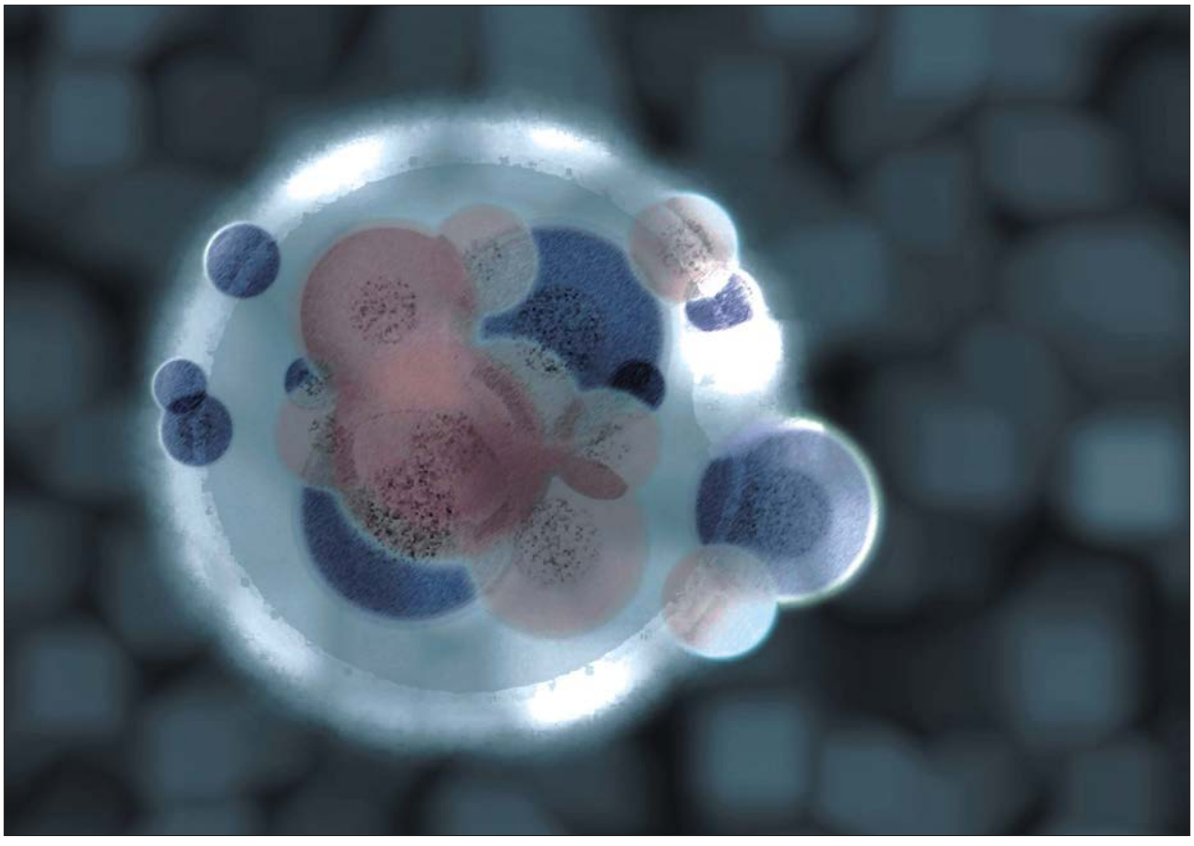

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Bioaccumulation and Elimination of Copper and Lead by Freshwater Prawn *Macrobrachium lanchesteri*

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Abstract: In this current investigation the accumulation and elimination of copper (Cu) and lead (Pb) by *Macrobrachium lanchesteri* were examined in the laboratory. Animals were exposed to different concentrations of metals for 96 h to allow uptake and then transferred to clean water for elimination (96 h). The accumulation of Cu and Pb in *M. lanchesteri* was observed to be rapid and bioaccumulation increased with increasing metal concentrations in the water and with exposure time. In the elimination study, Cu and Pb were found to be eliminate rapidly from *M. lanchesteri*. Metal accumulation and elimination patterns in this organism are discussed.

Key words: Accumulation, depuration, *Macrobrachium lanchesteri*, regulation, uptake, Bioconcentration Factor (BCF)

INTRODUCTION

Bioaccumulation is a general term describing the net uptake by all possible routes and sequestration of pollutants by organisms from their ambient environment (Spacie and Hamelink, 1985). Some metals such as copper are essential to aquatic organisms in trace amounts; others such as lead offer no known direct benefits. All aquatic invertebrates accumulate trace metals in their tissues, whether or not these metals are essential to metabolism. Metals accumulation studies, which focus on the aquatic environment, are important for various reasons. The rate at which metals are taken up by aquatic animals can be related to metal toxicity which is related to a threshold concentration of metabolically available metal. Toxicity occurs when the rate of metal uptake into the body exceeds the combined rate of excretion and detoxification of metabolically available metal (Rainbow, 2002). The ability of aquatic animals to maintain their internal chemical composition at a steady state level when variations in the chemical composition of the external medium occur varies from species to species and according to the physiological functions of trace elements (Amiard-Triquet *et al*., 1986). The best regulation is generally observed in the more highly evolved forms such as fishes and decapod crustaceans (Rainbow and White, 1990).

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. *M. lanchesteri* is common in still or slowly flowing water such as reservoirs, ponds, irrigation ditches and other artificial, enclosed freshwater bodies. It occurs in neutral, alkaline, and slightly acid water, but very acid and peaty water are unfavourable (Johnson, 1963; Cranbrook and Furtado, 1988). This prawn is gaining popularity as live food for aquarium and cultivated fish (Chong and Khoo, 1988). *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 (Chong and Khoo, 1988; Shuhaimi-Othman *et al*., 2003; Shuhaimi-Othman and Nurlailawati, 2004; Shuhaimi-Othman and Nor-Azwa, 2004). However toxicity testing with this species has not so far been investigated and only a few studies have been conducted (Suckcharoen, 1980; Anantha Raman *et al*., 1981; Anantha Raman *et al*., 1982). In this study, the net uptake and elimination patterns of copper and lead by *M. lanchesteri* were examined.

MATERIALS AND METHODS

M. lanchesteri were collected from a pond located 10 km from Bangi, Selangor, Malaysia (02.53°N;

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101.48°W). Prior to toxicity testing, prawns were held for one week under laboratory conditions (27-28°C with 12 h light: 12 h darkness) in 20 L stocking glass tanks using dechlorinated tap water (approximate water quality conditions: pH 6.7, temperature 27°C, conductivity 110 μS cm, hardness 3.8 mg L^{-1} as $CaCO_3$) and aerated through an air stone. During acclimation, prawns were fed with commercial fish food Aquadene®. This study was conducted in 2004 at the Faculty of Science and Technology, National University of Malaysia.

Toxicity tests were performed with control dilution water and four copper (10, 18, 32 and 56 μg Cu L^{-1}) and lead (10, 32, 56 and 100 μg Pb L^{-1}) concentrations. Concentrations selected were based on previous acute toxicity studies (Shuhaimi-Othman *et al*., 2003; Shuhaimi-Othman and Nor-Azwa, 2004). Copper and lead were selected because they are highly related to anthropogenic activities. The standard stock solution of copper (100 mg Cu L^{-1}) was prepared from $CuSO_4.5H_2O$ and for lead (100 mg Pb L^{-1}) from $PbCl_2$. Four replicates each containing 5 adults of *M. lanchesteri* (approximately 3-4 cm body length obtained from stocking tanks) in a 500 mL glass jar (10×8 cm) containing 400 mL of the appropriate solution. A total of 20 animals per treatment were used in the experiment and a total of 180 animals, were employed in the investigation. The tests were carried out under static conditions with renewal of the solution every 48 h. 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 1 h were removed using polyethylene hand pipette. The experiments were performed in a laboratory at room temperature of 27-28°C with photoperiod 12 h light: 12 h darkness, using fluorescent lights (334-376 lux). Water quality parameters (pH, conductivity and dissolved oxygen) were measured every 24 hours using portable meters and water hardness samples (0.45 μm filtered) were fixed with Aristar® nitric acid (70%) and measured by flame atomicabsorption spectrophotometry (AAS-Perkin Elmer AAnalyst 800).

The experiments were designed to allow up to 96 h accumulation where after survived animals were rinsed under dechlorinated tap water and transferred immediately to clean water (dechlorinated tap water) for 96 h depuration. Samples of water from the control and metal containers were taken every 48 h, before and after each solution renewal. Water samples during the elimination study were also taken at intervals. Samples were acidified to 1% with Aristar® nitric acid (to pH<2) before metal analysis by THGA graphite furnace atomic absorption spectrophotometry (AAS-Perkin Elmer Aanalyst 800). Animal samples were taken at intervals (every 48 h) throughout the uptake and elimination phases for metal analysis as described above. Each sample contained three replicates of two animals in a glass test tube and was oven dried (95°C) for at least 48 h before being weighed. Each replicate was digested in 1.0 mL Aristar® nitric acid (70%) in a block thermostat (80°C) for 2 h. The solutions were cooled down before 0.8 mL of hydrogen peroxide (30%) was added. The test tubes were put back on the block thermostat for another 1 h until the solutions looked clear and then made up to 25 mL with deionised water in 25 mL volumetric flasks. Accuracy of the digestion method was tested with reference material (mussel tissue SRM 2976, National Institute of Standard and Technology, USA) using the same digestion procedure. All values obtained were within 10% ranges of the reference values.

Statistical analyses of copper and lead uptake data were conducted by oneway ANOVA with Tukey-Kramer multiple comparison tests 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. Data were tested for normality (Shapiro-Wilk test) and homogeneity (Barlett's χ^2) and to meet these requirements, data were log10 or square root transformed. Data from the study were also fitted into a two-compartment (water-animal) model and the rate constant of uptake (K_1), rate constant of elimination (K_2) and the bioconcentration factor (BCF) were calculated as described by Xu and Pascoe (1993).

RESULTS

In all data analyses, the actual, rather than nominal copper and lead concentrations were used. The mean measured copper concentrations (with SE) for 10, 18, 32 and 56 μg Cu L^{-1} nominal copper exposures were 9.6 (0.8), 18.5 (2.4), 29.1 (3.4) and 50.2 (5.0) μg Cu L^{-1}, respectively and the mean measured lead concentrations (with SE) for 10, 32, 56 and 100 μg Pb L^{-1} nominal lead exposure were 9.5 (1.8), 28.7 (5.39), 56.1 (10.1) and 92.4 (10.5) μg Pb L^{-1}, respectively. The mean water quality parameters measured during the test were pH 6.8±0.1, conductivity 108.3±0.2 μS^{-1} cm, dissolved oxygen exceeding 70% (6.5 mg L^{-1}) of air saturation value and total hardness (Mg^{2+} and Ca^{2+}) 3.90±0.08 mg L^{-1} as $CaCO_3$.

Bioaccumulation of copper and lead in *M. lanchesteri* are shown in Fig. 1 and 2. Copper and lead bioaccumulation in *M. lanchesteri* increases with increasing concentrations exposure. Statistical analyses

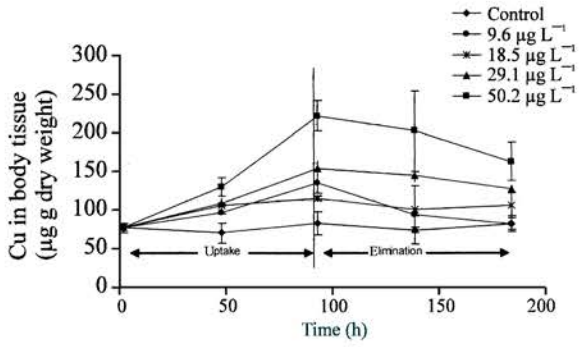

Fig. 1: Bioaccumulation of copper by *M. lanchesteri* after 96 h uptake and 96 h elimination

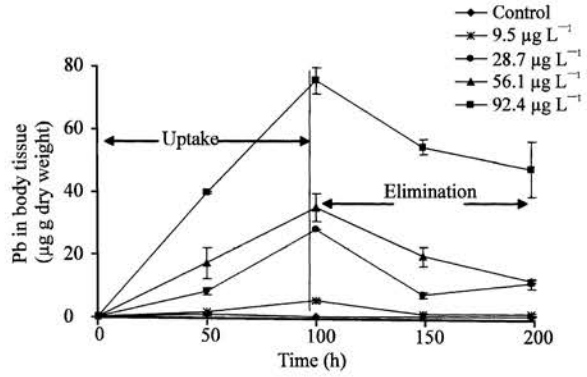

Fig. 2: Bioaccumulation of lead by *M. lanchesteri* after 96 h uptake and 96 h elimination

Table 1: The mean rates of accumulation, elimination and maximum concentration attained for animals exposed to copper and lead for 96 h

Copper concentration (µg Cu L^{-1})	9.6	18.5	29.1	50.2
Mean rate of accumulation (µg/g/h)	0.61	0.40	0.81	1.52
Mean rate of elimination (µg/g/h)	0.55	0.10	0.27	0.62
Maximum (SE) concentration attained (µg g^{-1} dry weight)	123.1 (13.5)	103.4 (2.7)	142.1 (10.5)	210.8 (19.4)
Lead concentration (µg Pb L^{-1})	9.5	28.7	56.1	92.4
Mean rate of accumulation (µg/g/h)	0.04	0.26	0.33	0.72
Mean rate of elimination (µg/g/h)	0.04	0.16	0.22	0.27
Maximum (SE) concentration attained (µg/g dry weight)	4.6 (0.5)	25.5 (0.1)	31.8 (4.0)	68.8 (3.9)

show that there are significant differences (ANOVA, $p<0.01$; Tukey-Kramer) in copper and lead bioaccumulation at all exposure concentrations compared with control water at the end of 96 h. Copper and lead elimination were rapid, especially in the first 24 h for high concentrations exposure. The mean rates of accumulation

Table 2: Uptake (K_1) and elimination (K_2) rate constants (mean±SD) and BCF for *M. lanchesteri* exposed to a) copper and b) lead concentrations (C_w)

a) Copper

C_w (µg L^{-1})	K_1 (h^{-1})	K_2 (h^{-1})	BCF
9.6	121.98±67.39	0.0258±0.0004	11500
18.2	33.26±11.64	0.0065±0.0049	9749
29.1	28.67±7.21	0.0034±0.0013	11031
50.2	31.46±10.6	0.0042±0.0017	8815

b) Lead

C_w (µg L^{-1})	K_1 (h^{-1})	K_2 (h^{-1})	BCF
9.5	8.56±4.03	0.0280±0.0112	363
28.7	12.15±3.30	0.0198±0.0136	878
56.1	8.76±1.51	0.0120±0.0004	733
92.4	9.66±0.2	0.0059±0.0014	1676

and elimination and maximum concentrations attained for copper and lead are shown in Table 1. The mean rate constant values for uptake (K_1) and elimination (K_2) and the bioconcentration factor (BCF) for *M. lanchesteri* are shown in Table 2. As copper is essential metal, basal levels of 64.8±6.2 µg Cu g^{-1} (dry weight of the animal) was detected in *M. lanchesteri* by analysing samples of control animals and was subtracted from the total body burden to correct for background metal levels in the calculations of BCF.

DISCUSSION

This study showed that copper and lead were accumulated in *M. lanchesteri* and were eliminated when transferred to clean water. Accumulation and elimination pattern of both metals were also depending on metal concentration and exposure time. Many studies have shown similar results either with prawn (Vijayram and Geraldine, 1996; Reddy *et al*., 2006) or other organisms such as freshwater amphipod (Xu and Pascoe, 1993; Shuhaimi-Othman and Pascoe, 2003), midge larvae (Krantzberg and Stokes, 1989) and green-lipped mussel (Yap *et al*., 2003). However, statistical analysis shows a different pattern on regulation of these metals in the prawn. Linear regression analysis (Table 3) revealed no significant regression ($p>0.05$) between the copper concentrations in the prawn tissues and copper concentrations in the solutions. However, for lead, a significant regression ($p<0.05$) could be plotted (Fig. 3). Higher accumulation of lead seems to be associated with net accumulation of non-essential metal, whilst the essential metal (copper) was thought to be normally regulated. Table 2 also revealed that for essential metal (Cu), the BCF shows no trend among different concentrations exposed; however for non-essential metal (Pb), there was an apparent trend of increasing in BCF with increasing exposure concentrations. The net

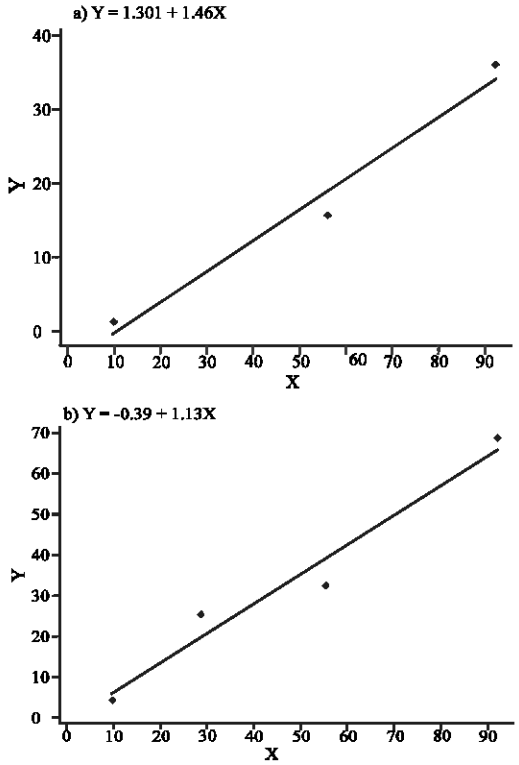

Fig. 3: Regression between different lead concentrations in solution (X) on lead concentrations (Y) in *M. lanchesteri* for (a) 48 h, (b) 96 h

Table 3: Regression equations for effect of different metal concentrations in solution (X) on copper or lead concentrations (Y) in *M. lanchesteri* tissues

Metal	Time (h)	t-value	Regression Eq. (Y on X)
Copper	48	4.10	Y = 1.73 + 0.192X
	96	1.80	Y = 1.69 + 0.340X
Lead	48	22.34**	Y = -1.31 + 1.46X
	96	6.66*	Y = -0.39 + 1.13X*

p<0.05, ** p<0.01

accumulation of lead occurs in *M. lanchesteri* may be useful as a biological monitor for this metal since it fulfills the necessary prerequisites of a biomonitor (Phillips, 1980).

The ability to regulate essential metals such as cobalt, manganese, copper and zinc has been shown for various species of mollusc, 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 (White and Rainbow, 1982; Krantzberg and Stokes, 1982; Kraak *et al*., 1992, 1994). A study by Vijayram and Geraldine (1996) with freshwater prawn *Macrobrachium malcolmsonii* showed that the prawn accumulated the non-essential metal (cadmium) at all exposure levels (6.3-157 μg L^{-1}) without any regulation. However, the prawn regulates essential metal (zinc) until a threshold level (373 μg L^{-1}) when regulation collapses and net accumulation begins. Borgmann *et al.* (1993) 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. Maximum copper concentrations attained by *M. lanchesteri* in this study ranged from 103-210 μg g^{-1} dry weight after exposure to 9.6-50.2 μg Cu L^{-1} and lead concentrations ranged from 4.6-68.8 μg g^{-1} dry weight after exposure to 9.5-92.4 μg Pb L^{-1}. There was no evidence of a steady state concentration being achieved within the short exposure period used for any of the metals.

Aquatic animals can assimilate metals by ingestion of particulate material suspended in water, ingestion of food, ion exchange and adsorption on tissue and membrane surfaces. Elimination consists of biotransformation and excretion processes. In addition, animals with exoskeleton may lose considerable amount of accumulated metals during molting (Phillips and Russo, 1978; Barron, 1995). Rainbow (2002) suggest that for invertebrates, accumulation pattern for essential metals can be divided into three i.e., regulation of body metal concentration, accumulation without excretion and accumulation with some excretion and for non-essential metal its can be divided into two i.e., accumulation without excretion and accumulation with some excretion. Present study show that the mean rates of elimination for animals which had been exposed to 9.6, 18.5, 29.1 and 50.2 μg L^{-1} copper were 0.55, 0.10, 0.27 and 0.62 (μg/g/h), respectively and mean rates of elimination for animals which had been exposed to 9.5, 28.7, 56.1 and 92.4 μg L^{-1} lead were 0.04, 0.16, 0.22 and 0.27 (μg/g/h), respectively. Results suggest that there was an apparent trend in the rate of elimination (increasing) with increasing in exposure concentration for lead but not for copper. Calculation of mean rate constant values for uptake (K_1) and elimination (K_2) in current study showed that the uptake rates were always greater than the elimination rates (Table 2).

Figure 1 and 2 show that concentrations of copper and lead in *M. lanchesteri* did not reached the background concentration after 96 h depuration. Many trace metals cannot be immediately excreted or detoxified, for they are required to play essential roles in metabolism (Rainbow, 2002). Metals accumulated can also cause harm in normal physiological function in animal body as shown by a study conducted with *M. carcinus*

exposure to zinc and copper resulted in reduction in respiration and ammonia excretion (Correa, 1987). Accumulated metals were also stored in different tissue in prawn body. Among tissues, most studies found that hepatopancreas accumulated maximum levels of metal followed by gill and muscle (Vijayaram and Geraldine, 1996; Reddy *et al*., 2006).

In conclusion, the accumulation of copper and lead in *M. lanchesteri* was observed to be rapid and bioaccumulation increased with increasing metal concentrations in the water and with exposure time. In the elimination study, copper and lead were found to be lost from *M. lanchesteri*. Further study with longer exposure times and lower concentrations are needed to see regulatory ability of metals in *M. lanchesteri*.

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