Sensitivity of freshwater organisms to zinc

M. Shuhaimi-Othman, N. Yakub, and N.A. Ramle

Abstract—A study was conducted in the laboratory to determine acute toxicity of heavy metal zinc (Zn) to six species of freshwater organisms i.e. one species of shrimp (Macrobrachium lanchesteri), two species of fish (Rasbora sumatrana (Seluang) and Poecilia reticulata (guppy)), one species of gastropod (Melanoides tuberculata), one species of ostracod Stenocypris major and one species of midge (larvae) Chironomus javanus. All organisms were exposed for a four-day period in the controlled laboratory condition to a range of Zn for 96 hours. Mortality was assessed and median lethal concentrations (LC50) were calculated. 96 hours LC50 for M. lanchesteri, R. sumatrana, P. reticulata, M. tuberculata, S. major and C. javanus of Zn were 0.52, 0.46, 1.05, 3.9, 1.19 and 5.57 mg/L, respectively. This study indicated that R. sumatrana was the most sensitive to Zn.

Keywords—Acute, freshwater, heavy metals, sensitivity, soft water, toxicity.

I. INTRODUCTION

Toxicity testing has been widely used as a tool to identify suitable organisms as a bioindicator and to derive water quality standards for chemicals. Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystems. Metal contamination has been shown to have serious effects on the both environment and humans. Malaysia, as a developing country, is no exception and faces metal pollution caused especially by anthropogenic activities such as manufacturing, agriculture, sewage and motor vehicle emissions [1], [2]. Metals such as Zn are released from natural sources as well as human activity. Despite the adverse effect of metal on the environment and organisms, Zn is essential to living organisms and plays an important role as an enzyme carbonic anhydrase.

Macroinvertebrate and fish as a test organisms in toxicity tests has several valuable characteristics such as its widespread distribution and common occurrence in freshwater, its ecological importance and ease of handling during testing, as well as its rapid growth, short life cycle and sensitivity to contaminants [3], [4]. Therefore, these organisms have the potential to act as a bioindicator of heavy metals pollution in an aquatic environment and as organisms for toxicity testing.

The purpose of this study was to determine the acute toxicity of Zn to six local freshwater organisms and to compare the sensitivity between them.

II. MATERIALS AND METHODS

A. Organisms and test chemicals

In this study six local freshwater organisms have been used in toxicity testing i.e. a prawn Macrobrachium lanchesteri, two fish Poecilia reticulata (guppy, family Poecillidae) and Rasbora sumatrana (family Cyprinidae), a snail (Gastropoda) Melanoides tuberculata (family Thiaridae), an ostracod Stenocypris major, and a midge larvae Chironomus javanus (Diptera, Chironomidae). M. lanchesteri and R. sumatrana were obtained from local pet stores. P. reticulata and M. tuberculata were collected from the field. S. major and C. javanus were collected from a fish pond filter system. Prior to toxicity testing, the organisms were acclimatized for one week under laboratory conditions (28-30°C with 12h light : 12h darkness) in 20-L stocking tanks using dechlorinated tap water (filtered by several layer of sand and activated carbon; T.C. Sediment Filter® (TK Multitrade, Seri Kembangan, Malaysia)) and aerated through an air stone. During acclimation the organisms were fed with commercial fish food Tetramin® (Tetrawerke, Germany). The standard stock solution (100 mg/L) of Zn was prepared from analytical grade metallic salts of ZnSO4·7H2O (Merck, Darmstadt, Germany). The stock solutions were prepared with deionized water in 1-L volumetric.

B. Acute toxicity test

Acute Zn toxicity experiments were performed for a four-day period (96-h) using adult animals or larvae (fourth instar midge larvae). Following a range finding test, five Zn concentrations were chosen. Metal solutions were prepared by dilution of a stock solution with dechlorinated tap water. A control with dechlorinated tap water only was also used. The tests were carried out under static conditions with renewal of the solution every two days. Control and metal treated groups each consisted of two to four replicates of five randomly allocated organisms. No significant stress was observed for the organisms in the solution indicated by 95-100% survival.
for the organism in the control water until the end of the study. For each species, a total of 10 to 20 animals per treatment (concentrations) were used in the experiment. Samples of water for metal analysis taken before and immediately after each solution renewal were acidified to 1% with ARISTAR™ nitric acid (65%) (BDH Inc, VWR International Ltd., England) before metal analysis by flame or furnace Atomic Absorption Spectrophotometer (AAS – Perkin Elmer (Massachusetts, USA), model Analyst 800) depending on the concentrations. To avoid possible contamination, all glassware and equipment used were acid-washed (20% HNO₃ (Dongbu Hitek Co. Ltd., Seoul, Korea, 68%)) and the accuracy of the analyses were checked against blanks. Procedural blanks and quality control samples made from standard solution for Cu, Cd, Al and Mn (Spectrosol, BDH, England) were analysed in every ten samples in order to check for samples accuracy. Percentage recoveries for metals analyses were between 85-105%.

During the toxicity test, organisms were not fed. The experiments were performed at room temperature of 28-30°C with photoperiod 12h light : 12h darkness, using fluorescent lights (334-376 lux). Water quality parameters (pH, conductivity, and dissolved oxygen) were measured every two days using portable meters (model Hydrolab Quanta®, Hach, Loveland, USA) and water hardness samples were fixed with ARISTAR™ nitric acid and measured by flame atomic absorption spectrophotometer (AAS– Perkin Elmer Analyst 800). Mortality was recorded every 3 to 4 hours for the first two days and then at 12 to 24 hour intervals throughout the test period. Any dead animals were removed immediately.

Median lethal concentrations (LC₅₀) for the animals exposed to Zn were calculated using measured metal concentrations. FORTRAN programs based on the methods of Litchfield [5] and Litchfield and Wilcoxon [6] were used to compute the LC₅₀.

### III. RESULTS AND DISCUSSION

The mean water quality parameters measured during the test were pH 6.68 ± 0.2, conductivity 180.3 ± 4.6 µS/cm, dissolved oxygen 6.25 ± 0.3 mg/L and total hardness (Mg²⁺ and Ca²⁺) 18.72 ± 1.72 mg/L as CaCO₃.

This study showed that 24-h and 96-h LC₅₀ of Zn for *M. lanchesteri*, *R. sumatrana*, *P. reticulata*, *M. tuberculata*, *S. major* and *C. javanus* were 1.87, 2.5, 6.4, 33.9, 2.6 and 14.4, and 0.52, 0.46, 1.05, 3.9, 1.19 and 5.57 mg/L, respectively (Table 1). Results of acute toxicity tests using six aquatic species showed that fish *R. sumatrana* and prawn *M. lanchesteri* were the most sensitive species to Zn, while midge *C. javanus* and mollusc *M. tuberculata* were the most resistant (Fig. 1). According to Von Der Ohe and Liess [7] 13 taxa belonging to Crustacea were among the most sensitive to metal compounds, and concluded that taxa belonging to Crustacea are similar to one another and to *Daphnia magna* in terms of sensitivity to organics and metals; and that Molluscs have an average sensitivity to metals. Mitchell et al. [8] reported that the snail has a tightly sealing operculum that allows it to withstand desiccation and apparently also increases its tolerance to chemicals. Brix et al. [9] also reported that warm water fish, crustaceans other than cladocers and other invertebrates were consistently of intermediate sensitivity and insects were the least sensitive taxonomic group evaluated for five metals (Cd, Cu, Pb, Ni, Zn). Resistant of insect midge larvae to metals have been reported elsewhere. Toxicity of metal to midge larvae were also influence by age as generally known that first instar larvae of *Chironomus* was more sensitive compare to the third and fourth instar stages [10]. Fish *R. sumatrana* was also found to be sensitive to Zn. Zakaria-Ismail and Fatimah [11] reported on the tolerance levels of common freshwater fish in Peninsular Malaysia and concluded that *R. sumatrana* has a medium tolerance level to pollutants with a value of 2.5 (value ranged from 0.5 being the most sensitive to 4.5 being the most tolerant species).

Comparison of toxicity between organisms in rank 1 (most sensitive) and 6 (most resistant) showed that for Zn, the toxicity was 12 times lower respectively. The difference seen

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**Table 1**

<table>
<thead>
<tr>
<th>Species</th>
<th>24h</th>
<th>48h</th>
<th>72h</th>
<th>96h</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. lanchesteri</em></td>
<td>1.87</td>
<td>0.72</td>
<td>0.62</td>
<td>0.52</td>
</tr>
<tr>
<td><em>R. sumatrana</em></td>
<td>2.5</td>
<td>1.1</td>
<td>0.8</td>
<td>0.46</td>
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<tr>
<td><em>P. reticulata</em></td>
<td>6.4</td>
<td>2.7</td>
<td>1.5</td>
<td>1.05</td>
</tr>
<tr>
<td><em>M. tuberculata</em></td>
<td>33.9</td>
<td>13.1</td>
<td>4.7</td>
<td>3.9</td>
</tr>
<tr>
<td><em>S. major</em></td>
<td>2.6</td>
<td>1.7</td>
<td>1.5</td>
<td>1.2</td>
</tr>
<tr>
<td><em>C. javanus</em></td>
<td>14.4</td>
<td>8.7</td>
<td>7.3</td>
<td>5.6</td>
</tr>
</tbody>
</table>

Fig. 1 The relationship between median lethal concentrations (LC₅₀) and exposure times for six freshwater organisms exposed to zinc.
for trace metals might be explained by metallothionein (MT) synthesis, which is believed to play a protective role against toxic metals in aquatic animals [12], [13]. Other studies also provided evidence that the hypothalamic-pituitary–adrenocortical (HPA) axis, crucial in vertebrates coping with stressors, is one of the metal targets in several animal species, including teleost fish [14], [15]. According to Luoma and Rainbow [16], the rank order of toxicity of metals will vary between organisms, and the factors that affect the rate of uptake of metals affect the toxicity of metal. Metal toxicity results when metals accumulate at an undesirable site(s) in the organisms and disrupts important molecular function. Toxicity ensues once the threshold of metal availability has been passed, indicating that the rate of uptake exceeds both the rate of excretion and detoxification. Metals also can inhibit the uptake of major ions (Na\(^+\), Ca\(^{2+}\), Mg\(^{2+}\), Cl\(^-\)) by freshwater organisms through either competitive or direct inhibition [17].

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REFERENCES


