

Toxicity Testing of Four Heavy Metals with Selected Marine Phytoplankton

by

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*To My Family
&
Friends*

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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 $28 \pm 1.0^\circ\text{C}$ and synthetic seawater ($\text{pH } 8.0 \pm 0.5$ and salinity $30 \pm 2.0 \text{ gL}^{-1}$), under continuous illumination of $50.4 \mu\text{mol photon m}^{-2}\text{s}^{-1}$. 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), IC₂₅ and IC₅₀ (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 IC₅₀ values and results similar to tests conducted in the conventional shake-flasks. In the absence of EDTA, the ranges

of 96h IC₅₀ 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 IC₅₀ 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 IC₅₀ 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 $28 \pm 1.0^{\circ}\text{C}$ dan menggunakan air laut sintetik ($\text{pH } 8.0 \pm 0.5$ and saliniti $30 \pm 2.0 \text{ gL}^{-1}$), di bawah cahaya berterusan $50.4 \mu\text{mol photon m}^{-2}\text{s}^{-1}$. 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), IC₂₅ and IC₅₀ (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 penggunaan 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 IC₅₀ 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 IC₅₀ 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 IC₅₀ 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 IC₅₀ 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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