274	*0.000788	*0.000175	*0.000169
C	{0.1}	*0.000158	0.068556		*0.020530	*0.014444	*0.000645	*0.000488
D	{1}	*0.000207	*0.001274	*0.020530402660369		0.519982	*0.036565	*0.028794
E	{10}	*0.000299	*0.000788	*0.014444	0.519982		0.051881	0.057211
F	{100}	*0.003385	*0.000175	*0.000645	*0.036565	0.051881		0.681594
G	{500}	*0.002934	*0.000169	*0.000488	*0.028794	0.057211	0.681594	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	18.34000	18.34000	20.00000	17.56000	16.84000	12.08000
A	{0}		*0.000150	*0.000158	*0.000174	*0.000196	*0.000180	*0.000179
B	{0.01	*0.000150	9565216	1	0.605728	0.655007	0.662236	*0.012132
C	{0.1}	*0.000158	1		0.347673	0.892299	0.816004	*0.018229
D	{1}	*0.000174	0.605728	0.347673		0.503181	0.385563	*0.004233
E	{10}	*0.000196	0.655007	0.892299	0.503181		0.679847	*0.016392
F	{100}	*0.000180	0.662236	0.816004	0.385563	0.679847		*0.014693
G	{500}	*0.000179	*0.012132	*0.018229	*0.004233	*0.016392	*0.014693	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	49.21000	48.67000	46.80000	42.30000	30.75000	0.000000
A	{0}		*0.001885	*0.001586	*0.001612	*0.002488	*0.013992	1
B	{0.01	*0.001885	5333328247	0.954825	0.964158	0.879547	0.325814	*0.001445
C	{0.1}	*0.001586	0.954825		0.844342	0.777725	0.264774	*0.001166
D	{1}	*0.001612	0.964158	0.844342		0.637603	0.233289	*0.001119
E	{10}	*0.002488	0.879547	0.777725	0.637603		0.236766	*0.001412
F	{100}	0.*013992	0.325814	0.264774	0.233289	0.236766		*0.005498
G	{500}	1	*0.001445	*0.001166	*0.001119	*0.001412	*0.005498	

APPENDIX 42: ANOVA: *Ventricaria ventricosa* : DNA Damage (RAPD-Appearance of new band)

* = Significant difference : p<0.05

ANOVA-APPEARANCE-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	52.85000	49.52000	39.50000	32.22000	26.30000	0.000000
A	{0}		*0.001127	*0.001516	*0.007043	*0.019693	*0.036871	1
B	{0.01}	*0.001127		0.730037	0.361631	0.176121	0.086607	*0.000872
C	{0.1}	*0.001516	0.730037		0.307252	0.196122	0.11161	*0.001116
D	{1}	*0.007043	0.361631	0.307252		0.454218	0.369395	*0.004662
E	{10}	*0.019693	0.176121	0.196122	0.454218		0.541404	*0.011191
F	{100}	*0.036871	0.086607	0.11161	0.369395	0.541404		*0.014845
G	{500}	1	*0.000872	*0.001116	*0.004662	*0.011191	*0.014845	

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	39.21000	34.33000	31.20000	27.07000	26.04000	6.250000
A	{0}		*0.002255	*0.005240	*0.008046	*0.014718	*0.010789	0.424695
B	{0.01}	*0.002255		0.531217	0.556595	0.411063	0.446503	*0.007262
C	{0.1}	*0.005240	0.531217		0.686745	0.615675	0.700513	*0.017238
D	{1}	*0.008046	0.556595	0.686745		0.595417	0.77927	*0.024874
E	{10}	*0.014718	0.411063	0.615675	0.595417		0.894229	*0.039868
F	{100}	*0.010789	0.446503	0.700513	0.77927	0.894229		*0.020936
G	{500}	0.424695	*0.007262	*0.017238	*0.024874	*0.039868	*0.020936	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	49.01000	48.69000	44.23000	41.54000	35.95000	0.000000
A	{0}		*0.000202	*0.000180	*0.000211	*0.000266	*0.000368	1
B	{0.01}	*0.000202		0.961704	0.748835	0.669406	0.314642	*0.000178
C	{0.1}	*0.000180	0.961704		0.505662	0.532254	0.2518	*0.000173
D	{1}	*0.000211	0.748835	0.505662		0.686578	0.434869	*0.000228
E	{10}	*0.000266	0.669406	0.532254	0.686578		0.406288	*0.000215
F	{100}	*0.000368	0.314642	0.2518	0.434869	0.406288		*0.000239
G	{500}	1	*0.000178	*0.000173	*0.000228	*0.000215	*0.000239	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	28.67000	42.53000	46.29000	43.10000	31.16000	0.000000
A	{0}		*0.003665	*0.000425	*0.000376	*0.000495	*0.003452	1
B	{0.01}	*0.003665		0.166477	0.157318	0.230254	0.733824	*0.001468
C	{0.1}	*0.000425	0.166477		0.861001	0.937899	0.135449	*0.000369
D	{1}	*0.000376	0.157318	0.861001		0.663494	0.197882	*0.000308
E	{10}	*0.000495	0.230254	0.937899	0.663494		0.252967	*0.000389
F	{100}	*0.003452	0.733824	0.135449	0.197882	0.252967		*0.001953
G	{500}	1	*0.001468	*0.000369	*0.000308	*0.000389	*0.001953	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	39.21000	48.47000	63.33000	63.07000	65.52000	60.81000
A	{0}		*0.000291	*0.000210	*0.000161	*0.000153	*0.000176	*0.000198
B	{0.01}	*0.000291		0.239236	*0.042788	*0.030878	*0.034038	*0.031350
C	{0.1}	*0.000210	0.239236		0.244008	0.164682	0.21354	0.123732
D	{1}	*0.000161	*0.042788	0.244008		0.973073	0.775602	0.940489
E	{10}	*0.000153	*0.030878	0.164682	0.973073		0.943656	0.768652
F	{100}	*0.000176	*0.034038	0.21354	0.775602	0.943656		0.922262
G	{500}	*0.000198	*0.031350	0.123732	0.940489	0.768652	0.922262	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	51.44000	42.61000	38.82000	34.09000	35.23000	31.95000
A	{0}		*0.000183	*0.000254	*0.000350	*0.000426	*0.000543	*0.000340
B	{0.01}	*0.000183		0.186331	0.152151	0.099014	0.094568	0.07292
C	{0.1}	*0.000254	0.186331		0.560405	0.553626	0.49405	0.476715
D	{1}	*0.000350	0.152151	0.560405		0.741754	0.581039	0.706011
E	{10}	*0.000426	0.099014	0.553626	0.741754		0.86026	0.741327
F	{100}	*0.000543	0.094568	0.49405	0.581039	0.86026		0.864754
G	{500}	*0.000340	0.07292	0.476715	0.706011	0.741327	0.864754	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	39.80000	32.40000	27.38000	23.57000	17.14000	16.36000
A	{0}		*0.000550	*0.002353	*0.006725	*0.013254	*0.048835	*0.025042
B	{0.01}	*0.000550		0.275127	0.173464	0.104989	*0.025758	*0.028109
C	{0.1}	*0.002353	0.275127		0.453861	0.389613	0.135281	0.155687
D	{1}	*0.006725	0.173464	0.453861		0.568046	0.289621	0.363857
E	{10}	*0.013254	0.104989	0.389613	0.568046		0.340457	0.525664
F	{100}	*0.048835	*0.025758	0.135281	0.289621	0.340457		0.906503
G	{500}	*0.025042	*0.028109	0.155687	0.363857	0.525664	0.906503	

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	41.52000	52.76000	51.77000	39.07000	34.10000	33.41000
A	{0}		*0.005405	*0.001316	*0.001181	*0.005724	*0.008519	*0.003838
B	{0.01}	*0.005405		0.489204	0.304022	0.802474	0.72537	0.832585
C	{0.1}	*0.001316	0.489204		0.919481	0.505057	0.340941	0.381643
D	{1}	*0.001181	0.304022	0.919481		0.406607	0.296651	0.355623
E	{10}	*0.005724	0.802474	0.505057	0.406607		0.613028	0.828055
F	{100}	*0.008519	0.72537	0.340941	0.296651	0.613028		0.943834
G	{500}	*0.003838	0.832585	0.381643	0.355623	0.828055	0.943834	

APPENDIX 42: ANOVA: *Ventricaria ventricosa* : DNA Damage (RAPD-Disappearance of band)

* = Significant difference : p<0.05

ANOVA-DISAPPEARANCE-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	89.24000	96.30000	97.86000	100.0000	100.0000	100.0000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		*0.025709	*0.022201	*0.009307	*0.014010	*0.019186
C	{0.1}	*0.000180	*0.025709		0.589818	0.413503	0.572466	0.690356
D	{1}	*0.000196	*0.022201	0.589818		0.461671	0.73444	0.872191
E	{10}	*0.000150	*0.009307	0.413503	0.461671		1	1
F	{100}	*0.000158	*0.014010	0.572466	0.73444	1		1
G	{500}	*0.000174	*0.019186	0.690356	0.872191	1	1	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	91.67000	92.59000	92.86000	95.49000	100.0000	100.0000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.764962	0.918335	0.59754	0.093918	0.12345
C	{0.1}	*0.000180	0.764962		0.930043	0.612069	0.111481	0.157135
D	{1}	*0.000196	0.918335	0.930043		0.3981	0.078945	0.129816
E	{10}	*0.000150	0.59754	0.612069	0.3981		0.157163	0.322854
F	{100}	*0.000158	0.093918	0.111481	0.078945	0.157163		1
G	{500}	*0.000174	0.12345	0.157135	0.129816	0.322854	1	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	67.71000	72.59000	75.11000	79.49000	85.54000	63.93000
A	{0}		*0.000203	*0.000214	*0.000178	*0.000171	*0.000179	*0.000192
B	{0.01	*0.000203		0.642614	0.756322	0.668971	0.446371	0.718841
C	{0.1}	*0.000214	0.642614		0.810124	0.783888	0.601913	0.684079
D	{1}	*0.000178	0.756322	0.810124		0.676808	0.580472	0.702847
E	{10}	*0.000171	0.668971	0.783888	0.676808		0.565876	0.57128
F	{100}	*0.000179	0.446371	0.601913	0.580472	0.565876		0.340297
G	{500}	*0.000192	0.718841	0.684079	0.702847	0.57128	0.340297	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	68.57000	69.38000	76.36000	80.12000	87.62000	86.00000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000174	*0.000158
B	{0.01	*0.000176		0.895111	0.422066	0.265347	0.061865	0.074693
C	{0.1}	*0.000180	0.895111		0.266216	0.211308	0.058783	0.065552
D	{1}	*0.000196	0.422066	0.266216		0.54281	0.284641	0.278223
E	{10}	*0.000150	0.265347	0.211308	0.54281		0.447877	0.345843
F	{100}	*0.000174	0.061865	0.058783	0.284641	0.447877		0.792087
G	{500}	*0.000158	0.074693	0.065552	0.278223	0.345843	0.792087	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	79.58000	81.33000	83.15000	89.17000	90.00000	91.67000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.79617	0.854487	0.494761	0.53891	0.484755
C	{0.1}	*0.000180	0.79617		0.788227	0.483641	0.574796	0.545879
D	{1}	*0.000196	0.854487	0.788227		0.380332	0.570296	0.588094
E	{10}	*0.000150	0.494761	0.483641	0.380332		0.902467	0.925393
F	{100}	*0.000158	0.53891	0.574796	0.570296	0.902467		0.805294
G	{500}	*0.000174	0.484755	0.545879	0.588094	0.925393	0.805294	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	54.44000	75.00000	75.56000	81.35000	86.57000	91.67000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		*0.000934	*0.001846	*0.000526	*0.000225	*0.000171
C	{0.1}	*0.000180	*0.000934		0.909402	0.410149	0.12346	*0.026991
D	{1}	*0.000196	*0.001846	0.909402		0.250343	0.092004	*0.022491
E	{10}	*0.000150	*0.000526	0.410149	0.250343		0.297894	0.117941
F	{100}	*0.000158	*0.000225	0.12346	0.092004	0.297894		0.30872
G	{500}	*0.000174	*0.000171	*0.026991	*0.022491	0.117941	0.30872	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	71.31000	76.86000	80.85000	83.18000	86.51000	100.0000
A	{0}		*0.000178	*0.000183	*0.000198	*0.000153	*0.000160	*0.000174
B	{0.01	*0.000178		0.586856	0.615147	0.642748	0.564901	0.101732
C	{0.1}	*0.000183	0.586856		0.695321	0.804454	0.769702	0.195709
D	{1}	*0.000198	0.615147	0.695321		0.81881	0.839397	0.264167
E	{10}	*0.000153	0.642748	0.804454	0.81881		0.743582	0.244809
F	{100}	*0.000160	0.564901	0.769702	0.839397	0.743582		0.197806
G	{500}	*0.000174	0.101732	0.195709	0.264167	0.244809	0.197806	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	84.07000	85.17000	85.83000	87.74000	89.22000	98.61000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.756693	0.869799	0.721311	0.590516	*0.009705
C	{0.1}	*0.000180	0.756693		0.852399	0.745267	0.658089	*0.012641
D	{1}	*0.000196	0.869799	0.852399		0.59184	0.604384	*0.011967
E	{10}	*0.000150	0.721311	0.745267	0.59184		0.677184	*0.019277
F	{100}	*0.000158	0.590516	0.658089	0.604384	0.677184		*0.017459
G	{500}	*0.000174	*0.009705	*0.012641	*0.011967	*0.019277	*0.017459	

APPENDIX 42: ANOVA: *Ventricaria ventricosa* : DNA Damage (RAPD-Disappearance of band)

* = Significant difference : p<0.05

ANOVA-DISAPPEARANCE-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	47.90000	53.35000	55.73000	60.13000	64.02000	100.0000
A	{0}		*0.000509	*0.000515	*0.000618	*0.000457	*0.000376	*0.000174
B	{0.01}	*0.000509	2024803161	0.603475	0.730595	0.641037	0.536746	*0.001959
C	{0.1}	*0.000515	0.603475		0.81991	0.789229	0.729229	*0.003587
D	{1}	*0.000618	0.730595	0.81991		0.674463	0.704057	*0.003615
E	{10}	*0.000457	0.641037	0.789229	0.674463		0.710191	*0.004485
F	{100}	*0.000376	0.536746	0.729229	0.704057	0.710191		*0.003622
G	{500}	*0.000174	*0.001959	*0.003587	*0.003615	*0.004485	*0.003622	

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	47.90000	56.24000	61.89000	65.96000	67.72000	100.0000
A	{0}		*0.000992	*0.000717	*0.000604	*0.000491	*0.000518	*0.000176
B	{0.01}	*0.000992	7749633785	0.474526	0.454119	0.414068	0.439093	*0.004585
C	{0.1}	*0.000717	0.474526		0.626309	0.675182	0.745313	*0.012759
D	{1}	*0.000604	0.454119	0.626309		0.72524	0.865925	*0.021585
E	{10}	*0.000491	0.414068	0.675182	0.72524		0.879035	*0.024378
F	{100}	*0.000518	0.439093	0.745313	0.865925	0.879035		*0.013120
G	{500}	*0.000176	*0.004585	*0.012759	*0.021585	0.02437*87	*0.013120	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	71.88000	73.13000	76.15000	76.97000	77.44000	100.0000
A	{0}		*0.000180	*0.000189	*0.000206	*0.000174	*0.000179	*0.000174
B	{0.01}	*0.000180	0656318664	0.905801	0.911281	0.959807	0.981908	0.133714
C	{0.1}	*0.000189	0.905801		0.775075	0.927539	0.974889	0.125294
D	{1}	*0.000206	0.911281	0.775075		0.938138	0.991574	0.144787
E	{10}	*0.000174	0.959807	0.927539	0.938138		0.964576	0.101855
F	{100}	*0.000179	0.981908	0.974889	0.991574	0.964576		*0.047162
G	{500}	*0.000174	0.133714	0.125294	0.144787	0.101855	*0.047162	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	74.44000	81.23000	81.27000	83.41000	87.50000	100.0000
A	{0}		*0.000176	*0.000181	*0.000196	*0.000151	*0.000158	*0.000174
B	{0.01}	*0.000176	9065856933	0.474451	0.744658	0.767561	0.629131	0.122189
C	{0.1}	*0.000181	0.474451		0.996704	0.969891	0.903396	0.301007
D	{1}	*0.000196	0.744658	0.996704		0.820208	0.781773	0.224383
E	{10}	*0.000151	0.767561	0.969891	0.820208		0.664724	0.206673
F	{100}	*0.000158	0.629131	0.903396	0.781773	0.664724		0.197407
G	{500}	*0.000174	0.122189	0.301007	0.224383	0.206673	0.197407	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	57.74000	70.29000	71.05000	75.60000	80.28000	78.91000
A	{0}		*0.000194	*0.000184	*0.000202	*0.000155	*0.000177	*0.000161
B	{0.01}	*0.000194	4303512573	0.20276	0.358985	0.271087	0.220579	0.216574
C	{0.1}	*0.000184	0.20276		0.936735	0.840351	0.821488	0.795787
D	{1}	*0.000202	0.358985	0.936735		0.635594	0.761228	0.686994
E	{10}	*0.000155	0.271087	0.840351	0.635594		0.873279	0.729816
F	{100}	*0.000177	0.220579	0.821488	0.761228	0.873279		0.886192
G	{500}	*0.000161	0.216574	0.795787	0.686994	0.729816	0.886192	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	59.70000	61.91000	67.06000	69.06000	71.63000	79.92000
A	{0}		*0.000197	*0.000220	*0.000226	*0.000192	*0.000189	*0.000182
B	{0.01}	*0.000197	4105834960	0.825676	0.739857	0.778443	0.744801	0.362188
C	{0.1}	*0.000220	0.825676		0.609075	0.752263	0.758697	0.395763
D	{1}	*0.000226	0.739857	0.609075		0.841994	0.888902	0.573834
E	{10}	*0.000192	0.778443	0.752263	0.841994		0.797902	0.527614
F	{100}	*0.000189	0.744801	0.758697	0.888902	0.797902		0.413899
G	{500}	*0.000182	0.362188	0.395763	0.573834	0.527614	0.413899	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	70.12000	74.88000	77.96000	81.78000	78.57000	85.66000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000158	*0.000150	*0.000174
B	{0.01}	*0.000176	6681671142	0.477959	0.471784	0.418217	0.580882	0.227222
C	{0.1}	*0.000180	0.477959		0.644376	0.719855	0.84042	0.491423
D	{1}	*0.000196	0.471784	0.644376		0.830142	0.926965	0.64871
E	{10}	*0.000158	0.418217	0.719855	0.830142		0.630565	0.561779
F	{100}	*0.000150	0.580882	0.84042	0.926965	0.630565		0.537633
G	{500}	*0.000174	0.227222	0.491423	0.64871	0.561779	0.537633	

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		0.000000	66.55000	73.08000	73.89000	74.93000	76.57000	81.04000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000151	*0.000158	*0.000174
B	{0.01}	*0.000176	7873764038	0.395482	0.597475	0.680626	0.669243	0.41663
C	{0.1}	*0.000180	0.395482		0.915036	0.966716	0.964774	0.81908
D	{1}	*0.000196	0.597475	0.915036		0.891047	0.931522	0.773576
E	{10}	*0.000151	0.680626	0.966716	0.891047		0.828975	0.69692
F	{100}	*0.000158	0.669243	0.964774	0.931522	0.828975		0.558035
G	{500}	*0.000174	0.41663	0.81908	0.773576	0.69692	0.558035	

APPENDIX 42: ANOVA: *Ventricaria ventricosa* : DNA Damage (RAPD-Similarity of band)

* = Significant difference : p<0.05

ANOVA-SIMILIAR-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	28.70000	11.00000	8.330000	0.000000	0.000000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000174	*0.000158	*0.000150
B	{0.01	*0.000176		*0.000210	*0.000198	*0.000158	*0.000151	*0.000196
C	{0.1}	*0.000180	*0.000210		0.394593	*0.019837	*0.013219	*0.007467
D	{1}	*0.000196	*0.000198	0.394593		0.067594	*0.039791	*0.016065
E	{10}	*0.000174	*0.000158	*0.019837	0.067594		1	1
F	{100}	*0.000158	*0.000151	*0.013219	*0.039791	1		1
G	{500}	*0.000150	*0.000196	*0.007467	*0.016065	1	1	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	33.33000	44.44000	52.78000	18.06000	0.000000	0.000000
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000174	*0.000158
B	{0.01	*0.000196		*0.004688	*0.000270	*0.000532	*0.000196	*0.000180
C	{0.1}	*0.000180	*0.004688		*0.024022	*0.000182	*0.000150	*0.000196
D	{1}	*0.000176	*0.000270	*0.024022		*0.000196	*0.000158	*0.000150
E	{10}	*0.000150	*0.000532	*0.000182	*0.000196		*0.000376	*0.000242
F	{100}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000376		1
G	{500}	*0.000158	*0.000180	*0.000196	*0.000150	*0.000242	1	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	39.82000	31.09000	22.50000	19.62000	5.560000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.216652	0.054922	*0.042612	*0.001439	*0.000563
C	{0.1}	*0.000180	0.216652		0.223717	0.239643	*0.009703	*0.003233
D	{1}	*0.000196	0.054922	0.223717		0.676041	0.060879	*0.022577
E	{10}	*0.000150	*0.042612	0.239643	0.676041		0.056057	*0.029042
F	{100}	*0.000158	*0.001439	*0.009703	0.060879	0.056057		0.423789
G	{500}	*0.000174	*0.000563	*0.003233	*0.022577	*0.029042	0.423789	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	36.67000	30.63000	29.55000	26.51000	21.55000	17.50000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.363962	0.525831	0.420832	0.186607	0.085154
C	{0.1}	*0.000180	0.363962		0.869214	0.800776	0.513188	0.297592
D	{1}	*0.000196	0.525831	0.869214		0.644005	0.448581	0.28293
E	{10}	*0.000150	0.420832	0.800776	0.644005		0.453738	0.367394
F	{100}	*0.000158	0.186607	0.513188	0.448581	0.453738		0.53933
G	{500}	*0.000174	0.085154	0.297592	0.28293	0.367394	0.53933	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	54.44000	46.67000	39.08000	28.89000	27.22000	20.83000
A	{0}		*0.000279	*0.000238	*0.000215	*0.000154	*0.000161	*0.000175
B	{0.01	*0.000279		0.38467	0.213832	*0.046070	*0.047510	*0.016681
C	{0.1}	*0.000238	0.38467		0.395478	0.135929	0.158455	0.063318
D	{1}	*0.000215	0.213832	0.395478		0.258796	0.382211	0.198
E	{10}	*0.000154	*0.046070	0.135929	0.258796		0.849882	0.630303
F	{100}	*0.000161	*0.047510	0.158455	0.382211	0.849882		0.472685
G	{500}	*0.000175	*0.016681	0.063318	0.198	0.630303	0.472685	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	45.56000	25.00000	23.33000	18.65000	14.24000	8.340000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		*0.000275	*0.000308	*0.000220	*0.000155	*0.000158
C	{0.1}	*0.000180	*0.000275		0.674389	0.265302	0.064655	*0.005821
D	{1}	*0.000196	*0.000308	0.674389		0.249043	0.083386	*0.008538
E	{10}	*0.000150	*0.000220	0.265302	0.249043		0.276131	*0.047167
F	{100}	*0.000158	*0.000155	0.064655	0.083386	0.276131		0.151759
G	{500}	*0.000174	*0.000158	*0.005821	*0.008538	*0.047167	0.151759	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	35.87000	37.02000	28.72000	26.91000	19.62000	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000180		0.737418	0.051743	*0.045789	*0.001475	*0.000150
C	{0.1}	*0.000176	0.737418		0.065644	*0.041539	*0.001227	*0.000158
D	{1}	*0.000196	0.051743	0.065644		0.598755	*0.042348	*0.000197
E	{10}	*0.000150	*0.045789	*0.041539	0.598755		*0.047893	*0.000182
F	{100}	*0.000158	*0.001475	*0.001227	*0.042348	*0.047893		*0.000209
G	{500}	*0.000174	*0.000150	*0.000158	*0.000197	*0.000182	*0.000209	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	23.90000	22.25000	21.77000	19.62000	18.81000	1.390000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.479115	0.625613	0.277218	0.220189	*0.000158
C	{0.1}	*0.000180	0.479115		0.835569	0.495382	0.454277	*0.000151
D	{1}	*0.000196	0.625613	0.835569		0.359447	0.415671	*0.000196
E	{10}	*0.000150	0.277218	0.495382	0.359447		0.726467	*0.000182
F	{100}	*0.000158	0.220189	0.454277	0.415671	0.726467		*0.000177
G	{500}	*0.000174	*0.000158	*0.000151	*0.000196	*0.000182	*0.000177	

APPENDIX 42: ANOVA: *Ventricaria ventricosa* : DNA Damage (RAPD-Similarity of band)

* = Significant difference : p<0.05

ANOVA-SIMILIAR-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	52.10000	46.65000	44.27000	39.87000	35.98000	0.000000
A	{0}		*0.000647	*0.000666	*0.000806	*0.000608	*0.000493	*0.000174
B	{0.01	*0.000647	485256195(	0.616934	0.747214	0.667198	0.570829	*0.002707
C	{0.1}	*0.000666	0.616934		0.826506	0.802793	0.75089	*0.004869
D	{1}	*0.000806	0.747214	0.826506		0.685882	0.721925	*0.004854
E	{10}	*0.000608	0.667198	0.802793	0.685882		0.720488	*0.005901
F	{100}	*0.000493	0.570829	0.75089	0.721925	0.720488		*0.004653
G	{500}	*0.000174	*0.002707	*0.004869	*0.004854	*0.005901	*0.004653	

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	52.10000	43.76000	38.11000	34.04000	32.28000	0.000000
A	{0}		*0.000186	*0.000184	*0.000197	*0.000152	*0.000159	*0.000174
B	{0.01	*0.000186	681747436	0.282129	0.181988	0.118123	0.112122	*0.000218
C	{0.1}	*0.000184	0.282129		0.461174	0.416145	0.441502	*0.000454
D	{1}	*0.000197	0.181988	0.461174		0.593764	0.719723	*0.000958
E	{10}	*0.000152	0.118123	0.416145	0.593764		0.816879	*0.001322
F	{100}	*0.000159	0.112122	0.441502	0.719723	0.816879		*0.000833
G	{500}	*0.000174	*0.000218	*0.000454	*0.000958	*0.001322	*0.000833	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	56.25000	43.00000	30.77000	27.31000	26.03000	0.000000
A	{0}		*0.001118	*0.000407	*0.000244	*0.000201	*0.000209	*0.000174
B	{0.01	*0.001118	6003684997	0.229393	0.072161	0.067039	0.078125	*0.001290
C	{0.1}	*0.000407	0.229393		0.265445	0.325876	0.404745	*0.008451
D	{1}	*0.000244	0.072161	0.265445		0.747695	0.895389	*0.048921
E	{10}	*0.000201	0.067039	0.325876	0.747695		0.905187	0.052606
F	{100}	*0.000209	0.078125	0.404745	0.895389	0.905187		*0.027142
G	{500}	*0.000174	*0.001290	*0.008451	*0.048921	0.052606	*0.027142	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	25.56000	27.52000	27.48000	22.79000	16.43000	0.000000
A	{0}		*0.000196	*0.000176	*0.000180	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000196	039676666	0.539742	0.30689	0.14836	*0.000635	*0.000196
C	{0.1}	*0.000176	0.539742		0.982792	0.084936	*0.000339	*0.000158
D	{1}	*0.000180	0.30689	0.982792		0.052612	*0.000311	*0.000150
E	{10}	*0.000150	0.14836	0.084936	0.052612		*0.003593	*0.000180
F	{100}	*0.000158	*0.000635	*0.000339	*0.000311	*0.003593	5640335083	*0.000176
G	{500}	*0.000174	*0.000196	*0.000158	*0.000150	*0.000180	*0.000176	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	56.35000	37.30000	30.32000	30.16000	26.30000	21.09000
A	{0}		*0.000188	*0.000180	*0.000196	*0.000151	*0.000158	*0.000174
B	{0.01	*0.000188	708305358	*0.015311	*0.005517	*0.009381	*0.005023	*0.001839
C	{0.1}	*0.000180	*0.015311	5391731262	0.328147	0.566991	0.411386	0.18552
D	{1}	*0.000196	*0.005517	0.328147		0.98191	0.830996	0.554268
E	{10}	*0.000151	*0.009381	0.566991	0.98191		0.584151	0.409417
F	{100}	*0.000158	*0.005023	0.411386	0.830996	0.584151		0.462062
G	{500}	*0.000174	*0.001839	0.18552	0.554268	0.409417	0.462062	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	40.30000	43.53000	41.32000	35.36000	35.03000	34.44000
A	{0}		*0.000273	*0.000201	*0.000233	*0.000205	*0.000230	*0.000255
B	{0.01	*0.000273	2276916503	0.937958	0.915678	0.609344	0.844263	0.923945
C	{0.1}	*0.000201	0.937958		0.818542	0.822688	0.89233	0.92254
D	{1}	*0.000233	0.915678	0.818542		0.805918	0.908233	0.946316
E	{10}	*0.000205	0.609344	0.822688	0.805918		0.972755	0.994871
F	{100}	*0.000230	0.844263	0.89233	0.908233	0.972755		0.951185
G	{500}	*0.000255	0.923945	0.92254	0.946316	0.994871	0.951185	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	29.88000	28.71000	27.09000	24.30000	22.86000	21.52000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176	6681671142	0.714596	0.655012	0.322453	0.221492	0.14435
C	{0.1}	*0.000180	0.714596		0.613392	0.363977	0.285409	0.20366
D	{1}	*0.000196	0.655012	0.613392		0.388514	0.392528	0.323884
E	{10}	*0.000150	0.322453	0.363977	0.388514		0.653054	0.656936
F	{100}	*0.000158	0.221492	0.285409	0.392528	0.653054		0.67557
G	{500}	*0.000174	0.14435	0.20366	0.323884	0.656936	0.67557	

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	39.03000	37.69000	34.82000	29.89000	23.43000	18.96000
A	{0}		*0.000193	*0.000218	*0.000240	*0.000187	*0.000170	*0.000181
B	{0.01	*0.000193	2978630065	0.893987	0.905182	0.79127	0.531264	0.371671
C	{0.1}	*0.000218	0.893987		0.775433	0.714552	0.493453	0.36161
D	{1}	*0.000240	0.905182	0.775433		0.625046	0.49797	0.405701
E	{10}	*0.000187	0.79127	0.714552	0.625046		0.523213	0.524851
F	{100}	*0.000170	0.531264	0.493453	0.49797	0.523213		0.657449
G	{500}	*0.000181	0.371671	0.36161	0.405701	0.524851	0.657449	

APPENDIX 42: ANOVA: *Ventricaria ventricosa* : DNA Damage (RAPD-Intensity of band)

* = Significant difference : p<0.05

ANOVA-INTENSITY-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	111.7300	154.3600	144.9200	0.000000	0.000000	0.000000
A	{0}		*0.011509	*0.000196	*0.000180	*0.000196	*0.000180	*0.000176
B	{0.01	*0.011509		*0.000180	*0.000176	*0.000150	*0.000196	*0.000180
C	{0.1}	*0.000196	*0.000180		*0.034480	*0.000174	*0.000158	*0.000150
D	{1}	*0.000180	*0.000176	*0.034480		*0.000158	*0.000150	*0.000196
E	{10}	*0.000196	*0.000150	*0.000174	*0.000158		1	1
F	{100}	*0.000180	*0.000196	*0.000158	*0.000150	1		1
G	{500}	*0.000176	*0.000180	*0.000150	*0.000196	1	1	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	145.8500	181.9900	172.5600	194.2000	107.9500	102.9500
A	{0}		*0.000338	*0.000158	*0.000151	*0.000174	0.560204	0.703303
B	{0.01	*0.000338		*0.000958	*0.003540	*0.000265	*0.000351	*0.000323
C	{0.1}	*0.000158	*0.000958		0.234294	0.129895	*0.000196	*0.000150
D	{1}	*0.000151	*0.003540	0.234294		*0.032249	*0.000181	*0.000196
E	{10}	*0.000174	*0.000265	0.129895	*0.032249		*0.000150	*0.000158
F	{100}	0.560204	*0.000351	*0.000196	*0.000181	*0.000150		0.520609
G	{500}	0.703303	*0.000323	*0.000150	*0.000196	*0.000158	0.520609	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	112.6200	121.6800	139.7300	218.5100	177.0200	162.6500
A	{0}		0.286747	0.1745	*0.016960	*0.000174	*0.000246	*0.000753
B	{0.01	0.286747		0.439886	0.077215	*0.000158	*0.000603	0.003167
C	{0.1}	0.1745	0.439886		0.135559	*0.000153	*0.001422	*0.007809
D	{1}	*0.016960	0.077215	0.135559		*0.000220	*0.014492	*0.064012
E	{10}	*0.000174	*0.000158	*0.000153	*0.000220		*0.002818	*0.000776
F	{100}	*0.000246	*0.000603	*0.001422	*0.014492	*0.002818		0.227893
G	{500}	*0.000753	*0.003167	*0.007809	0.064012	*0.000776	0.227893	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	137.3500	157.2800	194.1200	128.3300	120.2700	0.000000
A	{0}		*0.001327	*0.000171	*0.000158	*0.006203	*0.018822	*0.000176
B	{0.01	*0.001327		*0.020517	*0.000184	0.256413	0.098655	*0.000150
C	{0.1}	*0.000171	*0.020517		*0.000417	*0.005316	*0.001426	*0.000158
D	{1}	*0.000158	*0.000184	*0.000417		*0.000196	*0.000151	*0.000174
E	{10}	*0.006203	0.256413	*0.005316	*0.000196		0.308269	*0.000196
F	{100}	*0.018822	0.098655	*0.001426	*0.000151	0.308269		*0.000180
G	{500}	*0.000176	*0.000150	*0.000158	*0.000174	*0.000196	*0.000180	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	100.3100	122.1200	130.7700	116.6500	0.000000	0.000000
A	{0}		0.977704	0.220121	0.082105	0.304862	*0.000180	*0.000176
B	{0.01	0.977704		0.145851	0.059664	0.154034	*0.000196	*0.000180
C	{0.1}	0.220121	0.145851		0.438324	0.621775	*0.000158	*0.000150
D	{1}	0.082105	0.059664	0.438324		0.416839	*0.000174	*0.000158
E	{10}	0.304862	0.154034	0.621775	0.416839		*0.000150	*0.000196
F	{100}	*0.000180	*0.000196	*0.000158	*0.000174	*0.000150978565216		1
G	{500}	*0.000176	*0.000180	*0.000150	*0.000158	*0.000196	1	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	123.7400	148.7200	180.3400	162.8600	145.6100	120.7100
A	{0}		0.245494	*0.026834	*0.000957	*0.005819	*0.027197	0.16397
B	{0.01	0.245494		0.21462	*0.009527	0.063528	0.143184	0.832996
C	{0.1}	*0.026834	0.21462		0.09826	0.332902	0.828661	0.238835
D	{1}	*0.000957	*0.009527	0.09826		0.235385	0.110092	*0.008826
E	{10}	*0.005819	0.063528	0.332902	0.235385		0.45926	0.062749
F	{100}	*0.027197	0.143184	0.828661	0.110092	0.45926		0.216524
G	{500}	0.16397	0.832996	0.238835	*0.008826	0.062749	0.216524	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	113.6600	135.4300	147.2900	168.0400	143.2500	109.5400
A	{0}		0.470692	*0.033720	*0.009937	*0.000636	*0.013937	0.414998
B	{0.01	0.470692		0.075908	*0.045206	*0.002344	0.051125	0.722202
C	{0.1}	*0.033720	0.075908		0.562233	0.053284	0.502333	0.092062
D	{1}	*0.009937	*0.045206	0.562233		0.089093	0.727363	*0.034186
E	{10}	*0.000636	*0.002344	0.053284	0.089093		0.109018	*0.001741
F	{100}	*0.013937	0.051125	0.502333	0.727363	0.109018		*0.044625
G	{500}	0.414998	0.722202	0.092062	*0.034186	*0.001741	*0.044625	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	126.0000	146.5600	92.35000	86.37000	83.50000	0.000000
A	{0}		*0.006955	*0.000317	0.366792	0.253697	0.229893	*0.000150
B	{0.01	*0.006955		*0.025233	*0.003006	*0.001479	*0.001216	*0.000158
C	{0.1}	*0.000317	*0.025233		*0.000240	*0.000176	*0.000172	*0.000174
D	{1}	0.366792	*0.003006	*0.000240		0.477964	0.541734	*0.000196
E	{10}	0.253697	*0.001479	*0.000176	0.477964		0.731644	*0.000180
F	{100}	0.229893	*0.001216	*0.000172	0.541734	0.731644		*0.000176
G	{500}	*0.000150	*0.000158	*0.000174	*0.000196	*0.000180	*0.000176	

APPENDIX 42: ANOVA: *Ventricaria ventricosa* : DNA Damage (RAPD-Intensity of band)

* = Significant difference : p<0.05

ANOVA-INTENSITY-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	177.1100	203.6200	207.1500	198.3600	188.0000	0.000000
A	{0}		*0.000196	*0.000154	*0.000161	*0.000200	*0.000191	*0.000177
B	{0.01}	*0.000196		0.202553	0.179892	0.247612	0.404312	*0.000180
C	{0.1}	*0.000154	0.202553		0.784555	0.684189	0.453754	*0.000158
D	{1}	*0.000161	0.179892	0.784555		0.770672	0.45636	*0.000174
E	{10}	*0.000200	0.247612	0.684189	0.770672		0.427009	*0.000150
F	{100}	*0.000191	0.404312	0.453754	0.45636	0.427009		*0.000196
G	{500}	*0.000177	*0.000180	*0.000158	*0.000174	*0.000150	*0.000196	

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	110.9400	123.8200	112.2000	108.2400	94.22000	0.000000
A	{0}		0.742185	0.509587	0.839658	0.584262	0.700349	*0.000195
B	{0.01}	0.742185		0.663759	0.933039	0.857085	0.673913	*0.000171
C	{0.1}	0.509587	0.663759		0.442789	0.718656	0.382671	*0.000178
D	{1}	0.839658	0.933039	0.442789		0.961016	0.73913	*0.000174
E	{10}	0.584262	0.857085	0.718656	0.961016		0.617001	*0.000206
F	{100}	0.700349	0.673913	0.382671	0.73913	0.617001		*0.000187
G	{500}	*0.000195	*0.000171	*0.000178	*0.000174	*0.000206	*0.000187	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	145.6900	160.7800	101.0900	95.53000	89.42000	0.000000
A	{0}		*0.006616	*0.001328	0.931321	0.724051	0.677479	*0.000198
B	{0.01}	*0.006616		0.243963	*0.003071	*0.005982	*0.003674	*0.000158
C	{0.1}	*0.001328	0.243963		*0.000890	*0.001078	*0.000692	*0.000174
D	{1}	0.931321	*0.003071	*0.000890314579010		0.896015	0.783865	*0.000155
E	{10}	0.724051	*0.005982	*0.001078	0.896015		0.63005	*0.000183
F	{100}	0.677479	*0.003674	*0.000692	0.783865	0.63005		*0.000178
G	{500}	*0.000198	*0.000158	*0.000174	*0.000155	*0.000183	*0.000178	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	150.2200	173.3700	161.3200	160.8800	115.1900	0.000000
A	{0}		*0.000691	*0.000189	*0.000359	*0.000339	0.154136	*0.000176
B	{0.01}	*0.000691	8907165527	0.146014	0.5289	0.308233	*0.003861	*0.000196
C	{0.1}	*0.000189	0.146014		0.251823	0.45083	*0.000517	*0.000174
D	{1}	*0.000359	0.5289	0.251823		0.965909	*0.002285	*0.000158
E	{10}	*0.000339	0.308233	0.45083	0.965909		*0.001399	*0.000150
F	{100}	0.154136	*0.003861	*0.000517	*0.002285	*0.001399	4574546814	*0.000180
G	{500}	*0.000176	*0.000196	*0.000174	*0.000158	*0.000150	*0.000180	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	147.4900	154.2100	217.2600	222.6700	202.2800	201.7100
A	{0}		*0.001344	*0.001210	*0.000158	*0.000174	*0.000152	*0.000196
B	{0.01}	*0.001344	403907775	0.576071	*0.000416	*0.000321	*0.001946	*0.00120
C	{0.1}	*0.001210	0.576071		*0.000669	*0.000480	*0.003048	*0.001342
D	{1}	*0.000158	*0.000416	*0.000669240951538		0.651984	0.222618	0.40514
E	{10}	*0.000174	*0.000321	*0.000480	0.651984		0.226415	0.319948
F	{100}	*0.000152	*0.001946	*0.003048	0.222618	0.226415		0.962061
G	{500}	*0.000196	*0.001209	*0.001342	0.40514	0.319948	0.962061	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	157.8400	149.7600	147.5800	127.0600	113.3400	94.36000
A	{0}		*0.002242	*0.005600	*0.005250	0.082821	0.268038	0.63338
B	{0.01}	*0.002242	9823875427	0.496264	0.656785	0.077877	*0.012967	*0.001322
C	{0.1}	*0.005600	0.496264		0.853268	0.158144	*0.031952	*0.003224
D	{1}	*0.005250	0.656785	0.853268		0.097802	*0.026273	*0.003267
E	{10}	0.082821	0.077877	0.158144	0.097802		0.255242	0.057759
F	{100}	0.268038	*0.012967	*0.031952	*0.026273	0.255242		0.261681
G	{500}	0.63338	*0.001322	*0.003224	*0.003267	0.057759	0.261681	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	202.4100	215.2600	218.6000	200.3400	200.2500	195.6700
A	{0}		*0.000194	*0.000168	*0.000183	*0.000223	*0.000193	*0.000184
B	{0.01}	*0.000194	013118743	0.395034	0.5262	0.889694	0.988173	0.966493
C	{0.1}	*0.000168	0.395034		0.822939	0.577422	0.737857	0.673545
D	{1}	*0.000183	0.5262	0.822939		0.608853	0.721915	0.631199
E	{10}	*0.000223	0.889694	0.577422	0.608853		0.995296	0.945747
F	{100}	*0.000193	0.988173	0.737857	0.721915	0.995296		0.759127
G	{500}	*0.000184	0.966493	0.673545	0.631199	0.945747	0.759127	

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	138.6600	137.2900	139.5200	147.6200	173.0800	146.8900
A	{0}		*0.014857	*0.007319	*0.022691	*0.013084	*0.000517	*0.010669
B	{0.01}	*0.014857	2292175293	0.909776	0.943303	0.872845	0.073371	0.77085
C	{0.1}	*0.007319	0.909776		0.980808	0.902821	0.07959	0.848963
D	{1}	*0.022691	0.943303	0.980808		0.777044	0.057549	0.544384
E	{10}	*0.013084	0.872845	0.902821	0.777044		*0.049906	0.951881
F	{100}	*0.000517	0.073371	0.07959	0.057549	*0.049906	5396118	0.104404
G	{500}	*0.010669	0.77085	0.848963	0.544384	0.951881	0.104404	

APPENDIX 42: ANOVA: *Ventricaria ventricosa* : DNA Damage (RAPD-Genomic Template Stability)

* = Significant difference : p<0.05

ANOVA-GTS-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	20.37000	11.11000	8.330000	0.000000	0.000000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000174	*0.000158	*0.000150
B	{0.01	*0.000176		*0.005241	*0.002063	*0.000188	*0.000178	*0.000207
C	{0.1}	*0.000180	*0.005241		0.336434	*0.010192	*0.006751	*0.003784
D	{1}	*0.000196	*0.002063	0.336434		*0.043488	*0.025194	*0.010028
E	{10}	*0.000174	*0.000188	*0.010192	*0.043488		1	1
F	{100}	*0.000158	*0.000178	*0.006751	*0.025194	1		1
G	{500}	*0.000150	*0.000207	*0.003784	*0.010028	1	1	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	40.48000	39.59000	33.34000	31.48000	16.67000	14.49000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.896255	0.549289	0.551785	*0.022301	*0.016710
C	{0.1}	*0.000180	0.896255		0.366502	0.466126	*0.019116	*0.015590
D	{1}	*0.000196	0.549289	0.366502		0.785309	0.063258	0.059066
E	{10}	*0.000150	0.551785	0.466126	0.785309		*0.044194	0.057981
F	{100}	*0.000158	*0.022301	*0.019116	0.063258	*0.044194		0.749633
G	{500}	*0.000174	*0.016710	*0.015590	0.059066	0.057981	0.749633	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	35.55000	26.67000	23.61000	22.23000	17.50000	13.89000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.109274	0.088882	0.092192	*0.025807	*0.009800
C	{0.1}	*0.000180	0.109274		0.565	0.675998	0.328677	0.155714
D	{1}	*0.000196	0.088882	0.565		0.794317	0.485305	0.282945
E	{10}	*0.000150	0.092192	0.675998	0.794317		0.377716	0.275362
F	{100}	*0.000158	*0.025807	0.328677	0.485305	0.377716		0.498251
G	{500}	*0.000174	*0.009800	0.155714	0.282945	0.275362	0.498251	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	10.00000	13.21000	9.380000	8.930000	8.160000	0.000000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000180		*0.022314	0.627246	0.674965	0.477704	*0.000156
C	{0.1}	*0.000176	*0.022314		*0.021436	*0.018939	*0.009001	*0.000158
D	{1}	*0.000196	0.627246	*0.021436		0.723971	0.602516	*0.000203
E	{10}	*0.000150	0.674965	*0.018939	0.723971		0.547371	*0.000187
F	{100}	*0.000158	0.477704	*0.009001	0.602516	0.547371		*0.000184
G	{500}	*0.000174	*0.000156	*0.000158	*0.000203	*0.000187	*0.000184	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	33.33000	44.45000	47.23000	18.06000	0.000000	0.000000
A	{0}		*0.000196	*0.000180	*0.000176	*0.000150	*0.000174	*0.000158
B	{0.01	*0.000196		*0.004661	*0.002428	*0.000532	*0.000196	*0.000180
C	{0.1}	*0.000180	*0.004661		0.412805	*0.000182	*0.000150	*0.000196
D	{1}	*0.000176	*0.002428	0.412805		*0.000196	*0.000158	*0.000150
E	{10}	*0.000150	*0.000532	*0.000182	*0.000196		*0.000376	*0.000242
F	{100}	*0.000174	*0.000196	*0.000150	*0.000158	*0.000376		1
G	{500}	*0.000158	*0.000180	*0.000196	*0.000150	*0.000242	1	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	28.57000	27.98000	21.43000	16.19000	14.29000	3.130000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.679387	*0.000581	*0.000196	*0.000150	*0.000158
C	{0.1}	*0.000180	0.679387		*0.000499	*0.000181	*0.000196	*0.000150
D	{1}	*0.000196	*0.000581	*0.000499		*0.002308	*0.000581	*0.000196
E	{10}	*0.000150	*0.000196	*0.000181	*0.002308		0.195515	*0.000180
F	{100}	*0.000158	*0.000150	*0.000196	*0.000581	0.195515		*0.000177
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000177	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	36.39000	50.00000	50.00000	50.00000	41.67000	38.89000
A	{0}		*0.000260	*0.000624	*0.000399	*0.000250	*0.000281	*0.000268
B	{0.01	*0.000260		0.475807	0.592043	0.682847	0.836433	0.790169
C	{0.1}	*0.000624	0.475807		1	1	0.381322	0.469134
D	{1}	*0.000399	0.592043	1		1	0.646589	0.633262
E	{10}	*0.000250	0.682847	1	1		0.802893	0.748192
F	{100}	*0.000281	0.836433	0.381322	0.646589	0.802893		0.76738
G	{500}	*0.000268	0.790169	0.469134	0.633262	0.748192	0.76738	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	12.04000	12.50000	20.28000	12.50000	10.83000	0.000000
A	{0}		*0.000150	*0.000196	*0.000176	*0.000180	*0.000158	*0.000174
B	{0.01	*0.000150		0.89633	0.127101	0.990414	0.732075	*0.800980
C	{0.1}	*0.000196	0.89633		0.097664	1	0.880778	*0.013465
D	{1}	*0.000176	0.127101	0.097664		*0.041420	0.099375	*0.000598
E	{10}	*0.000180	0.990414	1	*0.041420		0.961824	*0.020200
F	{100}	*0.000158	0.732075	0.880778	0.099375	0.961824		*0.007564
G	{500}	*0.000174	*0.009806	*0.013465	*0.000598	*0.020200	*0.007564	

APPENDIX 42: ANOVA: *Ventricaria ventricosa* : DNA Damage (RAPD-Genomic Template Stability)

* = Significant difference : p<0.05

ANOVA-GTS-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	20.00000	16.67000	15.28000	10.28000	9.550000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.091949	0.055141	*0.000753	*0.000582	*0.000158
C	{0.1}	*0.000180	0.091949		0.462634	*0.009885	*0.008276	*0.000151
D	{1}	*0.000196	0.055141	0.462634		*0.016828	*0.019632	*0.000197
E	{10}	*0.000150	*0.000753	*0.009885	*0.016828		0.697647	*0.000342
F	{100}	*0.000158	*0.000582	*0.008276	*0.019632	0.697647		*0.000295
G	{500}	*0.000174	*0.000158	*0.000151	*0.000197	*0.000342	*0.000295	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	44.87000	39.74000	23.08000	15.38000	11.86000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.176562	*0.000250	*0.000198	*0.000151	*0.000158
C	{0.1}	*0.000180	0.176562		*0.000542	*0.000196	*0.000200	*0.000150
D	{1}	*0.000196	*0.000250	*0.000542	9983139038	0.050856	*0.019653	*0.000261
E	{10}	*0.000150	*0.000198	*0.000196	0.050856		0.345334	*0.002236
F	{100}	*0.000158	*0.000151	*0.000200	*0.019653	0.345334		*0.005500
G	{500}	*0.000174	*0.000158	*0.000150	*0.000261	*0.002236	*0.005500	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	26.67000	6.670000	0.000000	0.000000	0.000000	0.000000
A	{0}		*0.000176	*0.000180	*0.000174	*0.000158	*0.000150	*0.000196
B	{0.01	*0.000176		*0.000176	*0.000158	*0.000150	*0.000196	*0.000180
C	{0.1}	*0.000180	*0.000176		*0.000151	*0.000196	*0.000180	*0.000176
D	{1}	*0.000174	*0.000158	*0.000151	6342163085	1	1	1
E	{10}	*0.000158	*0.000150	*0.000196	1		1	1
F	{100}	*0.000150	*0.000196	*0.000180	1	1		1
G	{500}	*0.000196	*0.000180	*0.000176	1	1	1	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	34.07000	29.67000	30.00000	24.91000	23.15000	18.41000
A	{0}		*0.000176	*0.000196	*0.000180	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		0.525667	0.323381	0.144232	0.09635	*0.015047
C	{0.1}	*0.000196	0.525667		0.935121	0.251123	0.262207	0.057296
D	{1}	*0.000180	0.323381	0.935121		0.428603	0.348852	0.071711
E	{10}	*0.000150	0.144232	0.251123	0.428603		0.664841	0.264163
F	{100}	*0.000158	0.09635	0.262207	0.348852	0.664841		0.253026
G	{500}	*0.000174	*0.015047	0.057296	0.071711	0.264163	0.253026	

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	32.51000	26.58000	20.30000	15.88000	10.08000	0.000000
A	{0}		*0.000176	*0.000180	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000176		*0.003631	*0.000187	*0.000196	*0.000150	*0.000158
C	{0.1}	*0.000180	*0.003631234169		*0.002461	*0.000217	*0.000196	*0.000150
D	{1}	*0.000196	*0.000187	*0.002461254596710		*0.020530	*0.000251	*0.000196
E	{10}	*0.000150	*0.000196	*0.000217	*0.020530045032501		*0.004208	*0.000180
F	{100}	*0.000158	*0.000150	*0.000196	*0.000251	*0.004208		*0.000202
G	{500}	*0.000174	*0.000158	*0.000150	*0.000196	*0.000180	*0.000202	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	28.57000	28.57000	16.67000	17.62000	17.47000	0.000000
A	{0}		*0.000180	*0.000176	*0.000158	*0.000196	*0.000150	*0.000174
B	{0.01	*0.000180900096893		1	*0.000202	*0.000179	*0.000189	*0.000150
C	{0.1}	*0.000176	1		*0.000170	*0.000190	*0.000214	*0.000158
D	{1}	*0.000158	*0.000202	*0.000170111656188		0.819506	0.618163	*0.000176
E	{10}	*0.000196	*0.000179	*0.000190	0.819506		0.925293	*0.000196
F	{100}	*0.000150	*0.000189	*0.000214	0.618163	0.925293		*0.000180
G	{500}	*0.000174	*0.000150	*0.000158	*0.000176	*0.000196	*0.000180	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	28.57000	26.43000	27.62000	11.43000	8.330000	8.330000
A	{0}		*0.000176	*0.000196	*0.000180	*0.000150	*0.000174	*0.000158
B	{0.01	*0.000176		0.454849	0.593204	*0.000196	*0.000158	*0.000150
C	{0.1}	*0.000196	0.454849		0.504665	*0.000176	*0.000196	*0.000180
D	{1}	*0.000180	0.593204	0.504665		*0.000180	*0.000150	*0.000196
E	{10}	*0.000150	*0.000196	*0.000176	*0.000180959701538		0.210594	0.096141
F	{100}	*0.000174	*0.000158	*0.000196	*0.000150	0.210594		1
G	{500}	*0.000158	*0.000150	*0.000180	*0.000196	0.096141	1	

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		100.0000	37.40000	40.43000	32.64000	29.88000	22.95000	19.92000
A	{0}		*0.000180	*0.000176	*0.000196	*0.000150	*0.000158	*0.000174
B	{0.01	*0.000180900096893		0.137967	*0.026987	*0.004341	*0.000203	*0.000151
C	{0.1}	*0.000176	0.137967		*0.003341	*0.000586	*0.000151	*0.000158
D	{1}	*0.000196	*0.026987	*0.003341615200042		0.173742	*0.000644	*0.000240
E	{10}	*0.000150	*0.004341	*0.000586	0.173742		*0.003048	*0.000535
F	{100}	*0.000158	*0.000203	*0.000151	*0.000644	*0.003048		0.137967
G	{500}	*0.000174	*0.000151	*0.000158	*0.000240	*0.000535	0.137967	

APPENDIX 43:ANOVA: *Ventricaria ventricosa* : DNA Damage (AP-Site)

* = Significant difference : p<0.05

ANOVA-AP-SITE-VEN

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.022500	3.863000	4.037000	4.000000	6.000000	8.302000	5.923000
A	{0}		0.471109	0.807931	0.672213	0.154799	*0.0053833	0.133171
B	{0.01}	0.471109		0.987215	0.9057	0.368966	*0.015783C	0.306819
C	{0.1}	0.807931	0.987215		0.974573	0.228878	*0.0101616	0.11873
D	{1}	0.672213	0.9057	0.974573		0.330316	*0.0143871	0.241473
E	{10}	0.154799	0.368966	0.228878	0.330316		*0.0619944	0.946933
F	{100}	*0.0053833	*0.015783C	*0.0101616	*0.0143871	*0.0619944334030151		0.126405
G	{500}	0.133171	0.306819	0.11873	0.241473	0.946933	0.126405	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		3.022500	6.978000	7.000000	8.000000	4.298000	3.544000	2.523000
A	{0}		*0.0116022	*0.0167927	*0.0041957	0.478301	0.634336	0.648597
B	{0.01}	*0.0116022229194641		0.98403	0.61705	*0.025622C	*0.0165773	*0.007346
C	{0.1}	*0.0167927	0.98403		0.366958	0.059894	*0.0278443	*0.009756
D	{1}	*0.0041957	0.61705	0.366958		*0.0180985	*0.0073335	*0.002458
E	{10}	0.478301	*0.025622C	0.059894	*0.0180985331535339		0.493579	0.381641
F	{100}	0.634336	*0.0165773	*0.0278443	*0.0073335	0.493579		0.617612
G	{500}	0.648597	*0.0073461	*0.0097565	*0.0024583	0.381642	0.617612	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.030000	4.516000	9.223000	12.92500	15.62100	19.38400	19.94300
A	{0}		0.785597	*0.026104C	*0.0010086	*0.0002306	*0.0001598	*0.000175
B	{0.01}	0.785597		*0.017844S	*0.0009062	*0.0002701	*0.0001531	*0.000159
C	{0.1}	*0.026104C	*0.0178449153900146		0.053105	*0.0070163	*0.0004122	*0.000341
D	{1}	*0.0010086	*0.0009062	0.053105		0.146186	*0.0065631	*0.006411
E	{10}	*0.0002306	*0.0002701	*0.0070163	0.146186		*0.0497877	0.065914
F	{100}	*0.0001598	*0.0001531	*0.0004122	*0.0065631	*0.049787700176239		0.754455
G	{500}	*0.0001752	*0.0001597	*0.0003415	*0.0064110	0.065915	0.754455	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		4.030000	4.707000	5.000000	8.771000	8.566000	8.147000	8.538000
A	{0}		0.569569	0.688442	*0.0150975	*0.0160791	*0.0152810	*0.01225
B	{0.01}	0.569569		0.804728	*0.0338037	*0.0344676	*0.0263280	*0.024277
C	{0.1}	0.688442	0.804728		*0.0396014	*0.0371507	*0.0171399	*0.022423
D	{1}	*0.0150975	*0.0338037	*0.0396014451980591		0.862619	0.948572	0.978195
E	{10}	*0.0160791	*0.0344676	*0.0371507	0.862619		0.931282	0.981224
F	{100}	*0.0152810	*0.0263280	*0.0171399	0.948572	0.931282		0.741636
G	{500}	*0.0122543	*0.0242773	*0.0224239	0.978195	0.981224	0.741636	

APPENDIX 44: ANOVA: *Ventricaria ventricosa* : Superoxide dismutase (SOD) activity

* = Significant difference : p<0.05

ANOVA-SOD-VEN

Newman-Keuls test; CD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.698000	63.13600	53.67400	52.07100	48.12300	45.10500	39.49100
A	{0}		*0.000174(	*0.000158:	*0.000150!	*0.000196(	*0.000180!	*0.000176
B	{0.01}	*0.000174045562744(		*0.022379;	*0.024156(	*0.005622;	*0.001946(	*0.000317
C	{0.1}	*0.000158:	*0.022379219532012!		0.670035	0.317293	0.138772	*0.012880
D	{1}	*0.000150!	*0.024156(	0.670035		0.301808	0.177603	*0.019368
E	{10}	*0.000196(	*0.005622;	0.317293	0.301808		0.426258	0.082198
F	{100}	*0.000180!	*0.001946(	0.138772	0.177603	0.426258		0.149693
G	{500}	*0.000176;	*0.000317!	*0.012880(	*0.019368:	0.082198	0.149693	

Newman-Keuls test; CU_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.698000	29.06900	39.38600	79.31400	91.00900	102.9410	115.4840
A	{0}		*0.000176(	*0.000180!	*0.000196(	*0.000150!	*0.000158:	*0.000174
B	{0.01}	*0.000176668167114;		*0.000176;	*0.000180!	*0.000196(	*0.000150!	*0.000158
C	{0.1}	*0.000180!	*0.000176846981048!		*0.000176(	*0.000180!	*0.000196(	*0.000150
D	{1}	*0.000196(	*0.000180!	*0.000176668167114;		*0.000176;	*0.000180!	*0.000196
E	{10}	*0.000150!	*0.000196(	*0.000180!	*0.000176727771759(		*0.000176;	*0.000180
F	{100}	*0.000158:	*0.000150!	*0.000196(	*0.000180!	*0.000176727771759(		*0.000176
G	{500}	*0.000174(	*0.000158:	*0.000150!	*0.000196(	*0.000180!	*0.000176	

Newman-Keuls test; ZN_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.698000	88.50400	73.10600	61.78500	60.34300	59.42300	51.75700
A	{0}		*0.000174(	*0.000158:	*0.000150!	*0.000196(	*0.000180!	*0.000176
B	{0.01}	*0.000174045562744(		80.00018	*0.000180!	*0.000196(	*0.000150!	*0.000158
C	{0.1}	*0.000158:	*0.000184595584869;		*0.000429(	*0.000401;	*0.000407;	*0.000151
D	{1}	*0.000150!	*0.000180!	*0.000429689884185;		0.550086	0.586827	*0.004013
E	{10}	*0.000196(	*0.000196(	*0.000401;	0.550086		0.701913	*0.007083
F	{100}	*0.000180!	*0.000150!	*0.000407;	0.586827	0.701913		*0.005879
G	{500}	*0.000176(	*0.000158:	*0.000151(	*0.004013(	*0.007083!	*0.005879	

Newman-Keuls test; AD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.698000	30.32100	38.71000	31.41200	28.62900	26.48800	26.00200
A	{0}		*0.000150!	*0.000174(	*0.000158:	*0.000196(	*0.000180!	*0.000176
B	{0.01}	*0.000150978565216(		*0.000852!	0.539277	0.345594	0.103842	0.104889
C	{0.1}	*0.000174(	*0.000852584838867:		*0.001011;	*0.000406;	*0.000190;	*0.000186
D	{1}	*0.000158:	0.539277	*0.001011252403259;		0.275773	0.056399	*0.049558
E	{10}	*0.000196(	0.345594	*0.000406;	0.275773		0.237108	0.313713
F	{100}	*0.000180!	0.103842	*0.000190;	0.056399	0.237108		0.783364
G	{500}	*0.000176(	0.104889	*0.000186(	*0.049558(	0.313714	0.783364	

Newman-Keuls test; CD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		10.36800	13.24100	18.50600	14.00600	22.57400	29.44700	31.84900
A	{0}		0.212905	*0.011422!	0.257275	*0.000705(	*0.000160;	*0.000174
B	{0.01}	0.212905		0.075494	0.733451	*0.004159;	*0.000176;	*0.000161
C	{0.1}	*0.011422!	0.075494		0.060287	0.0859	*0.000703;	*0.000328
D	{1}	0.257275	0.733451	0.060287		0.00445	*0.000216;	*0.000155
E	{10}	*0.000705(	*0.004159;	0.0859	0.00445		*0.007636;	*0.002462
F	{100}	*0.000160;	*0.000176;	*0.000703;	*0.000216;	*0.007636487483978;		0.293636
G	{500}	*0.000174;	*0.000161;	*0.000328(	*0.000155;	*0.002462!	0.293637	

Newman-Keuls test; CU_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		10.36800	9.873000	14.48100	39.14100	74.60400	78.87200	94.27000
A	{0}		0.843051	0.115788	*0.000180!	*0.000196(	*0.000150!	*0.000158
B	{0.01}	0.843051		0.181344	*0.000196(	*0.000150!	*0.000158:	*0.000174
C	{0.1}	0.115788	0.181344		*0.000176;	*0.000180!	*0.000196(	*0.000150
D	{1}	*0.000180!	*0.000196(	*0.000176727771759(		*0.000176(	*0.000180!	*0.000196
E	{10}	*0.000196(	*0.000150!	*0.000180!	*0.000176668167114;		0.103819	*0.000182
F	{100}	*0.000150!	*0.000158:	*0.000196(	*0.000180!	0.103819		*0.000190
G	{500}	*0.000158:	*0.000174(	*0.000150!	*0.000196(	*0.000182(	*0.000190	

Newman-Keuls test; ZN_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		10.36800	43.95400	35.88500	34.46600	34.58700	21.05700	15.01200
A	{0}		*0.000174(	*0.000158:	*0.000196(	*0.000150!	*0.000266;	*0.021982
B	{0.01}	*0.000174045562744(		*0.000662!	*0.000767;	*0.000516;	*0.000150!	*0.000158
C	{0.1}	*0.000158:	*0.000662803649902;		0.716191	0.482981	*0.000197!	*0.000150
D	{1}	*0.000196(	*0.000767;	0.716191		0.947464	*0.000177!	*0.000180
E	{10}	*0.000150!	*0.000516;	0.482981	0.947464		*0.000184;	*0.000196
F	{100}	*0.000266;	*0.000150!	*0.000197!	*0.000177!	*0.000184297561645!		*0.004844
G	{500}	*0.021982;	*0.000158:	*0.000150!	*0.000180!	*0.000196(	*0.004844	

Newman-Keuls test; AD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		10.36800	35.04100	38.24700	34.37600	31.40900	20.60300	16.27300
A	{0}		*0.000158;	*0.000174(	*0.000151(	*0.000196;	*0.002240;	*0.027540
B	{0.01}	*0.000158309936523(		0.202751	0.785751	0.314399	*0.000344;	*0.000158
C	{0.1}	*0.000174(	0.202751		0.272528	0.055414	*0.000176;	*0.000158
D	{1}	*0.000151(	0.785751	0.272528		0.236524	*0.000302;	*0.000203
E	{10}	*0.000196;	0.314399	0.055414	0.236524		*0.000640;	*0.000218
F	{100}	*0.002240;	*0.000344;	*0.000176;	*0.000302;	*0.000640392303466;		0.092696
G	{500}	*0.027540;	*0.000158!	*0.000158(	*0.000203;	*0.000218!	0.092696	

APPENDIX 44: ANOVA: *Ventricaria ventricosa* : Superoxide dismutase (SOD) activity

* = Significant difference : p<0.05

ANOVA-SOD-VEN

Newman-Keuls test; BD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.698000	46.57500	51.35400	57.99500	50.71800	31.96300	29.63700
A	{0}		*0.000196	*0.000158	*0.000174	*0.000150	*0.000180	*0.000176
B	{0.01}	*0.000196		0.185966	*0.002840	0.128668	*0.000220	*0.000202
C	{0.1}	*0.000158	0.185966		*0.021560	0.807876	*0.000202	*0.000153
D	{1}	*0.000174	*0.002840	*0.021560		*0.033225	*0.000151	*0.000158
E	{10}	*0.000150	0.128668	0.807876	*0.033225		*0.000185	*0.000197
F	{100}	*0.000180	*0.000220	*0.000202	*0.000151	*0.000185		0.379963
G	{500}	*0.000176	*0.000202	*0.000153	*0.000158	*0.000197	0.379963	

Newman-Keuls test; BD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		10.36800	22.42500	30.31600	28.75900	19.85500	16.15700	16.75300
A	{0}		*0.001021	*0.000176	*0.000164	*0.004757	*0.023586	*0.035338
B	{0.01}	*0.001021		*0.010018	*0.014838	0.27805	0.066143	0.063119
C	{0.1}	*0.000176	*0.010018		0.505355	*0.002216	*0.000391	*0.000411
D	{1}	*0.000164	*0.014838	0.505355		*0.004303	*0.000716	*0.000763
E	{10}	*0.004757	0.27805	*0.002216	*0.004303		0.268414	0.19466
F	{100}	*0.023586	0.066143	*0.000391	*0.000716	0.268414		0.797419
G	{500}	*0.035338	0.063119	*0.000411	*0.000763	0.194661	0.797419	

Newman-Keuls test; MC_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.698000	41.53800	46.98400	37.93900	27.54600	20.34800	15.18100
A	{0}		*0.000158	*0.000174	*0.000150	*0.000196	*0.000189	*0.000577
B	{0.01}	*0.000158		*0.019948	0.104296	*0.000196	*0.000196	*0.000150
C	{0.1}	*0.000174	*0.019948		*0.001872	*0.000196	*0.000150	*0.000158
D	{1}	*0.000150	0.104296	*0.001872		*0.000343	*0.000181	*0.000196
E	{10}	*0.000196	*0.000196	*0.000196	*0.000343		*0.003861	*0.000261
F	{100}	*0.000189	*0.000196	*0.000150	*0.000181	*0.003861		*0.025854
G	{500}	*0.000577	*0.000150	*0.000158	*0.000196	*0.000261	*0.025854	

Newman-Keuls test; MC_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		10.36800	20.62400	31.02300	23.65100	15.35700	16.29100	5.285000
A	{0}		*0.029313	*0.000313	*0.007566	0.142479	0.191282	0.135665
B	{0.01}	*0.029313		*0.015429	0.361728	0.261787	0.198479	*0.002386
C	{0.1}	*0.000313	*0.015429		*0.037671	*0.001999	*0.002232	*0.000184
D	{1}	*0.007566	0.361728	*0.037671		0.089312	0.089993	*0.000721
E	{10}	0.142479	0.261787	*0.001999	0.089312		0.775406	*0.018745
F	{100}	0.191282	0.198479	*0.002232	0.089993	0.775406		*0.018908
G	{500}	0.135665	*0.002386	*0.000184	*0.000721	*0.018745	*0.018908	

Newman-Keuls test; DD_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.698000	34.04400	31.00900	31.18200	27.08500	26.19200	26.75400
A	{0}		*0.000174	*0.000150	*0.000158	*0.000196	*0.000176	*0.000180
B	{0.01}	*0.000174		0.404359	0.231516	*0.039038	*0.038048	*0.044048
C	{0.1}	*0.000150	0.404359		0.940885	0.108453	0.198922	0.186972
D	{1}	*0.000158	0.231516	0.940885		0.208502	0.242261	0.258059
E	{10}	*0.000196	*0.039038	0.108453	0.208502		0.919978	0.887139
F	{100}	*0.000176	*0.038048	0.198922	0.242261	0.919978		0.80963
G	{500}	*0.000180	*0.044048	0.186973	0.25806	0.887139	0.80963	

Newman-Keuls test; DD_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		10.36800	18.35000	18.11800	19.94700	23.06100	15.69700	14.06200
A	{0}		*0.000275	*0.000297	*0.000174	*0.000174	*0.002330	*0.010855
B	{0.01}	*0.000275		0.856143	0.224133	*0.005800	0.122991	*0.019406
C	{0.1}	*0.000297	0.856143		0.340343	*0.007295	0.074428	*0.015708
D	{1}	*0.000174	0.224133	0.340343		*0.026571	*0.020542	*0.002801
E	{10}	*0.000174	*0.005800	*0.007295	*0.026571		*0.000456	*0.000196
F	{100}	*0.002330	0.122991	0.074428	*0.020542	*0.000456		0.213876
G	{500}	*0.010855	*0.019406	*0.015708	*0.002801	*0.000196	0.213876	

Newman-Keuls test; MA_V_D4 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		5.698000	43.53000	39.81300	38.86200	29.57000	21.96900	20.57300
A	{0}		*0.000174	*0.000158	*0.000151	*0.000213	*0.000867	*0.000731
B	{0.01}	*0.000174		0.288639	0.374671	*0.004984	*0.000267	*0.000238
C	{0.1}	*0.000158	0.288639		0.781991	*0.022611	*0.000740	*0.000559
D	{1}	*0.000151	0.374671	0.781991		*0.015552	*0.000661	*0.000624
E	{10}	*0.000213	*0.004984	*0.022611	*0.015552		*0.040716	*0.045432
F	{100}	*0.000867	*0.000267	*0.000740	*0.000661	*0.040716		0.685023
G	{500}	*0.000731	*0.000238	*0.000559	*0.000624	*0.045432	0.685023	

Newman-Keuls test; MA_V_D10 (anova-ven.sta)
Probabilities for Post Hoc Tests
MAIN EFFECT: CONC

		{0 mg/L}	{0.01 mg/L}	{0.1 mg/L}	{1 mg/L}	{10 mg/L}	{100 mg/L}	{500 mg/L}
		10.36800	23.32200	25.67800	25.41700	28.91400	25.60800	13.15000
A	{0}		*0.000257	*0.000203	*0.000218	*0.000177	*0.000190	0.218832
B	{0.01}	*0.000257		0.700825	0.348773	0.12637	0.554276	*0.000486
C	{0.1}	*0.000203	0.700825		0.992065	0.156502	0.974742	*0.000501
D	{1}	*0.000218	0.348773	0.992065		0.400314	0.930909	*0.000317
E	{10}	*0.000177	0.12637	0.156502	0.400314		0.307404	*0.000188
F	{100}	*0.000190	0.554276	0.974742	0.930909	0.307404		*0.000433
G	{500}	0.218832	*0.000486	*0.000501	*0.000317	*0.000188	*0.000433	

APPENDIX 45:

Details results for RAPD: Quantification of genomic DNA concentration in algae, Suitable primers for the RAPD analysis and Annealing temperature for each primer used in the study

Table 8.1: Annealing Temperature for each primer used in the study

Algae	Primer	Optimum annealing temperature
Chlorella vulgaris UMACC 245	a) OPA13	40°C
	b) OPN13	42°C
	c) S17	42°C
	d) S67	44°C
	e) S112	44°C
	f) S118	44°C
	g) S124	44°C
	h) S125	44°C
Tetraselmis tetrahele UMACC 144	a) OPA13	40°C
	b) OPN13	44°C
	c) OPN16	44°C
	d) S17	40°C
	e) S67	44°C
	f) S68	44°C
	g) S87	44°C
	h) S118	44°C
Boergesenia forbesii	a) OPA13	40°C
	b) OPK14	38°C
	c) OPN6	38°C
	d) OPN13	38°C
	e) S17	40°C
	f) S67	44°C
	g) S105	38°C
	h) S124	38°C
Ventricaria ventricosa	a) OPA13	40°C
	b) OPK14	40°C
	c) OPN12	38°C
	d) OPN13	44°C
	e) S17	44°C
	f) S20	40°C
	g) S67	44°C
	h) S86	40°C

APPENDIX 45: Detailed Results of RAPD Assay

NO. OF PCR BAND PRODUCED USING 190 TYPES PRIMER

No.	Primer	Chlorella	Tetraselmis	Boergesenia	Ventricaria	No.	Primer	Chlorella	Tetraselmis	Boergesenia	Ventricaria
1	OPA 1	0	0	0	0	97	S 37	0	3	0	6
2	OPA 2	8	5	7	6	98	S 38	6	7	1	6
3	OPA 3	7	9	1	0	99	S 39	1	0	0	5
4	OPA 4	6	8	3	6	100	S 40	7	9	5	4
5	OPA 5	4	10	7	6	101	S 41	0	0	0	0
6	OPA 6	0	1	2	6	102	S 42	4	5	5	3
7	OPA 7	4	5	5	7	103	S 43	9	7	4	4
8	OPA 8	4	2	6	6	104	S 44	4	4	3	3
9	OPA 9	7	6	3	5	105	S 45	7	4	4	6
10	OPA 10	3	8	0	7	106	S 46	0	2	0	0
11	OPA 11	4	9	4	6	107	S 47	2	9	0	5
12	OPA 12	3	7	4	6	108	S 48	9	7	8	5
13	OPA 13	8	8	2	7	109	S 49	0	0	0	0
14	OPA 14	4	6	2	2	110	S 50	2	4	0	5
15	OPA 15	3	8	1	7	111	S 51	7	5	5	5
16	OPA 16	1	9	5	7	112	S 52	1	4	6	3
17	OPA 17	0	6	0	8	113	S 53	3	7	2	4
18	OPA 18	7	6	7	5	114	S 54	0	3	0	3
19	OPA 19	3	3	6	7	115	S 55	2	6	4	1
20	OPA 20	0	10	5	3	116	S 56	5	8	3	6
21	OPK 1	3	3	5	6	117	S 57	0	2	0	0
22	OPK 2	3	10	0	4	118	S 58	4	7	6	3
23	OPK 3	1	8	0	3	119	S 59	0	8	0	5
24	OPK 4	0	5	4	0	120	S 60	7	10	5	7
25	OPK 5	0	4	0	0	121	S 61	6	4	0	2
26	OPK 6	1	2	2	4	122	S 62	3	10	7	4
27	OPK 7	6	8	5	2	123	S 63	0	0	0	0
28	OPK 8	3	5	8	4	124	S 64	4	9	8	4
29	OPK 9	0	3	2	1	125	S 65	4	9	5	3
30	OPK 10	1	6	7	4	126	S 66	1	8	1	9
31	OPK 11	0	6	4	4	127	S 67	8	9	8	6
32	OPK 12	1	7	1	5	128	S 68	6	14	3	5
33	OPK 13	2	4	5	4	129	S 69	4	2	1	3
34	OPK 14	3	7	8	6	130	S 70	4	4	4	4
35	OPK 15	3	6	4	5	131	S 71	6	7	7	6
36	OPK 16	6	11	4	8	132	S 72	1	0	2	1
37	OPK 17	3	8	6	5	133	S 73	5	7	5	1
38	OPK 18	0	2	0	0	134	S 74	2	4	5	2
39	OPK 19	5	5	5	3	135	S 75	6	6	5	9
40	OPK 20	3	3	1	1	136	S 76	5	4	5	4
41	OPN 1	1	6	0	5	137	S 77	0	0	0	0
42	OPN 2	4	6	0	7	138	S 78	4	5	5	2
43	OPN 3	1	6	4	7	139	S 79	3	6	4	7
44	OPN 4	5	7	7	9	140	S 80	3	6	4	5
45	OPN 5	1	8	6	6	141	S 81	0	1	0	0
46	OPN 6	6	7	10	4	142	S 82	2	7	7	6
47	OPN 7	7	8	4	1	143	S 83	1	3	5	5
48	OPN 8	9	2	5	4	144	S 84	3	8	6	6
49	OPN 9	6	7	6	6	145	S 85	8	7	6	6
50	OPN 10	3	10	6	6	146	S 86	3	5	6	9
51	OPN 11	4	7	4	5	147	S 87	4	14	6	3
52	OPN 12	4	6	5	9	148	S 88	4	6	4	6
53	OPN 13	9	9	4	9	149	S 89	4	5	4	4
54	OPN 14	4	8	3	3	150	S 90	9	9	7	5
55	OPN 15	6	10	2	5	151	S 91	6	7	4	5
56	OPN 16	7	12	8	4	152	S 92	9	4	7	6
57	OPN 17	2	3	5	4	153	S 93	3	9	4	2
58	OPN 18	5	8	5	4	154	S 94	5	7	7	5
59	OPN 19	1	5	4	5	155	S 95	5	5	1	5
60	OPN 20	3	7	3	5	156	S 96	4	6	9	5
61	S 1	0	2	8	0	157	S 97	4	7	5	5
62	S 2	1	1	0	6	158	S 98	4	8	8	3
63	S 3	0	2	5	9	159	S 99	6	5	6	7
64	S 4	2	3	5	2	160	S 100	0	0	0	0
65	S 5	2	6	6	4	161	S 101	7	3	0	3
66	S 6	3	10	0	4	162	S 102	4	5	5	3
67	S 7	3	6	8	5	163	S 103	5	9	6	7
68	S 8	0	9	6	9	164	S 104	4	7	7	5
69	S 9	0	5	0	7	165	S 105	8	6	10	2
70	S 10	4	7	5	7	166	S 106	5	6	3	7
71	S 11	1	7	5	4	167	S 107	7	8	4	5
72	S 12	1	7	4	3	168	S 108	4	5	7	6
73	S 13	0	5	2	5	169	S 109	4	6	4	2
74	S 14	0	6	5	5	170	S 110	0	0	0	0
75	S 15	1	5	6	2	171	S 111	5	2	1	0
76	S 16	0	6	0	2	172	S 112	10	9	9	3
77	S 17	6	9	7	8	173	S 113	7	9	5	4
78	S 18	3	8	1	4	174	S 114	7	7	3	5
79	S 19	0	2	0	5	175	S 115	8	5	7	3
80	S 20	3	5	5	11	176	S 116	8	3	0	2
81	S 21	3	6	5	7	177	S 117	7	6	3	4
82	S 22	2	4	3	8	178	S 118	9	11	3	4
83	S 23	7	9	5	2	179	S 119	9	8	5	4
84	S 24	7	9	1	5	180	S 120	7	6	3	5
85	S 25	0	4	4	6	181	S 121	8	7	6	4
86	S 26	0	3	0	4	182	S 122	6	5	4	2
87	S 27	4	5	3	6	183	S 123	6	8	8	2
88	S 28	1	1	4	6	184	S 124	13	8	10	3
89	S 29	7	7	0	6	185	S 125	10	7	6	4
90	S 30	3	5	0	8	186	S 126	7	6	3	2
91	S 31	2	8	0	8	187	S 127	1	2	0	2
92	S 32	2	5	0	9	188	S 128	5	3	8	3
93	S 33	8	10	0	6	189	S 129	2	2	2	4
94	S 34	3	6	0	2	190	S 130	6	8	8	0
95	S 35	1	8	0	3						
96	S 36	2	10	0	7						

APPENDIX 45: Detailed Results of RAPD Assay

TEMPERATURE OPTIMIZATION FOR PRIMER-NO. OF PCR BAND

CHLORELLA

Temperature	OPA2	OPA3	OPA13	OPN13	OPN8	S18	S23	S43	S48	S68	S90	S124
34°C	5	12	12	5	2	8	6	5	3	5	9	10
36°C	4	3	14	6	3	8	5	5	4	7	8	9
38°C	5	9	8	4	5	2	5	4	5	4	9	10
40°C	0	0	0	1	0	1	0	0	0	1	1	0
42°C	5	6	7	8	6	9	7	4	3	4	9	10

TETRASELMIS

Temperature	OPA5	OPK16	OPN5	OPN13	S6	S40	S60	S62	S68	S90	S112	S123
34°C	6	13	8	6	10	6	9	4	7	7	4	6
36°C	4	15	10	8	9	11	9	6	4	4	4	7
38°C	5	11	7	8	12	9	9	6	7	8	7	8
40°C	0	0	0	0	0	0	9	7	7	8	7	8
42°C	0	10	6	8	8	7	6	3	3	4	8	6

BOERGESENIA

Temperature	OPA18	OPK8	OPN5	OPN6	S84	S86	S90	S96	S105	S124	S125	S130
34°C	5	5	4	3	5	1	5	9	10	11	4	8
36°C	5	6	5	9	6	1	6	10	12	10	7	9
38°C	7	10	5	9	8	7	7	8	11	11	6	6
40°C	0	0	0	0	0	0	0	0	0	0	0	0
42°C	0	0	3	1	6	5	6	8	9	8	3	3

VENTRICARIA

Temperature	OPA5	OPA8	OPA10	OPK12	OPN4	OPN5	OPN12	S25	S30	S75	S84	S86
34°C	4	2	4	7	6	5	4	4	4	5	8	7
36°C	4	3	3	7	6	3	5	4	4	7	8	7
38°C	5	4	4	9	10	8	8	3	4	8	7	7
40°C	0	0	0	0	0	0	0	0	0	0	0	0
42°C	3	3	2	8	6	5	4	4	5	6	5	7