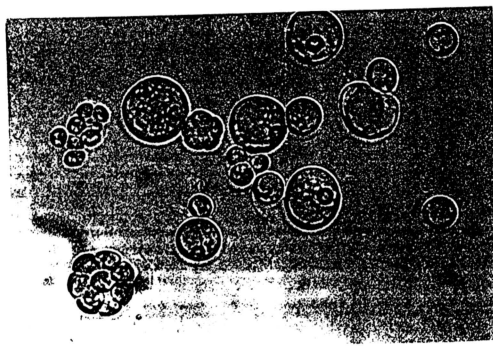
CHAPTER 5

DISCUSSION



Chlorococcum sp. (isolate 110)

CHAPTER 5 : DISCUSSION

5.0 Discussion

5.1 Preliminary Toxicity Test

Algal species which are sensitive to heavy metals make useful indicators of the metals and may be used as the test organisms in toxicity tests to generate data for use in the formulation of water quality criteria and standards. Of eleven microalgal species tested, *Chlorella vulgaris* (isolate 001), *Scenedesmus* sp. (isolate 039) and *Synechococcus* sp. (isolate 075) may used as indicator for copper (Cu) because the three algae were most sensitive to Cu toxicity (Table 14, Chapter 4, page 66). *Mougeotia* sp. (isolate 069) may be use as indicator for zinc (Zn), while *Ulothrix* sp. (isolate 071) and *Euglena* sp. (isolate 058) are sensitive to manganese (Mn). *Chlorella* sp. (isolate 078), *Chlorococuum* sp. (isolate 110) and *Oocystis polymorpha* UTEX LB 1645 (isolate 153) may be indicators for iron (Fe), *Ankistrodesmus convolutus* (isolate 101) indicator for Cobalt (Co) and *A. arcuatus* UTEX LB 1379 (isolate 170) as indicator for chromium (Cr).

Tolerant species may be used for long term biomonitoring, as they can bioaccumulate high level of metals without being damaged or killed. Of the eleven species *C. vulgaris*, *Scenedesmus* sp., *Mougeotia* sp., *Synechococcus* sp., *Chlorella* sp. (isolate 078) and *A. arcuatus* UTEX LB 1379 may be used to monitor Mn. *Chlorococuum* sp., *Euglena* sp., *Oocystis polymorpha* UTEX

LB 1645 are tolerant of Cd, *Ulothrix* sp. tolerant of Cr and *A. convolutus* tolerant of Cu.

These results present a useful reference point for further detailed investigations on selection of appropriate species to be used as indicators or biomonitors of heavy metals.

A. convolutus was selected for detailed investigation, for five reasons: (i) of the eleven microalgae, *A. convolutus* was most sensitive to Co and relatively sensitive to Cr, Cd , Mn and Fe (Table 13, page 64). This makes it a useful sensitive species for heavy metal toxicity testing. (ii) it has high tolerance to Cu which is one of the toxic compounds found in abundance in Malaysian waters. (iii) it is unicellular. (iv) the cells are large (length: 19.8 – 22.0 μm and width / diameter: 2.2 – 3.3 μm). (v) it grows fast and easily (growth rate: 0.32 ± 0.01 μ, day^{-1} ; Chu *et al.* ,1992). According to Chu *et al.*, (1992 and 1995a), *A. convolutus* has high carotenoid content (8.6 mg g^{-1} DW) and appreciable amounts of lipids (17.0 ± 0.6 % DW) and may be a potential poultry feed. *A. convolutus* therefore also has economic significance. No distinct morphological changes occur in *A. convolutus* under 12 : 12 h LD or continuous illumination (Chu *et al.*, 1995b). It has stable morphological characters which therefore would not interfere with its use in bioassays or toxicity tests. This fact is important to make sure that morphological characteristics do not change under different cultural conditions .

5.2 Toxicity Tests Of Different Metals On *Ankistrodesmus convolutus*

Single species toxicity tests have provided the great majority of data used in evaluating the hazards of waste materials and are an unsurpassed tool for studies of relative sensitivity of organisms, relative toxicity of chemicals or effluents, or effects on population level responses such as growth or reproduction (Cair & Niederlehner, 1987). Therefore in the present study, *A. convolutus* was chosen for detailed studies. This project is an important milestone for the management of wastes in Malaysia and is an effort towards promoting systematic and environmentally sound waste management.

Heavy metals in general, observed by Clijsters & Van Assche (1985), inhibit plant growth and overall physiological processes including photosynthesis. Heavy metals also have the ability to denature proteins (Gadd & Griffith, 1978) and stimulate the hydrolytic activity of protease, RNAase, and DNAase (Lee *et al.*, 1976).

Observations based on growth or its inhibition is an a appropriate method to investigate the effect of heavy metal toxicity on *A. convolutus*.

With this is mind, the culture medium, like nearly all laboratory media in routine use, shows substantial qualitative and quantitative chemical differences from the composition of most natural waters. Thus, care must be taken in directly extrapolating toxicity levels, determined for a single algal strain under a single set of laboratory environmental conditions, to actual situations in nature.

(i) Iron (Fe): Among the heavy metals studied, Fe was the most toxic with the lowest 96 h IC_{50} of 0.52 mgL^{-1}. However, Borovik (1989), classed Fe as one of the most essential mirconutrients. In algae a very high concentration of Fe is needed. Upitis *et al.* (1989), gave between 80 to 100 mgL^{-1} of Fe for normal proportions growth in *Chlorella* sp. In the normal criteria of Fe nutrition in *Chlorella* sp., about 200 to 800 mgL^{-1} are needed and concentrations of 900 to 1500 mgL^{-1} are needed for abundant growth. Less than 150 mgL^{-1} will cause deficiency. This contrasts to results obtained in the present study. *A. convolutus* might be explained in several ways. Considerable morphological and physiological differences exist between *Ankistrodesmus* and *Chlorella*. Secondly, the illumination and temperature conditions utilised by Upitis *et al.* (1989) were not mentioned and may be different substantially from those employed in the present study. Therefore, further studies are required to understand this interesting though contradictory observation.

(ii) Cobalt (Co): Co was the second heavy metal which was toxic to *A. convolutus*. The present study indicates that *A. convolutus* is very sensitive to Co with 96 h IC_{50} and IC_{25} values of 0.58 and 0.29 mgL^{-1} (see Table 24, page 88). Co affects algal growth because it inhibits synthesis of the photosynthesis pigment. This was reported by Issa *et al.* (1995) who worked with *Kirchneriella lunaris* culture which had a 50% loss of chlorophyll and carotenoids when incubated in 10^{-4} M of Co.

(iii) Chromium (Cr): Very few studies have been done concerning Cr toxicity. The present study showed that *A. convolutus* can grow at lower concentration of Cr, but at high Cr concentration growth is inhibited. The 96 h IC_{50} and IC_{25} values were 7.29 and 0.97 mg L^{-1} Cr (see Table 24, page 88). Effect of Cr toxicity to algae is not well documented especially in *A. convolutus*.

(iv) Copper (Cu): The present study showed that *A. convolutus* is moderately tolerant to Cu compared to the other metals. The 96 h IC_{50} and IC_{25} values were 11.14 and 4.66 mgL^{-1} Cu (see Table 24, page 88). Stauber & Florence (1986) observed that the growth of *Nitzschia closterium* was inhibited when treated with 20 μgL^{-1} of Cu. Garvey *et al.* (1991) in a study on *Chlamydomonas reinhardtii* (Chlorophyceae), observed that encysted cells first appeared in cultures exposed to 14 µg Cu L^{-1} and encystment significantly increased in cells exposed to 48 and 64 µg Cu L^{-1}, showing that stress from exposure to Cu occurred.

Cu has been reported to inhibit photosystem II in *A. falcatus* (Shioi *et al.*, 1978), but it depends on the algal species as to whether Cu inhibits primarily photosynthesis rather than other functions such as respiration, pigment formation or cell division (Stauber & Florence, 1987).

Cu is classed as one of the most essential micronutrients (Borovik, 1989). In algae, Cu is required for both photosynthesis and respiration

(Sandman, 1985). Upitis *et al.* (1989) stated that the normal proportions criteria of mineral nutrition of algae for example in *Chlorella* sp. is 1 mgL^{-1}. The normal criteria is 4 to 15 mgL^{-1} and 15 to 25 mgL^{-1} is the range for abundant growth. If less than 2 mgL^{-1} a deficiency occurs, but at levels above that required for optimal growth, Cu is known to be toxic. Thus, more work is necessary to elucidate the role of Cu as an essential micronutrient and its toxicity, in *A. convolutus*.

Cu according to Hellawell (1986), readily forms complexes with a wide range of other substances commonly found either in clean or polluted waters. Cu is also readily adsorbed onto suspended solids. In algae, the toxicity of Cu varies widely. Gavis *et al.* (1981), reported this may due to the interspecific and clonal variations in algae. It is also related to various factors such as hardness and the presence of organic matter, temperature and oxygen concentration (Hellawell, 1986) and composition and pH of growth media and the physiological condition of algal cells (Gavis *et al.*, 1981). However, pH effects on Cu toxicity are somewhat contradictory. Rai *et al.* (1981), observed that toxicity of Cu increased at lowered pH, while according to Steemann Nielsen & Kamp-Nielsen (1970), the toxicity of Cu is increased with increasing pH. The reduction of metal uptake at lowered pH, may due to binding site competition between H^+ and free metallic ions at the cell membrane (Harrison *et al.*, 1986). Metal toxicity may be regulated to some extent by macronutrient availability (Bates *et al.*,1985). Li (1979) and Say &

Whitton (1977) observed that the Cu toxicity decreased with increase in phosphorus (P) concentration. Hall *et al.* (1989b), observed that *Chlamydomonas geitleri* and *C. vulgaris*, were more sensitive to Cu under P-limitation than under nitrogen (N)-limitation. A possible explanation for the increased sensitivity to cellular Cu concentration of P-limited cultures compared with N-limited cultures may lie in a mechanism for detoxification of metals within the cell proposed by Sicko-Goad & Stoermer (1979) and Petersson *et al.* (1985). They proposed that metals may be compelled by polyphosphates within the osmotically inert polyphosphate bodies and therefore be detoxified. Petterson *et al.* (1985) showed a similar effect for Al in *Anabaena cylindrica* as did Sicko-Goad & Stoermer (1979) for Pb in *Diatoma tenue*. Since the formation of polyphosphate bodies is dependent on P availability (Rhee, 1973), the availability of P may influence the cell's ability to detoxify Cu, and hence result in a greater sensitivity to Cu in P-limited cultures. Thus, much work is necessary to elucidate the roles of medium composition, organic chelations, light, temperature and pH in Cu toxicity and accumulation in *A. convolutus*.

(v) Cadmium (Cd): In the present study, addition of Cd as $CdCl_2$ in concentration of 1 mgL^{-1}, in the definitive test of *A. convolutus* cultures, was found to stimulate growth as expressed in an increase in cell number. *Chlorella ellipsoidea* have also been observed to be stimulated in growth

when exposed to 0.56 mgL^{-1} Cd concentration in the nutrient solution (Lue-Kim *et al.*,1980).

Increased concentration of Cd caused growth inhibition. The 96 h IC_{50} and IC_{25} values were 12.02 and 6.52 mgL^{-1} Cd (see Table 24, page 88). Several other algal species have been shown to be less tolerant to Cd. Cd as low as 1 μgL^{-1} has been reported to inhibit growth in the marine diatom *Cylindrotheca closterium*. Concentration above 5 mgL^{-1} Cd was lethal to *A. falcatus*, *Scenedesmus obliquus* and 10 mgL^{-1} for *Chlorococcum* spp. (Devi Prasad & Devi Prasad, 1982).

The most dramatic effect of Cd toxicity is on cell division (Lue-Kim *et al.*,1980). In *C. ellipsoidea* cultures which had been exposed to 14 h light and treated with Cd at the end of this period or 2 or 4 h into the dark period, failed to reach the cell number count of control cultures. According to them, a threshold value of 2.9 times the initial dry weight, in the form of synthesised organic substances, is required before some degree of cell division can occur.

The toxicity of Cd to microalgae is due to its effects on various metabolic processes. Low concentrations of Cd can inhibit photosynthesis and respiration in *Cylindrotheca closterium*. Cd at concentration of 50 µM was toxic to cell division and inhibited chlorophyll synthesis in *C. ellipsoidea* (Lue-Kim *et al.*,1980). In *Thalassiosira aestivalis* Cd interfered with cell division (Thomas *et al.*, 1980). Fernandez-Leborans & Novillo (1996) reported that Cd can inactivate various enzymes in eukaryotic algae and

cyanobacteria. This could be due to its binding to the sulfhydryl groups of protein and amino acid (Trevors *et al.*, 1986). Cd toxicity causes decrease of cellular volume, growth rate and the levels of photosynthetic pigments in a marine phytoflagellate (*Olisthodiscus luteus*), observed by Fernandez-Leborans & Novillo (1996). Reduction of cell number was also observed in *Chlamydomonas reinhardii* at concentration of 7.5 to 20 μg Cd L^{-1} (Lawrence *et al.* ,1989) and in *Selenastrum capricornutum* at a concentration of 30 to 100 μg Cd L^{-1} (Thompson & Couture, 1991).

Cd toxicity due to concentration and duration of exposure not only inhibited growth rate but also led to disturbances in cell division. Massalski *et al.* (1981) reported on the growth of *A. braunii* exposed to increasing amount of Cd ions which led to the appearance of larger proportions of electron-dense cells whose organelles were less well defined than those of untreated cells. Similar findings by Rosko & Rachlin (1977) from *C.vulgaris* cells exposed to 0.32 mgL^{-1} of Cd for 16 days which were larger than control cells and this effect could still be seen after 33 days. The observed reduction in cell number may be a reflection of depressed synthesis of both chlorophyll and dry weight components. It is suggested here that long term exposure *A. convolutus* to metals should be investigated.

Devi Prasad & Devi Prasad (1982), observed that Cd can cause stimulation of growth in *A. falcatus*, *Chlorococcum* sp. and *S. obliquus*.This

could be a result of presence of specific enzymes as an daptation to Cd tolerance, or stimulation of general cellular metabolism. Cd has also been observed to increase growth rate in the diatom *Skeletonema costatum* and *S. capricornutum* (Thompson & Couture,1991). Thompson & Couture (1991) attribute the observation to a transitory physiological compensation in response to chemical stress, as part of the process of species acclimatisation. This response is known as "hormesis", and can also be observed as a consequence of natural pressures.

A major effect of Cd is the inhibition of cell division, although different species may exhibit different levels of tolerance to this toxicant. It is also possible that Cd affects nucleic acid duplication.

(vi) Zinc (Zn): The present study shows that of the metals tested, *A. convolutus* is most tolerant to Zn. The 96 h IC_{50} and IC_{25} values are 16.14 and 8.57 mgL^{-1} Zn (see Table 24, page 88). Rachlin & Farran (1974) observed that *C. vulgaris* treated with 2.4 mgL^{-1} Zn in 96 h had its growth rate reduced by approximately 50%.

According to Hellawell (1986), even though Zn has low toxicity to man , it is highly toxic (around 5 mgL^{-1}) to many species of fish. Whereas, Borovik (1989) in his study, classify Zn as one of the most essential micronutrients. Zn is one of the metals needed by algae to carry out physiological activities (Guanzon *et al.*,1994). Upitis *et al.* (1989) suggested

2 to 3 mgL^{-1} Zn as requirement for the minimal growth of *Chlorella sp.*; 20 to 30 mgL^{-1} for normal growth and 35 to 40 mgL^{-1} for enhanced growth. Whereas a level lower than 15 mgL^{-1} is considered as a deficiency in Zn.

Starodub *et al.* (1987) showed that in *Scenedesmus quadricauda*, Zn toxicity is reduced by production of extracellular ligands which can bind Zn. An example of a ligand is polyphosphate. Bates *et al.* (1985) observed that the concentration of cellular polyphosphate in the cell declines with increasing culture age. When the quantity of intracellular Zn exceeds a critical threshold, the phosphorous metabolism is disrupted, interfering with cell division and this will cause a decrease in cell yield.

The tolerance of microalgae to Zn varies between the species. In Chlorophyta, Takamura *et al.* (1989) reported that 17 Chlorophyta isolated from polluted areas, were tolerant to a range of 8.4 to more than 98.1 mgL^{-1} of Zn.

(vii) Manganese (Mn): Generally, Mn is not a significance pollutant and thus, few studies have been done concerning the toxicity of Mn (Hellawell, 1986). Mn is also an essential micronutrient (Borovik, 1989) and very high concentrations are needed for growth. According to Upitis *et al.* (1989 *Chlorella* sp., need about 8 to 10 mgL^{-1} of Mn for normal proportions, 30 to 80 mgL^{-1} for normal criteria, 100 to 350 mgL^{-1} for abundant and if less than 20 mgL^{-1} will cause a deficiency. Present study showed that *A. convolutus* is

very tolerant of Mn compared to the other metals with 96 h IC_{50} and IC_{25} values were 16.14 and 8.57 mg L^{-1} Mn (see Table 24, page 88). This suggest that Mn is one of the essential micronutrients for *A. convolutus*.

The growth rate of *Kirchneriella lunaris* (Chlorophyta) was affected by Mn. According to Issa *et al.* (1995), *Kirchneriella lunaris* when treated with 10^{-4} M of Mn, lost 63% (0.39 gL^{-1}) of dry weight compared to the control (1.05 gL^{-1}). However, with the same concentration of Mn the production of protein (23.30 mgg^{-1} DW) was inhibited compared to the control (53.67 mgg^{-1} DW). According to Sabnis *et al.* (1969), Mn can cause chlorophyll damage, with a location on the thylakoid membranes showing an affinity for heavy metals.

In summary the tolerance of *A. convolutus* to the heavy metals in increasing order is as follows:- Fe < Co < Cr < Cu < Cd < Zn = Mn (96 h IC_{50} : Fe = 0.52, Co = 0.58, Cr = 7.29, Cu = 11.14, Cd = 12.02, Zn = 16.14 and Mn =16.14 mgL^{-1}).

5.3 Bioaccumulation Studies

Many studies conducted to date have shown that heavy metals have detrimental effects on aquatic organisms and in general the effects of heavy metals on aquatic animals are better known than the effects on aquatic plants. In Malaysia for example, more studies have been done on marine macroalgae than on either freshwater or marine microalgae. Since phytoplankton form the

basis of food chains, they are potentially capable of transferring heavy metals to higher trophic levels. The extent to which phytoplankton transfer heavy metal through the food chain depends, in part, on their ability to accumulate and to a large extent, tolerate high concentrations of the metal before they themselves become obviously affected.

Studies conducted to date with *A. convolutus* freshwater algae have generally focused either on heavy metal such as Cd, Co, Zn, Mn, Cr, Cu or Fe phytotoxicity and bioaccumulation. However, certain aspects of heavy metal accumulation by can only be studied after first establishing, under controlled experimental conditions, levels of a particular metal which adversely affect growth. With this in mind, this laboratory study was undertaken only to demonstrate the effects of various concentration of such metals and efficiency of bioaccumulation by *A. convolutus*. This study also determined whether the incubation time and age of the cell population influences heavy metal accumulation in this organism.

Another purpose is to establish the efficiency of metal bioaccumulation in *A. convolutus*.

Bioaccumulation of metals by algae may present a feasible method for remediating contaminated waste waters (Darnall *et al.*, 1986). Generally, according to Wolterbeek *et al.* (1995), uptake is considered to comprise an initial rapid phase, which is independent of light, temperature and the presence of metabolic inhibitors (cell wall adsorption) (Ting *et al.*, 1991; Garnham *et*

al., 1992) and subsequent slow uptake due to active membrane transport of the metals into the cells. However, active transport is not always found.

Essentially, metal accumulation should be regarded as the result of simultaneous influx and efflux reaching an equilibrium or steady state in the case of endogenous cellular constituents of low or high molecular weight (Okamura & Aoyama, 1994).

According to Stauber & Florence (1986), following Cu penetration into the cell, Cu may react with -SH enzyme groups and thiols, disrupting enzyme active sites, and leading to a lowering of the GSH-GSSG ratio and suppression of mitosis . These was shown when only 1.68 nmol SH 10^{-1} cells were detected in *Nitzschia closterium* when treated with 175 gL^{-1} of Cu, compared to 3.68 nmol SH 10^{-1} cells in Cu-untreated cells. The same occurred with *Chlorella sorokiniana*, where exposure to Cu decreased the -SH content from 0.051 µmol SH μg^{-1} chlorophyll to 0.028 µmol SH μg^{-1} chlorophyll (Stauber & Florence, 1986). This suggests that Cu may exert its toxicity by oxidizing intracellular thiols, and that this process is reversible through thiol-exchange reactions.

Above certain metal levels, photosynthesis processes in plankton may decrease (Davies & Sleep, 1980). A second aspect of metal accumulation by plankton is the transport of metal into food chains with possible bioaccumulation. The metal content of organisms depends on the metal

concentration in the ambient water as well as metal bioavailability (Davies, 1978 in Wolter 1984; Canterford & Canterford, 1980 and Rai *et al*, 1978).

According to Vymazal (1984), the metal uptake pattern may divided approximately into three groups (i) rapid uptake occurred in the first hour of exposure and then only sligh uptake appears; or (ii) rapid uptake during first two hours of exposure appears and then only slight uptake takes place; or (iii) continous uptake appears during the entire hour of exposure. Similar uptake pattern also observed in the present study, approximately four groups were observed (i) rapid uptake during the first hour of exposure and then followed by a gradual release; or (ii) continuous uptake appears during the entire hour exposure till equilibrium; or (iii) rapid uptake during the first hour of exposure and followed by a gradual release till equilibrium; or (iv) alternating uptake – release pattern.

According to Fehrmann & Pohl (1993) , there were two steps of heavy metal uptake in algae. The first is a rapid and reversible reaction called 'physical adsorption' or 'biosorption' to the cell surface. The second involves uptake of heavy metals into the cells. It is slower and called 'chemisorption' or 'bioaccumulation' (Aksu & Kutsal, 1990). About 90% of the total amount of heavy metal taken up is adsorbed by 'biosorption' within the fist 5 - 40 min and only 10% of the total amount is absorbed by 'bioaccumulation' within the following hours of days (Geisweid & Urbach, 1983 and Khummongkol *et al*.,

1982). The organism will finally die due to the toxic effect of the accumulated heavy metal ions and can be used only once for heavy metal elimination.

Bioaccumulation may decrease as a result of a diminution of permeability, active accumulation and characteristics of absorption surfaces, while active excretion may also have an important role (Albergoni *et al.*, 1980). In addition, intracellular chelators may be observed in algae in the presence of the metal. Albergoni *et al.*(1980) and Gipps & Coller (1980) have established that *Euglena gracilis* is able to high concentrations of Cd. According to Fernandez-Leborans & Novillo (1996), a greater reduction of chlorophyll levels was observed in the 500 μg Cd L^{-1} treatment, with about 80.6% (184.8 mg m^{-3}) reduction with respect to the control (953.6 mg m^{-3}), while in the 10 μg Cd L^{-1} treatment this reduction was 33.59% (633.3 mg m^{-3}). These pronounced differences in the levels of chlorophyll may be a result of the increased internal concentrations of Cd, which is highest in the 500μg Cd L^{-1} treatment. It is well known that Cd, in concentrations of up to 40 μg Cd L^{-1}, can cause disorganisation of chloroplasts, and at concentration of 10 - 100 μg Cd L^{-1} the mitochondria structure is modified (Soyer & Prevot, 1981) and the photosystems (De Fillippis *et al.*, 1981). This ultrastructural damage can be reflected in a reduction of the photosynthetic pigments.

5.3.1 Effect of Concentration

Present results show that generally the accumulation Cd, Co, Zn, Mn and Fe

in the *Ankistrodesmus convolutus* cells increased proportional to metal concentration in the medium (see Table 25, page 148 and 149). Table 26 shows the correlation between metal concentration in medium and the bioaccumulation factor of the metals in *A. convolutus*. The analysis was done with data from the logarithmic growth cells only. In general, very good correlation were obtained (r ranging from 0.001 to 0.9968, $p < 0.01$) for Cd, Zn, Co, Mn, Cu and Fe. Poor correlation was obtained for Cr. This was also observed by Costa & Leite (1991), in studies using *Chlorella* and *Scenedesmus* species where the uptake of Cd and Zn was dependent on the external concentration of the metal, but up a to level at which, the toxic effects will lead to reduction in accumulation. Okamura *et al.* (1994) observed that *Chlorella ellipsoidea* cells accumulated Cr up to 300 fg/cell ($f = 10^{-15}$) after 1 day exposure to the highest concentration of Cr used. Cr in the medium was increasingly accumulated in algal cells with increasing Cr concentration Sakaguchi *et al.* (1979) working with *C. vulgaris* also reported that amounts of Cd absorbed increased as the external Cd concentration increased.

This present investigation has demonstrated that the intracellular Cd content is directly proportional to the concentration of Cd in the medium and confirms the observation that Cd is accumulated *by A. convolutus* . Similar observation was reported by Bartlett & Rabe (1973), in *C. pyrenoidosa* cultures treated with Cd.

Table 26: Correlation factor (r) of analysis to show effect of metal concentration on metal accumulation

Metal	r $p < 0.01$
Cd	0.9968
Fe	0.9945
Zn	0.9608
Co	0.9157
Mn	0.8937
Cu	0.7568
Cr	0.001

Exposure to combinations of metals should be studied. Cd accumulation in *Chlorella pyrenoidosa* is not affected by the concentration of calcium (Ca), magnesium (Mg), molybdenum (Mo), Cu, Zn or Co in the growth medium (Hart & Scaife, 1977), whereas a level of Mn equal to 0.2 mgL^{-1} completely blocks Cd accumulation; Fe may also play a role in regulating Cd accumulation. Similar observations were also reported by Stauber & Florence (1985), in the marine diatom (*N. caldarium*) where dissolved Fe is immobilised on the cell surface as colloidal ferric hydroxide which binds to the cell membrane. This colloid also adsorbs Cu and prevents Cu penetration into the cell. Fe-colloid covered diatom cells reduce the toxicity of Cu ions in seawater.

5.3.2 Effect of Time

According to Fernandez-Leborans & Novillo (1996), time is an important factor to be considered for metal bioaccumulation study. *Olisthodiscus luteus* was shown to be less able to accumulate Cd in the final stages of the experiment, even though the uptake was dependent on the concentration of metal in the external medium. The present results also show that the maximum Cd, Co, Cr, Cu, Fe, Mn, and Zn uptake happened during the first hour of exposure. Winter *et al.* (1994), for *Ectocarpus siliculosus* (brown alga) as well as Geisweid & Urbach (1983), also found that 80% of added Cd were adsorbed by *Asterionella formosa*, *Fragilaria crotonensis* and *Chlorella*

vulgaris after 5 minutes of exposure. Sakaguchi *et al.* (1979), reported that Cd was absorbed by *C. vulgaris* was very rapidly during the first 30 minutes following exposure.

According to Vymazal (1984), the four hour periods were suitable for studying metal bioaccumulation considering that the maximum time of retention in a continuous-flow system would not exceed this period.

In some cases the uptake can occur for more then 1 h. For example Fernandez-Leborans & Novillo (1996) using *Olisthodiscus luteus* exposed to Cd for 12 h found that 13% of Cd was lost with concentration of 10 μgL^{-1} test solution, 86% with test solution of 50 μgL^{-1} and 91% with test solution of 500 μgL^{-1}. Whereas Cd concentration in the *O. luteus* cells after 12 hr was between 0.90 to 56.42 fg $cell^{-1}$ for Cd concentration in the range 38 - 56 μgL^{-1}, 0.57 to 17.15 fg $cell^{-1}$ for concentration from 2 to 6 μgL^{-1}, in the respective test solutions.

The accumulation of metal ions in growing algal cells, should consider both the initial rapid (passive) and the subsequent slow (active) uptake, while longer-term accumulation is interpreted as the result of simultaneous influx and efflux (Wolterbeek *et al.*, 1995). Short-term effects of metal pollutants may result in inhibition of cell growth, cell division and metabolic functions (Thompson & Couture, 1991).

Vymazal (1987) concludes that the uptake of the metal is linear within a certain range, but when biomass decreases relative to the amount of metal

available, proportional accumulation decreases hyperbolically, as a result of weight dilution. This was shown by Aoyama & Okamura (1993), where Cd concentration in the medium continued to decrease even after 24 h when *Chlorella* sp. was exposed to a concentration of 10 μequiv L^{-1} Cd. This was explained by the fact that uptake by *Chlorella* sp. did not reach saturation, since no reduction in the number of cells was observed. However, at the concentration of 20 μequiv L^{-1} Cd, *Chlorella* sp. reached saturation during the first 24 h.

5.3.3 Effect of Age

Present results show that the logarithmic phase of *A. convolutus* accumulate Cd, Co, Cr, Cu, Fe, Mn, and Zn more actively compared to the stationary phase. *Chlorella ellipsoidea* reported by Okamura *et al.* (1994) also accumulated Cd up to 1400 fg/cell ($f = 10^{-15}$) after 1 day exposure and decreased the Cd concentration to less than 200 fg/cell in the stationary growth phase. Sakaguchi, *et al.* (1979), observed that as the algal cell concentration increased the total amount of Cd absorbed in *C. vulgaris* cells increased. At high concentration of algal cells (OD_{620} nm = 2.0), 82.5% of Cd existing in the external solution was accumulated by *C. vulgaris* cells.

After uptake into a cell, chemicals may remain unchanged in solution, they may be deposited, or they may become reversibly or irreversibly bound to

endogenous cellular constituents of low or high molecular weight (Okamura & Aoyama, 1994).

The facts that physiological changes associated with aging of cell population can effect metal accumulation and that organisms differ in this regard, are significant for those contemplating use of algae in removal of metals from wastewater. Thus, further research should be conducted to understand the interaction between *A. convolutus* and heavy metals.