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Cadmium, Copper, and Zinc Toxicity to the Clam, *Donax faba* C., and the Blood Cockle, *Anadara granosa* L.

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Metal pollution has become a problem for local management authority in Malaysia especially in estuaries and coastal marine waters which are ultimately the repositories of contaminants from anthropogenic activities. Although studies on heavy metals in the coastal waters of Malaysia have been carried out for more than 20 years, available data and publications are still quite limited. Some of the studies that have been carried out include metal content in fish and shellfish (Babji et al. 1978, Jamaliah 1998), and level of metals in the environment (Seng et al. 1987, Din 1995). While studies on trace-metal contents in the local marine environment have been increasingly progressive, knowledge on biological effects of the metals on tropical marine organisms is still very scarce with only a handful of studies carried out including those of Ong and Din (1995), Shazili (1995), Din et al. (1997), and Lim et al. (1998). Use of organisms as bioindicators to assess the effects of toxicants on aquatic communities has become popular recently and for heavy metal pollution, bivalve molluscs have been found to be suitable (Cunningham 1979).

The objectives of this study were: (1) to compare the toxicity of cadmium (Cd), copper (Cu) and zinc (Zn) to the clam, *Donax faba* and spats of the blood cockle, *Anadara granosa* and (2) to select a suitable inorganic reference toxicant for each test organism from the three test materials used. The clams and cockles were selected as test organisms because of their wide distribution in Malaysia, their importance to the local commercial fisheries and the ease of maintaining them in the laboratory. The metals were chosen because of their common occurrence in industrial waste effluents discharged around the country. For this study, the static 96-h acute toxicity test method was used to provide rapid, relatively inexpensive and reproducible evaluation of the metals toxicity to the test organisms.

MATERIALS AND METHODS

Seawater used during the study was pumped from approximately 0.5 km offshore from the testing laboratory, sandfiltered, followed by filtration through a series of filters of mesh size 10µm, 5µm and 1.0 µm. The seawater was usually filtered at least a day before use. The filtered seawater was aerated at all times during storage. The dilution water used in this study met with the biological and chemical

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specifications as described by Rand and Petrocelli (1985) and APHA (1992). All glass and plasticwares used in this study were cleaned following the procedures outlined by Rand and Petrocelli (1985).

The adult clams, *D. faba*, were dug out from the intertidal area along the beach overlooking the laboratory. The range of mean wet weight and shell length of the clams used were 0.1- 0.2 g and 1.2 - 2.0 cm, respectively. Spats of the blood cockle, *A. granosa*, were collected from Kuala Jalan Baru, Penang Island. The mean wet weight and shell length of the cockles used for all the tests ranged from 0.01 - 0.02 g and 0.6 - 1.1 cm, respectively. Each batch of about 1000 clams and cockle spats collected were kept separately in two 20-L glass tanks. Seawater for each holding tank was changed daily and aerated constantly at room temperature for about 3 days.

During the course of the study, room temperature fluctuations were less than ± 1.5°C. Constant photoperiod of 12:12 h light:darkness was closely maintained. The clams and cockle spats were fed daily with *Isochrysis* sp. until about 24-h before test initiation. During the 3-d acclimation period, no mortality was recorded for the clams and <5% mortality was recorded for the spats.

Rand and Petrocelli (1985) and EPA (1991) recommended a maximum loading capacity (i.e. ratio of test organism biomass to the volume of test solution used) of 0.3 to 0.8 g/L for bioassay tests involving various marine organisms. For this study we decided to be more conservative and chose a loading capacity of less than 0.3 g/L for the cockle spats, and less than 0.2 g/L for the clams. This choice is based on our preliminary study (unpublished) which showed that for both species, a loading capacity of 0.4 g/L or more will result in a significant drop in the dissolved oxygen after 48-h of exposure. This then will influence the mortality rate of the animals.

One litre of Cd, Cu and Zn stock solution (1,000 mg/L) was prepared by diluting one ampoule of Titrisol Cadmium (No. 9960), Copper (No. 9987) and Zinc (No. 9953) Merck Standard Solution each with deionized distilled water.

Glass tanks of 20-L and 2-L capacities were used as test containers for the clams and spats, respectively.The range of Cd, Cu and Zn concentrations used in the definitive test was determined earlier with a range finding test as recommended by Buikema et al. (1982) and CPMS-II (1992).

For the definitive tests, the clams and cockle spats were exposed to three replicates of five nominal concentrations and a control for each test material. These tests were repeated 5 or more times with a different batch of test organisms. Ten test organisms were randomly distributed into the respective test tanks after checking the water quality parameters (DO, pH, temperature and salinity) suitability. All test chambers were covered during the test period. Dissolved oxygen (DO) (mg/L), temperature (°C) and pH were recorded daily during the test period while salinity ($^{0}/_{00}$) was recorded at 0-h and 96-h only.

Dissolved oxygen and temperature were measured using a Model 59 YSI Dissolved Oxygen-Meter while pH and salinity were measured with a Model 25 ORION pH meter and a refractometer, respectively. Mortality was recorded every 24-h and dead animals were removed. The clams and the cockle spats were considered dead when they failed to response to gentle prodding (Buikema et al. 1982; Rand and Petrocelli 1985). The shells of the dead animals were usually opened wider than usual and the muscle exposed slightly frayed. All tests were terminated after 96-h of exposure.

In order to monitor the health and sensitivity of each batch of clams and cockle spats used for each test, a standard reference toxicant was used. Cadmium was chosen in this case. Thus, cadmium testing was also carried out every time a toxicity test involving copper or zinc was conducted. Whenever the results of a cadmium test deviates significantly from the regressed LC_{50} values, that particular batch of organisms will not be used.

Samples for acid-soluble metal analysis were obtained by placing 20 mL of samples in polyethylene vials and preserving them with concentrated nitric acid to a pH $\leq$ 2.0 (EPA 1987) at 0-h and 96-h of each exposure period. The average of 0-h and 96-h metal concentration was used for the calculation of the LC_{50} value.

Concentration verification of each test for acid-soluble Cd, Cu and Zn concentration was determined by flame atomic absorption spectrophotometry (FAAS) with background correction. Preservation of samples and analysis by FAAS followed the procedure as recommended by the U.S. Environmental Protection Agency (EPA 1990).

Statistical analyses were conducted using EFFL statistical software which calculates the 96-h LC_{50} values using Trimmed Spearman-Karber method (Hamilton et al. 1977), and the method as described in Stephan (1977) which includes probit and moving average analysis. Selection of method used for the calculation of LC_{50} values depended on data suitability (EPA 1993).

RESULTS AND DISCUSSION

The concentrations of dissolved oxygen at test termination were found to be always above 40% saturation, which is the recommended level as suggested by APHA (1992) and Rand and Petrocelli (1985). Mean DO concentration at test termination for all clam exposures was 5.91 $\pm$ 0.59 mg/L with a minimum value of 3.0 mg/L (44.6% saturation). For all cockle exposures, mean DO concentration was 5.83 $\pm$ 0.53 mg/L with a minimum value of 3.3 mg/L (48.3% saturation).

Mean test temperatures recorded during the course of this study for the cockle spats and the clams were 27.2 $\pm$ 0.80°C and 26.2 $\pm$ 0.84°C, respectively. The maximum temperature fluctuations of 1.32°C and 1.44°C measured for all cockle spats and clams, respectively, are higher than the maximum of 1.0°C recommended by Rand and Petrocelli (1985) and APHA (1992) for tropical conditions. However, these conditions were found to be tolerable in our local situation, as evident from the < 10% mortality found in the control tanks.

pH and salinity values recorded during the tests carried out for *D. faba* were 8.3 ± 0.08 unit and 29.9 ± 0.1 ‰, respectively. The same parameters recorded for *A. granosa* tests were 8.3 ± 0.1 and 29.5 ± 0.08 ‰, respectively.

The 96-h LC_{50} of Cu to *D. faba* ranged from 0.17 to 0.25 mg/L with a mean LC_{50} value of 0.20 ± 0.03 mg/L (n =5). *A. granosa* proved to have similar sensitivity to Cu as *D. faba*. The 96-h LC_{50} of Cu to the cockle spats ranged from 0.17 to 0.29 mg/L with a mean value of 0.23 ± 0.06 mg/L (n = 5). Clams exposed to Cd generated 96-h LC_{50} values ranging from 0.75 to 1.24 mg/L with a mean value of 0.99 ± 0.18 mg/L (n = 10). Again, the cockles showed similar sensitivity as the clams with the 96-h LC_{50} ranging from 0.64 to 1.32 mg/L, and a mean value of 0.94 ± 0.25 mg/L (n = 7). The 96-h LC_{50} response of *D. faba* to Zn ranged from 1.94 to 4.74 mg/L with a mean value of 3.61 ± 1.04 mg/L (n = 5). For this metal, the cockle spats were found to be only half as sensitive with the 96-h LC_{50} values ranging from 3.68 to 11.81 mg/L and a mean value of 7.76 ± 3.32 mg/L (n = 5).

Due to limited availability of local data, the response of *D. faba* and *A. granosa* to Cu, Cd and Zn can only be compared to tests using bivalve species in temperate conditions. Watling (1978) working with various life stages of the oyster, *Crassostrea gigas*, recorded 96-h LC_{50} values for Cd ranging from 0.05 to 2.0 mg/L. For comparison, the sensitivity of *D. faba* and *A. granosa* spat is similar to the 25-d old oyster spat. Arkshell, *Anadara broughtonii* of about 2.1 cm in shell length, was found to have a 96-h LC_{50} value for Cd of 1.86 mg/L (Park and Kim 1978) which is almost twice as high as what was found for both *D. faba* and *A. granosa*. *Mytilus edulis*, the much tested blue mussel, showed similar sensitivity to Cd as the arkshells with a mean 96-h LC_{50} value of 1.62 mg/L (Ahsanullah 1976). Calabrese et al. (1977) found that for the arkshell, *Anadara broughtonii*, the 72-h LC_{50} for Cu was 0.31 ppm which suggests that they might be as sensitive to Cu as both *D. faba* and *A. granosa*. As an overall comparison of all tests reviewed on different testing organisms, the rank order of toxicity for the three metals are Cu > Cd > Zn.

Of the three metals studied, Cu was found to be most toxic to the clams. The animals were 5 times more sensitive to this metal than Cd and 15 times more than Zn (Fig. 1). Cu was also found to be most toxic to the cockle spats. This metal was found to be 4 times more toxic than Cd and 30 times more toxic than Zn. Sensitivity of the adult clams and cockle spats to Cu and Cd were found to be similar but as shown in Fig.1, the clams were about 2 times more sensitive to Zn.

It is quite surprising that the clams were more sensitive or as sensitive to the metals than the spats. Apart from being at a more mature stage, the clams used were twice as big. On the basis of their sensitivity to the 3 metals, both the clams and the spats are suitable as test organisms. However, the clam is a better choice due to its ready availability throughout the year; in comparison, spat-size *Anadara*

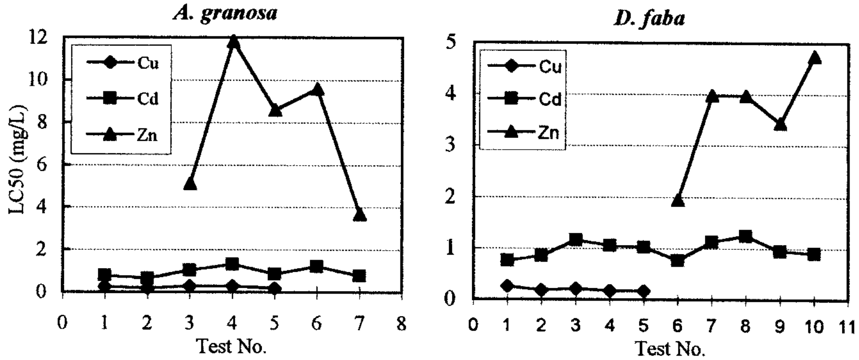

Figure 1. Sensitivity of *A. granosa* and *D. faba* to Cu, Cd and Zn expressed in terms of their LC_{50} values.

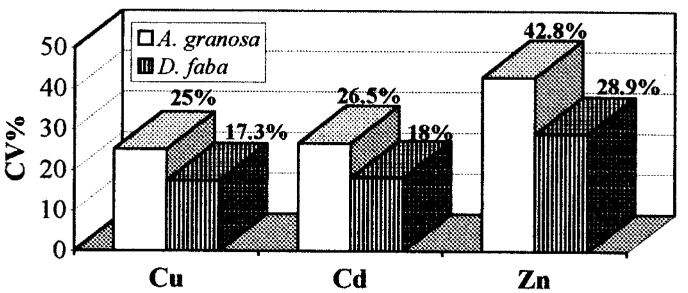

Figure 2. Consistency of Cu, Cd and Zn test results for *A. granosa* and *D. faba* measured by using coeffiecient variation percentage.

granosa can only be obtained from December to May annually.

When using any organism in a toxicity test it is essential, as a quality assurance and quality control step, to established a standard reference toxicant for that particular test organism. A reference toxicant is used to provide an understanding of changes in sensitivity that may occur due to improper acclimation procedure, disease, loading density or handling stress. An established reference toxicant can then be used to evaluate the reproducibility and validity of test data over time and to perform intralaboratory calibrations. An ideal reference toxicant should be toxic at low concentrations, rapidly lethal, stable, nonselective, detectable by known analytical techniques (Rand and Petrocelli 1985) and able to provide relatively consistent test results in any given laboratory (EC 1990).

Based on the relative toxicity results as reported in this study, Zn being the least sensitive is considered least suitable as a reference toxicant. In addition, as shown in Fig. 2, both Cu and Cd provided higher consistencies as reflected by their lower coefficient of variation percentages (% CV=100 SD/x, where SD is standard deviation and x is the number of 96-h LC_{50} values determined). For the Cu and Cd

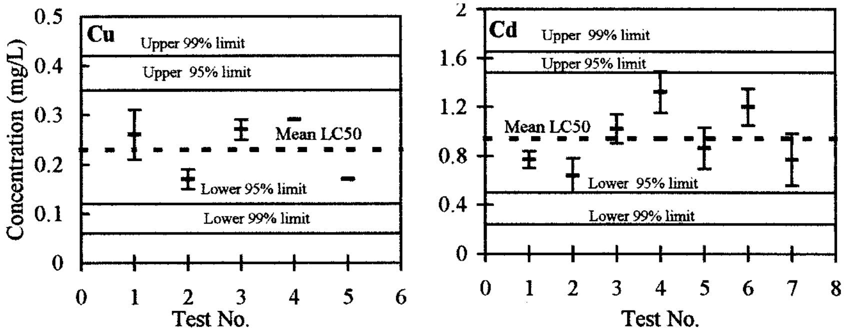

Figure 3. Warning limits chart of *D. faba* reference toxicant (Cu & Cd) using the 96-h LC_{50} value and the 95% confidence limit as the upper and lower limit.

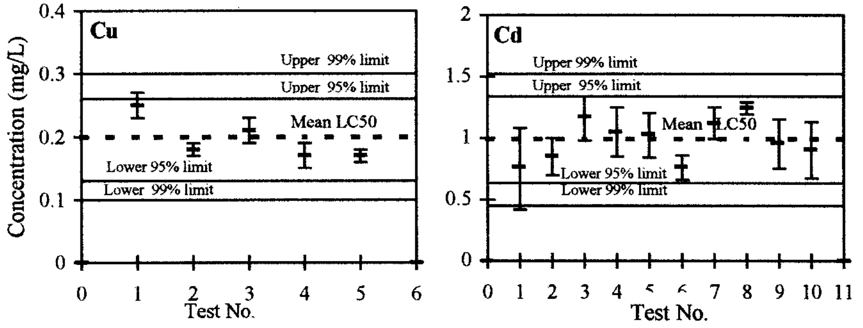

Figure 4. Warning limits chart of *A. granosa* reference toxicant (Cu & Cd) using the 96-h LC_{50} value and the 95% confidence limit as the upper and lower limit.

tests on *D. faba*, the % CV are 17.3% and 18.0%, respectively, while for the cockle spats, the % CV were calculated to be 25% and 26.5%, respectively. The % CV for Zn test results for both *A. granosa* and *D. faba* showed much higher values. EC (1990) suggested that a % CV of up to 30% could be considered realistic for most tests

Mortality of the adult clams and cockle spats exposed to Cu and Cd was found to be consistent from the 24th to 48th hour of the test period. Concentrations of Cu and Cd during the 96-h exposure period in this study were also found to be quite stable with ≤10% difference in concentration from 0-h to 96-h. Thus, based on their relative toxicity, consistency and stability as well as the rate of lethality on both animals and stability of the test materials, both Cu and Cd seem to be suitable as inorganic reference toxicants for these two test animals.

The mean and warning limits chart using both Cu and Cd as reference toxicants *to* *D. faba* and *A. granosa* have been prepared to the specification of EC (1990) and Weber et al. (1989) and are shown in Figs. 3 and 4.

The results of this study detail the toxicity of Cu, Cd and Zn to adult *D. faba* and spats of *A. granosa* using the static 96-h acute toxicity tests. *Donax faba* is considered a better choice for use as test organisms for the 3 metals because of of its higher sensitivity and easy accessibility. Both Cu and Cd are found to be suitable to be used as reference toxicants for the two animals.

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