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Accumulation and Depuration of Heavy Metals in The Hard Clam (*Meretrix meretrix)* under Laboratory Conditions

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Abstrak: Penumpukan dan penyingkiran logam berat mungkin memberi kesan terhadap keberkesanan penggunaan *Meretrix meretrix* sebagai organisma pemantauan biologi dalam penilaian kualiti air. Sehubungan itu kajian ini dijalankan untuk menilai kesan penumpukan dan penyingkiran logam berat terhadap *M. meretrix* dengan mendedahkannya dalam larutan Kuprum (Cu), Zink (Zn) dan Plumbum (Pb) di makmal. Hasil kajian menunjukkan bahawa *M. meretrix* berupaya menumpuk Cu, Zn dan Pb masing-masing pada kadar 0.99, 21.80 dan 0.57 µg/g sehari dan menyingkir pada kadar 0.42, 23.55 dan 1.01 µg/g sehari. Keputusan ini juga menunjukkan bahawa *M. meretrix* boleh digunakan sebagai organisma pemantauan biologi yang berkesan untuk Cu kerana kadar penumpukannya lebih tinggi secara signifikan ($p \leq 0.05$) berbanding kadar penyingkirannya. Walau bagaimanapun keadaan ini tidak berlaku bagi Zn kerana kadar penumpukan adalah hampir sama dengan kadar penyingkiran, manakala bagi Pb, tiada penumpukan dan penyingkiran yang berlaku dalam *M. meretrix*.

Kata kunci: *M. meretrix*, Logam Berat, Pemantauan Biologi

Abstract: Heavy metal accumulation and depuration may alter the effectiveness of *Meretrix meretrix* as a biomonitoring organism for water quality assessment. Therefore, this study was conducted to evaluate the effects of heavy metal accumulation and depuration on *M. meretrix,* by immersing it in Copper (Cu), Zinc (Zn), and Lead (Pb) solutions under laboratory conditions. The results showed that *M. meretrix* is able to accumulate Cu, Zn, and Pb at the rate of 0.99, 21.80, and 0.57 µg/g per day, respectively, and depurates at the rate of 0.42, 23.55, and 1.01 µg/g per day, respectively. These results indicate that *M. meretrix* could be effectively used as a biomonitoring organism for Cu because the accumulation rate is significantly ($p \leq 0.05$) higher than the depuration rate. However, this was not the case for Zn because the accumulation rate was almost similar to the depuration rate, while for Pb, accumulation or depuration did not occur in *M. meretrix*.

Keywords: *M. meretrix*, Heavy Metals, Biomonitoring

INTRODUCTION

Conventionally, heavy metal monitoring of water has been carried out by analyzing the concentration of heavy metals in water and sediments. However,

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information obtained through this method could be inaccurate, as heavy metals tend to be dispersed into the aquatic environment or distributed into the biota (Barsyte-Lovejoy 1999; Kennish 2000; Issam *et al.* 2003). Inaccurate information creates misleading decision-making for water quality assessment.

To overcome this problem, the idea has been proposed to use a biomonitoring organism to indicate heavy metal pollution. Compared to conventional methods, the ability of a biomonitoring organism to accumulate heavy metal within a certain time period can be used to monitor pollutants more effectively (Goldberg 1975; Bayne 1985; Szefer & Szefer 1985; Micallef & Tyler 1989; Rainbow 1995; Andersen *et al*. 1996; Luoma & Fisher 1997; Park & Presley 1997; Ruangwises & Ruangwises 1998; Barsyte-Lovejoy 1999; Hung *et al.* 2001; Storelli & Marcotrigiano 2001).

During the past few decades, many species have been studied to determine their potential as a biomonitoring organism, and mollusca have become a popular choice for heavy metal monitoring (Phillips 1980; Wilson 1980, 1982; Bryan *et al*. 1985; Hung *et al.* 2001). However, Viarengo (1985), Kägi (1993), Mason and Jenkins (1995), Barsyte-Lovejoy (1999), and Ruelas-Inzunza and Páez-Osuna (2000) have reported that molluscs have a depuration mechanism to reduce heavy metal toxicity in their body. This mechanism might diminish the effectiveness of molluscs as biomonitoring organism, as the concentration of heavy metal in the mollusc may not accurately reflect the concentration in the environment (Bryan *et al*. 1985; Langston & Spence 1995).

Therefore, there is a need to evaluate the effects of heavy metal accumulation and depuration in the biomonitoring organism. This study focused on the hard clam, *M. meretrix*, which is abundant in the estuarine area of Sabah, Malaysia (Ridzwan 1993). Studies have shown that *M. meretrix* is able to accumulate Cu, Zn, and Pb in the natural environment and this species has the potential to be used as a biomonitoring organism (Jovita 2005; Wang *et al*. 2005). However, no study has been carried out to determine the ability of *M. meretrix* to depurate heavy metal under laboratory conditions.

MATERIALS AND METHODS

Sample Collection and Analysis

Ninety individuals of *M. meretrix* were collected from the Likas estuary of Sabah in June, 2004. After washing, all specimens were transported to the laboratory and acclimatized for two days. Following acclimatization, 5 mg/l of Cu, Zn, and Pb solutions were added once into three different tanks. All experiments were conducted in experimental aquaria. Thirty specimens of similar size (5–6 cm) were placed in each tank and fed with commercial algae. Sampling was conducted at day 5, 10, 15, and 20. Prior to the metal exposure, six specimens were collected for analysis of background metals. The test solutions (40 l) were semi-static but constantly aerated and at room temperature (26°C–29°C). Salinity was 15%–20%, dissolved oxygen was above 5 mg/l, and pH ranged from 7.5–8.5.

Sample Preparation

Soft body tissues of all specimens were removed from shells, dried, weighed, and digested individually in 30 ml concentrated HNO_3 (APHA 1989; O'Leary & Breen 1997). The specimens were diluted into 100 ml and filtered through an Advantec 0.45 µm membrane filter. Heavy metals were measured with an atomic absorption spectrophotometer equipped with a polarized Zeeman background correction device. Concentration of heavy metals in *M. meretrix* samples were expressed in µg/g dry weight. The calculation of metal accumulation and the depuration rate was adapted from Yap *et al.* (2003):

$$\text{Metal accumulation rate} = \frac{\text{Metal level}_{\text{end of metal accumulation}} - \text{Metal level}_{\text{control}}}{\text{Day(s) of metal exposure}}$$

$$\text{Metal depuration rate} = \frac{\text{Metal level}_{\text{end of metal accumulation}} - \text{Metal level}_{\text{end of metal depuration}}}{\text{Day(s) of metal exposure}}$$

Statistical Analysis

Significant differences in heavy metals concentration between specimens was determined by t-test analysis using SPSS. Significant differences between the accumulation and depuration rates for each heavy metal, was determined using the paired t-test. Statistical significance was determined at the 95% confidence level.

RESULTS AND DISCUSSION

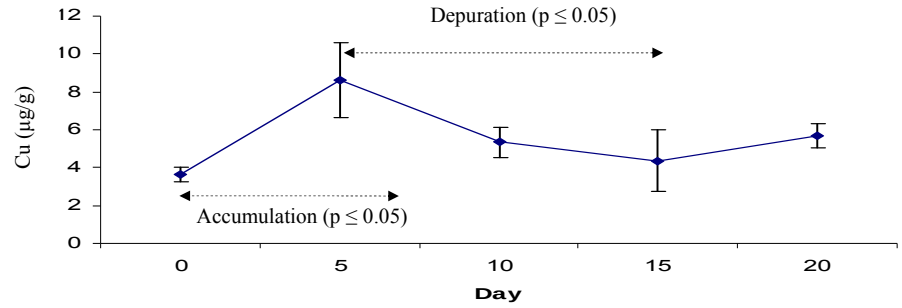
Table 1 shows the mean concentration ± standard deviation of Cu, Zn, and Pb in *M. meretrix* specimens throughout the experiment. The specimens' exposure to Cu was carried out for 20 days. Exposure to Zn and Pb was reduced to 15 and 10 days, respectively, because the specimens did not survive after these periods. A previous study by Ridzwan and Kaswandi (1995) showed that the concentration of Zn and Pb in *M. meretrix* from an unpolluted area of Semporna, Sabah were 7.10 and 0.35 µg/g dry weight respectively. However the concentrations of Zn and Pb in specimens from this study were 18 and 20 times higher, respectively, than those reported by Ridzwan and Kaswandi (1995). The specimens might have experienced toxic effects due to Zn and Pb before the experiment was completed. According to Chin and Chen (1993), under unfavourable environmental conditions, bivalves will close their shell to stop the penetration of unwanted chemicals into their body. By doing this, *M. meretrix* will experience starvation and might inevitably cause their own death. This is supported by a study by Wahi *et al*. (2005), which indicated that *M. meretrix* could not survive after 10 days without sufficient food.

Table 1: Metal concentrations (mean ± standard deviation as µg/g dry weight) of Cu, Zn, and Pb in *M. meretrix*.

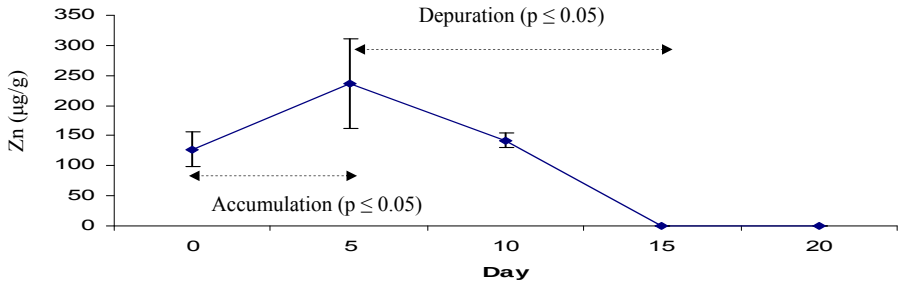
Metals	Days				
	0	5	10	15	20
Cu	3.65 ± 0.37	8.61 ± 1.96	5.33 ± 0.78	4.37 ± 1.65	5.69 ± 0.64
Zn	126.91 ± 28.68	235.90 ± 74.84	142.23 ± 12.68	0.40 ± 0.09	n.a
Pb	7.13 ± 2.29	9.98 ± 3.12	4.95 ± 2.10	n.a	n.a

n.a.: not available

Figure 1 shows the accumulation and depuration patterns of Cu, Zn, and Pb in *M. meretrix* throughout the experiment. The concentration of heavy metals increased during the accumulation phase and decreased during depuration. For Cu, the accumulation and depuration was significant ($p \leq 0.05$), occurring at the rate of 0.99 and 0.42 µg/g per day, respectively. This indicates that the rate of Cu accumulation is higher in *M. meretrix*, compared to its depuration rate. These data suggest that *M. meretrix* can be used as an effective biomonitoring organism for Cu.

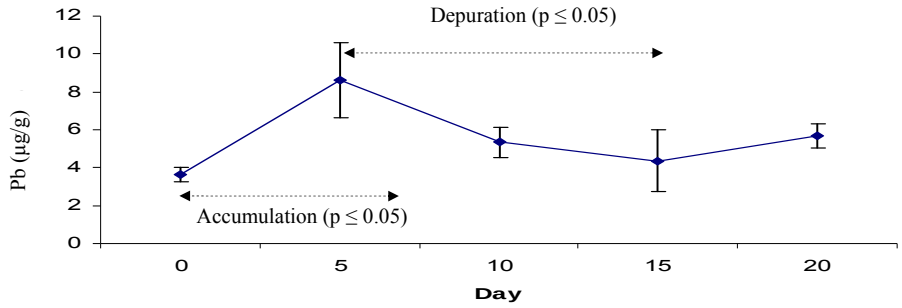


(a)



(b)

Figure 1: Patterns of accumulation and depuration of (a) Cu, (b) Zn, and (c) Pb in *M. meretrix (continued on next page).*



(c)

Figure 1: *(continued)*

For Zn, the accumulation and depuration was significant ($p \leq 0.05$), occurring at the rate of 21.80 and 23.55 µg/g per day, respectively. Throughout the experiment, the concentration of Zn in *M. meretrix* from day 0 was increased up to 87.1% at day 5, then decreased to 67.1% at day 10, and continued to decrease until day 15. This indicates that Zn has been regulated in *M. meretrix*. This observation was consistent with that of Yap *et al*. (2003), which reported that *Perna viridis* also regulates Zn after exposure. According to Viarengo *et al*. (1985), Zn is important for metabolism, however, it might also be regulated (Phillips 1985) in the bivalve organism. The ability of *M. meretrix* to regulate Zn has reduced their effectiveness to be used as a biomonitoring organism for Zn.

The accumulation and depuration for Pb occurred at the rates of 0.57 and 1.01 µg/g per day, respectively. However there was no significant difference ($p \geq 0.05$) before and after exposure. These data indicate that accumulation and depuration of Pb did not occur in the specimens. This result contradicts those of Jovita (2005) and Wang *et al*. (2005), who reported that *M. meretrix* accumulates Pb. However this difference could be due to the high concentration of Pb in the specimens of this study, which originates from their natural environment. The specimens did not accumulate the additional Pb provided in the laboratory to reduce the level of toxicity. Based on the accumulation and depuration rate for Pb reported herein, it could be concluded that *M. meretrix* would not be an effective biomonitoring organism for Pb. However if the concentration of Pb is as reported in other studies, its use would be more apparent.

CONCLUSION

The accumulation rate of Cu in the current study is higher than the depuration rate of this heavy metal, suggesting that *M. meretrix* is suitable to be used as a biomonitoring organism for Cu. Although *M. meretrix* is able to accumulate Zn, its active regulation of this metal does not support a role as biomonitoring organism for this heavy metal. The exposure of *M. meretrix* to Pb did not affect the

concentration of this heavy metal in the organism, suggesting *M. meretrix* would not be an effective blomonitoring organism for Pb. However, further studies should be carried out to determine the ability of *M. meretrix* to accumulate and depurate Pb within a lower range of Pb concentration.

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