# Toxicity Testing of Four Heavy Metals with Selected Marine Phytoplankton

by

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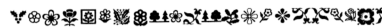
**Dissertation submitted to**
**The Institute of Postgraduate Studies and Research, University of Malaya, 50603 Kuala Lumpur, Malaysia, in partial fulfilment of the requirements for the Degree of Master of Philosophy**
**November 1998**



*To My Family*

*&*

*Friends*

# *Acknowledgments*

First and foremost, I would like to express my utmost gratitude and appreciation to *Assoc. Prof. Phang Siew Moi* for being a wonderful project supervisor throughout the period of my studentship in the Algae Lab. I thank her for the constant encouragement, understanding and undying patience. I am also grateful to Dr. Phang for the oppurtunities given to attend local and overseas training courses, workshops and conferences which have contributed a lot to this work, and also for the chance to participate in the Bukit Nenas consultancy project and the Seaweed expeditions with the Japanese. I am very grateful to my Co-Supervisor, *Assoc. Prof. Tong Soo Loong*, and thank him for his invaluable ideas, advice and useful discussions especially on the heavy metal work.

I gratefully acknowledge the following organisations and individuals who had contributed towards the realisation of this work :

* *The National Prawn Fry Production and Research Centre (NAPFPRE)* in Pulau Sayak, Kedah, and the *Fisheries Research Institute (FRI)*, Penang, for kindly providing the marine phytoplankton cultures. Special thanks are due especially to *Puan Faazaz Abdul Latiff* (formerly at NAPFPRE) and *Puan Devakie Nair* (FRI)
* *Assoc. Prof. Sam Choon Kook* who had kindly provided the use of the Multiskan machine
* *The Working Group on Environmental Biotechnology, National Biotechnology Committee*, Ministry of Science, Technology and the Environment, Malaysia, which had supported this research
* *ASEAN-Canada Cooperative Programme on Marine Science Phase II (CPMS-II)*, under which a grant had supported this work, and toxicity testing workshops organised at Burapha University, Thailand, and FRI, Penang
* *Miss Cathy McPherson* and *Mr. Armando Tang* from the Canadian Executing Agency, EVS Environmental Consultants, Vancouver, who under CPMS-II had provided the trainings on 'hands-on' toxicity testing with tropical marine organisms. I thank them for their advice, ideas and useful discussions pertaining to my work.

* *Tabung IPT* which had kindly provided some funds and enabled me to attend relevant training courses including the Asia-Pacific Conference on Science and Management of Coastal Environment in Hong Kong for the presentation of this work

* *The Commonwealth Science Council (CSC)* which had kindly provided a travel grant for the presentation of this work at the International Conference on Plants and Environmental Pollution in Lucknow, India

* *Bantuan UM,* and later the *PASCA SISWAZAH scheme* for the financial support during my studentship

I take this opportunity to also thank the lecturers who taught me during the one-semester course-work. I am also very grateful and thank the devoted staffs of IPSP especially *Cik Zubaidah, Pn. Rodziah, Mr. Gana* and *En. Razali.* I thank *En. Jasni* for his kind assistance with the ICP analyses, *Miss Molly* and her helpful library staffs and *En. Wan Yusoff* who helped with the photography.

I've been blessed with wonderful friends, old and new, at the Algae Lab, and thank them for their moral support and friendship, and all the enjoyable moments we've shared : *Chu WL, Muru TL, Rohani A, W Ching Lee, Samina H, Albert R, Saras E, Suresh K, N Seok Min, Zuraini AR, Sudesh K, C Kah Yoke, Zainab Z, Noraishah H, Sheila R, Susila J, Ahsan H, A Baset AI, Thaya, T Eng Lai, Annie G, Gan SY, L Phaik Eem, S Miah, Quamarul A, Ong GS* and *VG B.*

I thank my other friends who have been very helpful and supportive : *Amir Feisal Merican, L Pek Boon and Sanarth K, Shahnaz MD,* and *Nazimah AH. Sahilah AM, Suhaidah I, Hann Ling* and *L Yim Kheng* who'd once shared the ups and downs and one common dream. *Anu S, Y Chan Choo, Salmah I* and *Komala P* for their moral support. *Put O Ang Jr* and *A Tajuddin MN,* who have encouraged and inspired me to pursue my ambition.

I would like to express my sincerest gratitude to my aunt *Julaina* for her kindness and generosity in providing me a home and wonderful 'office' during the write-up and to my uncle *Zainal* for the Canon which has been very useful.

Lastly, but not least, I wish to thank the most important people in my life, my family, who have provided their undying support in every way and made this work possible. I am especially indebted to my *Parents,* who encouraged me to pursue my Master's Degree and interests, and I thank them for their love, trust, and patience. I thank my sister, *Melati,* for the constant 'push' and sacrificing some time from her hols to help me with the thesis. *Hatta, Mawar* and *Ayu* who have contributed in their own way... *Tok Milah* who kept me company while writing....

## ABSTRACT

Toxicity testing of four heavy metals (cadmium, copper, manganese and arsenic) with four species of marine phytoplankton, *Chaetoceros calcitrans, Isochrysis galbana, Tetraselmis tetrahele* and *Tetraselmis* sp., were carried out in Multiwell plates with test volumes of 2 mL at 28±1.0°C and synthetic seawater (pH 8.0 ± 0.5 and salinity 30 ± 2.0 $gL^{-1}$), under continuous illumination of 50.4 µmol photon $m^{-2}s^{-1}$. Tests were conducted both in the presence and absence of EDTA. NOEC (No Observed Effect Concentration; the highest test concentration which did not cause significant inhibition in growth, relative to the control), LOEC (Lowest Observed Effect Concentration; the lowest test concentration which caused significant inhibition in growth, relative to the control), $IC_{25}$ and $IC_{50}$ (inhibition concentrations estimated to inhibit 25% and 50% of growth, respectively, of the phytoplankton relative to the control) values were determined after 96 hours. Good correlations and regressions between O.D. readings (measured in Elisa microplates by the Multiskan) and cell counts determined prior to the study, enabled the use of O.D. measurements to determine growth (cell counts) by calculation via the regression equations at the end of the tests. Actual cell counts conducted on random test samples also showed good correlation between O.D. readings and actual cell counts. Therefore O.D. measurements using the Multiskan provides a rapid method for the determination of cell counts (growth) in place of the time-consuming direct cell counting. The multiwell plate provides a simple, economical, practical and rapid method of toxicity testing with good reproducibility of $IC_{50}$ values and results similar to tests conducted in the conventional shake-flasks. In the absence of EDTA, the ranges

of 96h $IC_{50}$ values were : Cd, 0.06±0.01 (*C. calcitrans* and *I. galbana*) to 5.7±1.6 (*T.* sp.) $mgL^{-1}$; Cu, 0.04±0.01 (*I. galbana*) to 0.37±0.04 (*T.* sp.) $mgL^{-1}$; Mn, 7.2±1.8 (*I. galbana*) to 21.4±3.2 (*T.* sp.) $mgL^{-1}$; and As, 33.9±8.9 (*T.* sp.) to 319.3±21.1 (*I. galbana*) $mgL^{-1}$. In the presence of EDTA, the ranges of 96h $IC_{50}$ values were : Cd, 4.7±1.0 (*I. galbana*) to 18.1±0.8 (*T. tetrahele*) $mgL^{-1}$; Cu, 6.0±0.5 (*T.* sp.) to 6.6±0.4 (*I. galbana*) $mgL^{-1}$; Mn, 38.1±9.6 (*I. galbana*) to 80.1±9.1 (*T. tetrahele*) $mgL^{-1}$; and As, 74.4±18.0 (*T. tetrahele*) to 307.1±20.1 (*C. calcitrans*) $mgL^{-1}$. As the existing Interim Standards for Marine Water Quality for Cd and Cu are higher than the $IC_{50}$ values for the most sensitive test species, *I. galbana*, it is recommended that the Standards for Cd and Cu be reviewed and lowered to 0.01 $mgL^{-1}$.

## ABSTRAK

Ujian toksisiti ke atas empat logam berat (kadmium, kuprum, mangan dan arsenik) dengan empat spesis fitoplankton marin, *Chaetoceros calcitrans, Isochrysis galbana, Tetraselmis tetrahele* and *Tetraselmis* sp., telah dijalankan di dalam plet-plet Multiwell dengan isipadu ujian sebanyak 2 mL pada suhu 28±1.0°C dan menggunakan air laut sintetik (pH 8.0 ± 0.5 and saliniti 30 ± 2.0 $gL^{-1}$), di bawah cahaya berterusan 50.4 µmol photon $m^{-2}s^{-1}$. Ujian-ujian ini dijalankan dengan kehadiran dan juga ketidakhadiran EDTA. Nilai-nilai NOEC (kepekatan tertinggi yang diuji yang tidak menyekat pertumbuhan secara signifikan berbanding dengan kawalan), LOEC (kepekatan terendah yang diuji yang menyekat pertumbuhan secara signifikan berbanding dengan kawalan), $IC_{25}$ and $IC_{50}$ (kepekatan-kepekatan yang dianggarkan akan menyekat 25% dan 50% pertumbuhan fitoplankton, masing-masingnya, relatif kepada kawalan) telah ditentukan selepas 96 jam. Korelasi yang baik serta regresi di antara bacaan-bacaan ketumpatan optik (O.D.) (yang telah diukur menggunakan plet-plet mikro Elisa dan Multiskan) dan bilangan sel, yang telah ditentukan sebelum kajian ini, telah membolehkan pengunaan ukuran O.D. untuk menentukan pertumbuhan (bilangan sel) melalui penghitungan berdasarkan persamaan-persamaan regresi pada akhir ujian-ujian itu. Pembilangan sel yang sebenar yang dijalankan ke atas sampel-sample ujian rawak juga menunjukkan korelasi yang baik di antara bacaan-baccan O.D. dan bilangan sel sebenar. Oleh itu, ukuran-ukuran O.D. menggunakan Multiskan menyediakan suatu kaedah yang pantas bagi menentukan bilangan sel (pertumbuhan) sebagai pengganti kepada pembilangan sel secara terus yang memakan masa. Plet Multiwell

menyediakan suatu kaedah menjalankan ujian toksisiti yang mudah, ekonomi, praktikal dan pantas yang memberikan nilai-nilai $IC_{50}$ yang boleh dihasilkan semula serta keputusan yang serupa dengan ujian-ujian yang dijalankan di dalam kelalang-kelalang goncang konvensional. Di dalam ketidakhadiran EDTA, renj-renj bagi nilai-nilai $IC_{50}$ 96 jam adalah seperti berikut : Cd, 0.06±0.01 (*C. calcitrans* dan *I. galbana*) hingga 5.7±1.6 (*T.* sp.) $mgL^{-1}$; Cu, 0.04±0.01 (*I. galbana*) hingga 0.37±0.04 (*T.* sp.) $mgL^{-1}$; Mn, 7.2±1.8 (*I. galbana*) hingga 21.4±3.2 (*T.* sp.) $mgL^{-1}$; dan As, 33.9±8.9 (*T.* sp.) hingga 319.3±21.1 (*I. galbana*) $mgL^{-1}$. Di dalam kehadiran EDTA, renj-renj bagi nilai-nilai $IC_{50}$ 96 jam adalah seperti berikut : Cd, 4.7±1.0 (*I. galbana*) hingga 18.1±0.8 (*T. tetrahele*) $mgL^{-1}$; Cu, 6.0±0.5 (*T.* sp.) hingga 6.6±0.4 (*I. galbana*) $mgL^{-1}$; Mn, 38.1±9.6 (*I. galbana*) hingga 80.1±9.1 (*T. tetrahele*) $mgL^{-1}$; and As, 74.4±18.0 (*T. tetrahele*) hingga 307.1±20.1 (*C. calcitrans*) $mgL^{-1}$. Oleh kerana Piawaian Interim bagi Kualiti Air Marin bagi Cd dan Cu yang wujud pada masa ini lebih tinggi daripada nilai-nilai $IC_{50}$ yang diperolehi dengan spesis paling sensitif, *I. galbana*, adalah dicadangkan bahawa Piawaian bagi Cd dan Cu dikajisemula dan diturunkan ke nilai 0.01 $mgL^{-1}$.

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