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Acute Toxicity and Bioaccumulation of Zinc and Lead in the Freshwater Prawn *Macrobrachium lanchesteri*

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ABSTRACT Adults of *Macobrachium lanchesteri* were exposed for a nine-day period under laboratory conditions to a range of zinc and lead concentrations. Mortality was assessed and median lethal times (LT50) and concentrations (LC50) were calculated. At the end of nine-day period, prawns that survived were used to determine bioaccumulation of the metals. LT50 and LC50 increased with decrease in mean exposure concentrations and times respectively for the two metals (Zn and Pb). LC50s for 24, 48 and 96 hours for zinc were 1230, 1054 and 395 μg/L and for lead 269, 194 and 123 μg/L respectively. Zinc and lead bioaccumulation in *M. lanchesteri* increased with increasing concentration of exposure and lead was more toxic than zinc to *M. lanchesteri* (Pb>Zn).

ABSTRAK *Macrobrachium lanchesteri* dewasa telah didedahkan selama sembilan hari di dalam makmal kepada satu siri kepekatan logam zink (Zn) dan plumbum (Pb). Masa kematian pertengahan (LT50) dan kepekatan kematian pertengahan (LC50) dikira berdasarkan data kematian yang diperolehi. Di akhir hari yang ke-9, udang yang masih hidup digunakan untuk penentuan bioakumulasi logam. Nilai LT50 dan LC50 didapati meningkat dengan pengurangan kepekatan dan masa pendedahan bagi kedua-dua logam (Zn dan Pb). Nilai LC50 untuk 24, 48 dan 96 jam bagi zink adalah 1230, 1054 dan 395 μg/L dan bagi plumbum 269, 194 dan 123 μg/L masing-masing. Bioakumulasi logam zink dan plumbum oleh *M. lanchesteri* meningkat dengan peningkatan kepekatan pendedahan dan logam plumbum didapati lebih toksik terhadap *M. Lanchesteri* berbanding logam zink (Pb>Zn).

(heavy metals, acute, *Macrobrachium lanchesteri*, bioaccumulation)

INTRODUCTION

Zinc and lead are heavy metals released from natural sources as well as human activity. Some heavy metals such as zinc and copper are essential metals for living organisms at low concentrations and some such as lead and cadmium are non-essential metals at all concentrations. Toxicity testing is an essential tool for assessing the effect and fate of toxicants in aquatic ecosystems. Acute toxicity testing has become a very important tool since the late 1950s in monitoring pollution effects. The tests are considered ecologically significant, scientifically and legally defensible, simple and cost effective [1]. Aquatic toxicity tests have generally been conducted using fish because they are presumed to be the best understood organism in the aquatic environment and perceived as most valuable by the majority of laymen. However, testing and protecting other members of the community is necessary and some, such as the macro invertebrates may be more sensitive to toxicants than fish and therefore water quality standards based solely upon fish toxicity studies will almost certainly be inadequate to protect the entire community in aquatic ecosystems [1, 2].

Bioaccumulation is a general term describing the net uptake by any or all the possible routes and sequestration of pollutants by organisms from their ambient environment [3]. Bioaccumulation of metals in organisms can be used as indicator of metal contamination in the environment. However, more data are needed from laboratory experiments and field studies before further

applications can be made. The dominant freshwater prawns of South East Asia are Palaemonidae, among which *Macrobrachium* is the principal genus. In peninsular Malaysia, 13 species have been recognized. *Macrobrachium lanchesteri* is common in reservoirs, ponds, irrigation ditches and other artificial, enclosed freshwater bodies [4]. This prawn is gaining popularity as live food for aquarium and cultivated fish [5]. *M. lanchesteri* as a test organism in toxicity testing has several valuable characteristic such as widespread and common occurrence in freshwater, ease of handling during testing and sampling and sensitivity to contaminants [5, 6 and 7].

The purpose of this study was to determine the acute toxicity of zinc and lead to adult *M. lanchesteri* and to examine bioaccumulation of these metals in the body after nine days exposure.

MATERIALS AND METHODS

Macrobrachium lanchesteri were collected from an unpolluted pond (unpublished data) located 10 km from Bangi, Selangor, Malaysia (02.53°N 101.48°W). Prior to toxicity testing, the prawns were acclimatized for one week under laboratory conditions (26-27°C with 12h light : 12h darkness) in 20 L stocking tanks using dechlorinated tap water and aerated through an air stone. During acclimation the prawns were fed with commercial fish food Aquadene®. The standard stock solution of zinc (100 mg/L) was prepared from $ZnSO_4.7H_2O$ and for lead (100 mg/L) from $PbCl_2$. The stock solutions were prepared with deionised water in 1L volumetric flask. Acute zinc and lead toxicity experiments were performed for a nine-day period using adult animals (approximately 2.5-4 cm body length) obtained from stocking tanks. Following a range finding test, five zinc concentrations (0.18, 0.56, 1.0, 3.2 and 10 mg/L) and five lead concentrations (0.01, 0.1, 0.18, 0.32 and 0.56 mg/L) 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 three days. Control and metal treated groups each consisted of four replicates of five randomly allocated prawns in a 500 mL glass jar (10 x 8 cm) containing 400 mL of the appropriate solution. No stress was observed to the prawns in the solution where this was indicated by 100% survival of the prawns in the control water until end of the study. A total of 20 animals per treatment were used in the experiment and a total of 220 animals, were employed in the investigation. Samples of water for metal analysis taken before and immediately after each solution renewal were acidified to 1% with ARISTAR® nitric acid (65%) before metal analysis by flame or furnace Atomic Absorption Spectrophotometer (AAS – Perkin Elmer model 3300) depend on the concentrations.

During the toxicity test, prawns were fed *ad libitum* with commercial fish food Aquadene® as required. Excess food that not consumed by the prawns after three hours were removed using polyethylene hand pipette. The experiments were performed at room temperature of 26-27°C with photoperiod 12 hours light: 12 hours darkness, using fluorescent lights (334-376 lux). Water quality parameters (pH, conductivity, and dissolved oxygen) were measured every three days using portable meters (model YSI 556) and water hardness samples (0.45µm filtered) were fixed with ARISTAR® nitric acid and measured by flame atomic absorption spectrophotometer (AAS– Perkin Elmer model 3300). Mortality was recorded every three to four hours for the first three days and then at 12 to 24 hour intervals throughout the test period. The criteria used to determine mortality were failure to respond to gentle physical stimulation and the absence of any beating pleopods. Any dead animals were removed immediately.

At the end of day nine, the survived prawns were used to determine bioaccumulation of the metals in whole body according to the concentrations used. The prawns were rinsed with distilled water and each sample contained three replicates of one to three animals in a glass test tube (depend on how many survived animal's left), and was oven dried (80°C) for at least 48 hours before being weighed. Each replicate was digested (whole organism) in 1.0 mL Aristar® nitric acid (65%) in a block thermostat (80°C) for two hours. Upon cooling, 0.8 mL of hydrogen peroxide (30%) was added to the solutions. The test tubes were put back on the block thermostat for another 1 hour until the solutions became clear. The solutions were then made up to 25 mL with addition of

deionised water in 25 mL volumetric flasks [8]. Efficiency of the digestion method was evaluated using mussel tissue reference material (SRM 2976, National Institute of Standard and Technology, USA). Efficiencies obtained were within 10% range of the reference values.

Median lethal times (LT50) and median lethal concentrations (LC50) for the prawns exposed to zinc and lead were calculated using measured metal concentrations. FORTRAN programs based on the methods of Litchfield [9] and Litchfield and Wilcoxon [10] were used to compute and compare the LT50 and LC50. Data were analyzed using both time/response (TR) and concentration/response (CR) methods by plotting cumulative percentage mortality against time and concentration on logarithmic-probit paper. Statistical analysis for bioaccumulation data was conducted by oneway ANOVA with Tukey-Kramer multiple comparison tests and t-test (for exposure that has only one replicate) using Minitab software (vers. 12). Bioaccumulation of metals in prawn tissues was also subjected to linear regression analysis. Student's *t* test was employed to test the significance of regression coefficients. All data were normally distributed (Shapiro-Wilk test) and homogenous (Bartlett's χ^2) after log10 transformation.

RESULTS AND DISCUSSION

In all data analyses, the actual, rather than nominal zinc and lead concentrations were used (Table 1). The mean water quality parameters measured during the test were pH 6.82 ± 0.14, conductivity 108.3 ± 0.2 µS/cm, dissolved oxygen 6.1 ± 0.3 mg/L and total hardness (Mg^{2+} and Ca^{2+}) 3.90 ± 0.08 mg/L as $CaCO_3$.

All control animals maintained in dechlorinated water survived throughout the experiment. Median lethal times (LT50) and concentrations (LC50) increased with decrease in mean exposure concentrations and times respectively for both metals (Tables 1 and 2) but the lethal threshold concentration/time could not be determined since the toxicity curves (Figures 1 and 2) did not become asymptotic to the time or concentration axis within the test period. Figures 1 and 2 also show that lead was more toxic than zinc to *M. lanchesteri.* Similar results were obtained by Borgmann et al. [11] and Shuhaimi-Othman and Pascoe [12] with amphipod *Hyalella azteca* and Keppler and Ringwood [13], with juvenile clams *Mercenaria mercenaria.*

Shazili and Ali [14] reported that estimated 48-hr LC50s of zinc and lead to juvenile Malaysian giant prawn *Macrobrachium rosenbergii* were 160 and 120 µg/L respectively. Vijayram and Geraldine [15] reported that with freshwater prawn *Macrobrachium malcolmsonii*, the LC50 (96-hr) for zinc was 2600 µg/L. The differences in zinc toxicity reported by other studies [14, 15] compare to this study probably due to different of species used, age and size of the prawn as this can affect toxicity [16, 17]. Shuhaimi-Othman and Pascoe [12] showed that with freshwater amphipod *Hyalella azteca*, cadmium was the most toxic to the amphipod followed by copper and zinc, and the 96-hr LC50 of zinc was 1613 µg/L. A study by Frias-Espericueta et al. [18] with postlarval whiteleg shrimp (*Litopenaeus vannamei*) showed that mercury was the most toxic among the three non-essential metals, i.e., cadmium, mercury and lead, followed by cadmium and lead. The LC50 (96-hr) for lead was 134 mg/L. Shazili and Ali [14] reported that cadmium, lead and zinc have similar toxicities to juvenile freshwater giant prawn *Macrobrachium rosenbergii.*

Bioaccumulation of zinc and lead in surviving *M. lanchesteri* are as shown in Figure 3. Bioaccumulation data for surviving prawns were obtained from three zinc concentrations exposure (154, 571 and 1052 µg/L µg/L) and four lead concentrations exposure (22, 94, 172 and 327 µg/L). Zinc and lead bioaccumulation in *M. lanchesteri* increases with increasing concentrations exposure. Statistical analyses show that there are significant differences (ANOVA, $P<0.01$; Tukey-Kramer, $P<0.05$; t-test, $P<0.001$) in zinc and lead bioaccumulation at all exposure concentrations compared with control water at the end of day nine. However, zinc accumulation was slightly less and slower compared to lead accumulation indicating some regulation and sequestering of the metal in the prawn (Table 3 and 4). Linear regression analysis (Table 4) revealed no significant regression ($p>0.05$) between the zinc concentrations in the prawn tissues and zinc concentrations in the solutions.

Table 1. Median lethal times (LT50) and slope functions (s) for *M. lanchesteri* exposed to different concentrations of zinc and lead.

Measured Concentration Zinc (µg/L)	LT50 (h)	s
154	176	2.5
571	74	3.6
1053	46	2.7
2928	13	1.4
12744	6	1.2

Measured Concentration Lead (µg/L)	LT50 (h)	s
22	227	2.2
94	203	2.6
172	171	5.3
327	15	1.7
628	11	1.5

Table 2. Median lethal concentrations (LC50) and slope functions (s) for *M. lanchesteri* at different exposure times for zinc and lead.

Time (h)	LC50 (µg/L) for Zinc	s	LC50 (µg/L) for Lead	s
24	1230	1.7	269	1.2
48	1054	3.0	194	1.4
72	630	3.9	148	2.7
96	395	3.2	123	2.8
120	318	3.1	95	3.1
144	279	2.9	76	3.5
168	178	3.8	69	3.5
192	127	3.5	55	4.2
216	101	6.0	41	5.1

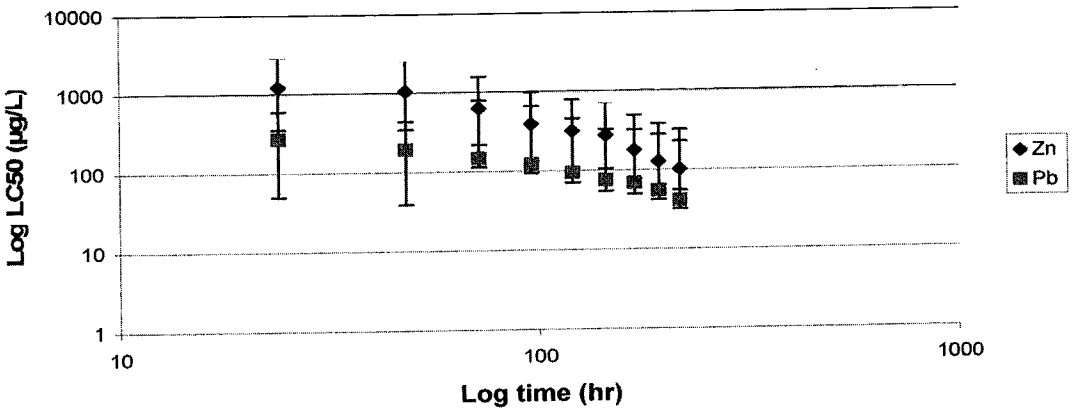

Figure 1. The relationship between median lethal concentration (LC50) and exposure times (with 95% confidence limits) for *M. lanchesteri*.

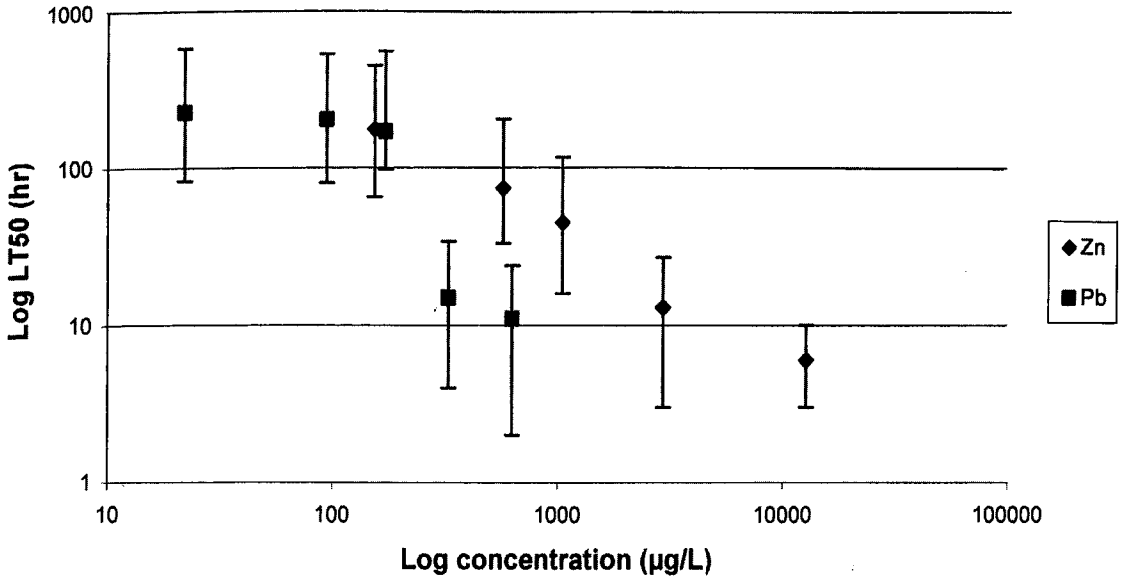

Figure 2. The relationship between median lethal time (LT50) and metal concentrations (with 95% confidence limits) for *M. lanchesteri*.

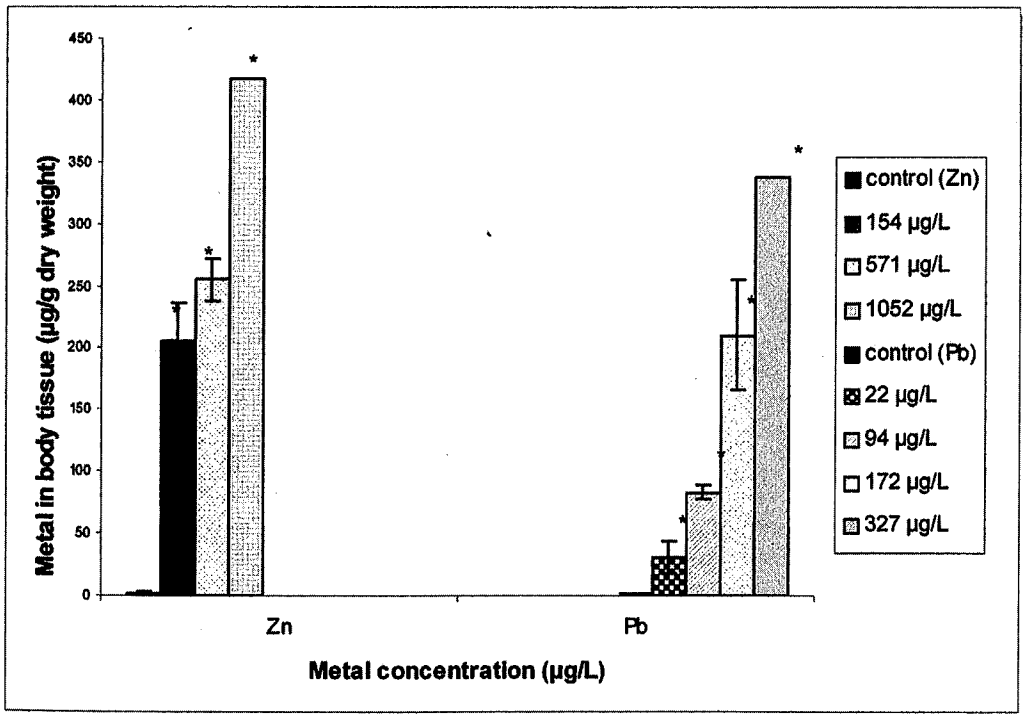

Figure 3. Bioaccumulation of Zn and Pb (mean ± S.E) in *M. lanchesteri* after nine day exposure to different concentrations of Zn and Pb. Asterisk (*) indicates significant different from control water ($P<0.001$).

Table 3. The mean rates of accumulation and maximum concentration attained by *M. lanchesteri* after exposure to zinc and lead for 9 days.

Zinc	154 µg/L	571 µg/L	1052 µg/L
Mean rate of accumulation (µg/g/hr)	0.95	1.18	1.94
Maximum (S.E) concentration attained (µg/g dry weight)	205 (32)	255 (18)	418

Lead	22 µg/L	94 µg/L	172 µg/L	327 µg/L
Mean rate of accumulation (µg/g/hr)	0.14	0.38	0.98	1.56
Maximum (S.E) concentration attained (µg/g dry weight)	31 (14)	83 (6)	211 (45)	338

Table 4. Regression equations for effect of different metal concentrations in solution (X) on zinc or lead concentrations (Y) in *M. lanchesteri* tissues.

Metal	t value	Regression equation (Y on X)
Zinc	2.20	$Y = 1.55 + 0.338X$
Lead	8.62*	$Y = 0.241 + 0.905X$

* $p < 0.05$

However, for lead, a significant regression ($p<0.05$) could be plotted. Higher accumulation of lead seems to be associated with net accumulation of non-essential metal, whilst the essential metal (zinc) was thought to be normally regulated. This can be seen from Table 3 where increasing zinc exposure from 154 to 571 µg/L only increased metal concentration in the prawn from 205 to 255 µg/g.

Metals accumulated in animals can be stored without excretion leading to high body concentrations (accumulators) or the metal levels in the body can be maintained at a low constant body concentration (regulators) by balancing the uptake with controlled rates of excretion [19]. The ability to regulate essential metals such as cobalt, manganese, copper and zinc has been shown for various species of mollusk, crustacean and fish, whereas the concentration of non-essential metals such as cadmium, mercury, silver and lead in organisms depends on their concentrations in the environment [20, 21, 22 and 23]. A study by Vijayram and Geraldine [15] with freshwater prawn *Macrobrachium malcolmsonii* showed that the prawn accumulated the non-essential metal (cadmium) at all exposure level (6.3-157 µg/L) without any regulation. However, the prawn regulates essential metal (zinc) until a threshold level (373 µg/L) when regulation collapses and net accumulation begins. Borgmann et al. [11] showed that amphipod *Hyalella azteca* was capable of regulating copper but unable to regulate zinc as effectively and did not regulate mercury, cadmium and lead. Among the five metals studied (cadmium, copper, zinc and lead), lead was the least accumulated by the amphipods.

All these observations showed that different organisms and metals have different patterns in metal accumulation and toxicity, which depends on various factors such as species, physiological and environmental conditions. Therefore, data gained from laboratory experiments for each species are important in helping to understand the relationship between metal concentrations in the environment and toxic effect to the organisms before the organisms can be used as a bioindicator. Further study with longer exposure times and lower concentrations are needed to see regulatory ability of essential metals in *M. lanchesteri*.

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