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Levels of Heavy Metals in Green-Lipped Mussel *Perna veridis* (Linnaeus) from Muar Estuary, Johore, Malaysia

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Abstract: Muscle and feather in tissue of 40 juveniles and 40 adult green-lipped mussel *Perna veridis* (L.) collected from Muar Estuary, Johor were analyzed for copper (Cu), cadmium (Cd), lead (Pb) and zinc (Zn) concentration using a fast and sensitive Inductively Coupled Plasma Mass Spectrometer (ICP-MS). In this study, the average concentration of Cu was 8.96 μg g^{-1} dry weights, Cd with 0.58 μg g^{-1} dry weight, Pb averaging 2.28 μg g^{-1} dry weights and Zn averaged to 86.73 μg g^{-1} dry weight. The highest accumulation of metal studied was found in feather sample compared to the muscle. The positive relationship of Cu, Cd, Pb and Zn with *P. virdis* length suggesting that the accumulation of these metals were formed in the mussel. In all cases, metal levels found were lower than the guideline of international standards of reference and the examined bivalve were not associated with enhanced metal content in their tissues and were safe within the limits for human consumption.

Key words: Green-lipped mussel, *P. veridis* (L.), copper, cadmium, lead, zinc, ICP-MS

INTRODUCTION

The wide diversity of human activities that introduce pollutants into the environment, as well as their magnitudes make the assessment of environmental impact a subject of utmost interest. Contamination of aquatic environments with potentially harmful substances, in particular non-degradable heavy metals and its subsequent impact on organism, is more dramatic within estuaries and semi-closed coastal zones, especially when they are near highly populated or industrial areas. Heavy metals may enter an estuary from different natural and anthropogenic sources, including industrial or domestic sewage, storm runoff, leaching from landfills, shipping activities and atmospheric deposits (Goodwin *et al*., 2003; Saifullah *et al*., 2002).

The green-lipped mussel *Perna veridis* (L.) is widely distributed in the coastal waters of the Asia-Pacific region (Tanabe, 2000) and therefore its use as a biomonitoring agent has been interesting for scientific purposes. The marine mussels can act as a quantitative indicator to reflect the heavy metal contamination in the coastal areas (Phillips, 1995; Yap *et al*., 2003). Another reason that mussels are often chosen for biomonitoring studies, are they are sedentary organisms, long-lived, easily identified and sampled, reasonably abundant and available throughout the year, tolerant of natural environmental fluctuations and pollution. Besides, they have good net accumulation capacities and they are important ecologically (Yap *et al*., 2004).

Some metals such as Cu, Cd, Pb and Zn have long been known to accumulate within the aquatic food chain. These metals are widely distributed in the coastal environment, both from natural source and anthropogenic activities, thus the metals are readily accumulated in the soft tissue of *P. veridis*. Mussels are well known to accumulate a wide range of contaminant in their tissues and have been suggested as a potential biomonitoring agent of heavy metals in many countries (Phillips, 1995; Yap *et al*., 2003). In recent years, the study area especially for the first km along the Muar River has been heavily impacted by discharges from municipal and industrial outflows. This was due to the rapid development of the area via expansion of the industrialization area as well as

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the increase in population. The aim of this study is to obtain the background data on levels of heavy metals (Cu, Cd, Pb and Zn) in muscle and feather of *P. virdis* that were found abundant in the study areas.

MATERIALS AND METHODS

Description of the study area: Muar estuary located in southern of peninsular Malaysia, encompasses an estimated wide of 150 to 300 m. It flows through from north to southwest of Johor and entering the Malacca Straight (Fig. 1). The indigenous people inhabiting Muar River are mostly fisherman, factory worker and grocer. This river is intensively exploited by the surrounding community not only as a route for fisherman but also as a port for shipyard, route of industrial wastes and also an active area for sand mining activities. Nevertheless, this river has a diverse ecosystem and also highly utilizable natural resources such as oyster, crab, prawn and some mangrove species. In recent years, the study area especially for the first km along the Muar River has been heavily impacted by discharges from municipal and industrial outflows especially near Muar City and Tanjung Agas areas. This was due to the rapid development of the area via expansion of the industrialization area as well as the increase in population. Electronics and petro-chemicals are the main industry in the area and is the catalyst for other supportive industries to develop around the same area. Bivalve species that were dominantly caught in 11th September 2006 were selected; green-lipped mussel *Perna veridis* (L.).

Analytical procedures: Forty juveniles and 40 adults *P. veridis* were collected randomly with a sufficient number of individuals along Muar estuary, especially near the fish culture areas. The samples were transported to the laboratory and stored at -20°C prior to analysis. Then the samples were thawed at room temperature and their length was recorded using the callipers and 3 decimal points of measuring weight, respectively. They were dissected using stainless steel scalpels and Teflon forceps using a laminar flow bench. A part of the mussel (muscle and feather) were removed and transferred in polypropylene vials. Before acid digestion, a porcelain mortar was employed to grind and to homogenise the dry tissue samples.

Briefly, the digestion method involved the heating of 1.0 g of sample in a teflon beaker with mixed concentrated acids of HNO_3 and H_2SO_4 in the ratio of 1:1. The teflon beakers were kept at 100°C for 2-3 h. After cooling, a H_2O_2 solution was added in order to break down any recalcitrant lipid material in the tissue and a clear solution with no residue should be obtained at this stage. Deionized water was added and allowed to evaporate a

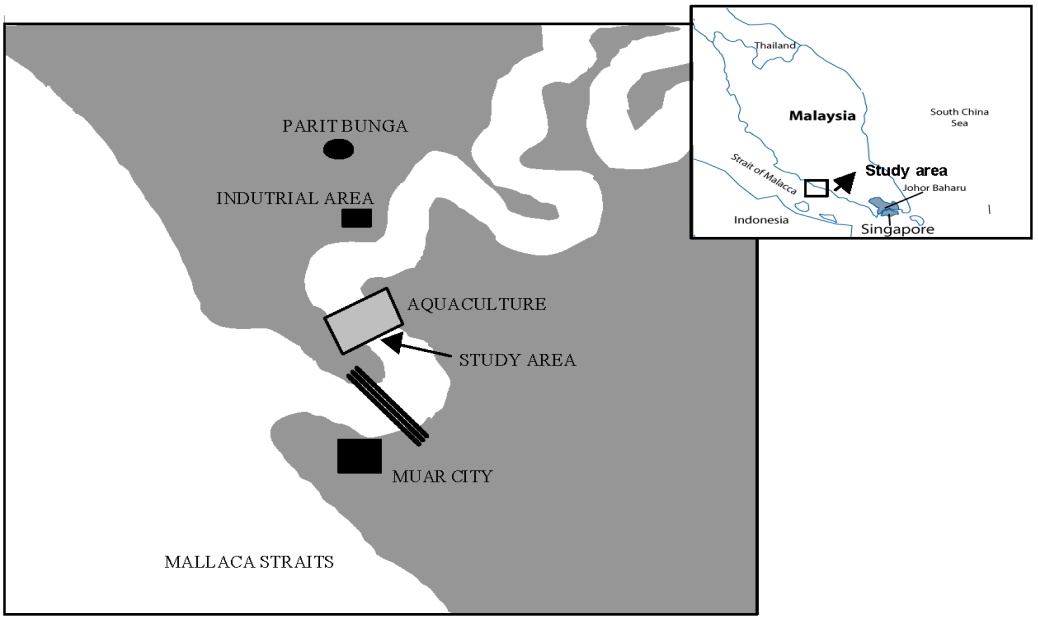

Fig. 1: The study area and sampling locations at Muar River

few times to dryness and finally the digested tissues were transferred into a 50 mL volumetric flask. An inductively coupled plasma mass spectrometer (ICP-MS) was used, for the quick and precise determination of Cu, Cd, Pb and Zn in the tissue samples. The precision assessed by replicate analyses was within 3%. The accuracy was also examined by analyzing a blank and a material standard from the National Research Council of Canada standard (NBS DORM 2) and the results coincided with the certified values within a difference of ±3%.

Statistical analysis: Statistics were performed using a two factor analysis of variance. This method was based on the procedure of general linear models, where samples were examined for potential influence on Cu, Cd, Pb and Zn. Differences between level means per factor were treated using Tukey's multiple comparisons of means. When ANOVA assumptions such as sample normality and homoscedasticity were not respected, multiple (n>2), sample comparisons were performed by nonparametric Kruskal-Wallis tests. An ANOVA paired tests were then used for two-sample comparisons. A $p \leq 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

A total of 80 *P. veridis* (40 juveniles and 40 adults) were analyzed for Cu, Cd, Pb and Zn concentration with a mean length size from 38 to 129 mm. Figure 2 shows the mean values of the measured heavy metals (Cu, Cd, Pb and Zn) in muscle and feather of *P. veridis*. There are significant differences between the samples within the same area. The concentration of Cu in muscle from the juvenile (38-80 mm) to the adult size (81-130 mm) of *P. veridis* was varied within the average of

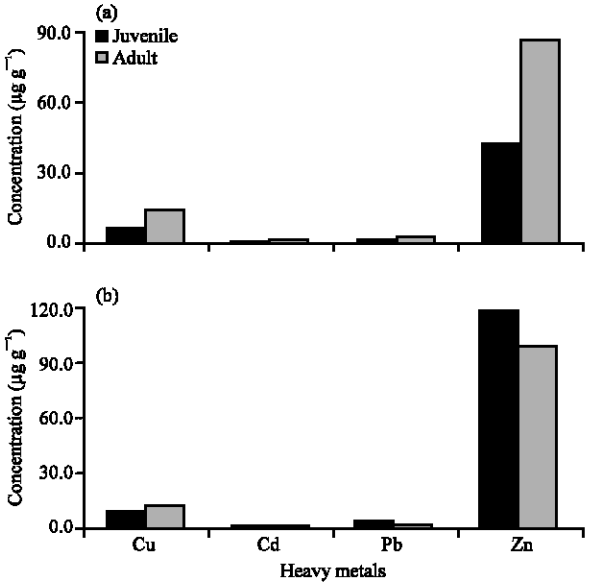

Fig. 2: The concentration of heavy metal in the muscle and feather content of *P. veridis* from Muar River, Johor, (a) Muscle and (b) Feather

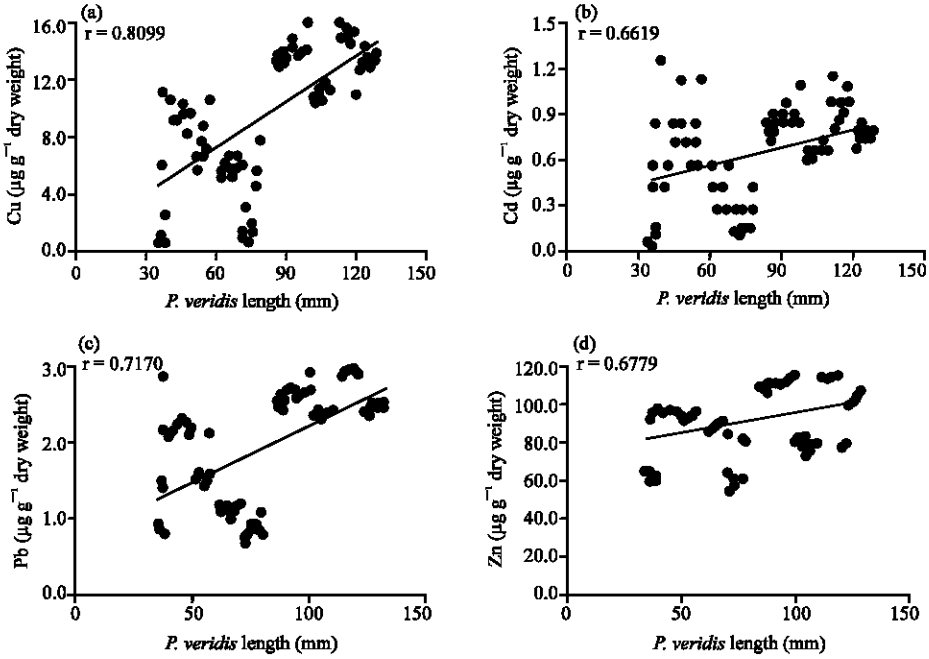

Fig. 3: Relationship of Cu, Cd, Pb and Zn concentrations in *P. veridis* versus mussel length

6.12 and 8.16 μg g^{-1} dry weight, respectively. Feather indicates the concentration of Cu was relatively high compared to the muscle concentration which was 11.20 and 10.36 μg g^{-1} dry weight, respectively. For Cd, the concentration in adult *P. veridis* muscle was 0.56 μg g^{-1} dry weight and juvenile was 0.42 μg g^{-1} dry weight. The Cd concentration in *P. veridis* feather was higher in juvenile mussel (0.85 μg g^{-1} dry weight) compared to the adult mussel (0.50 μg g^{-1} dry weight). Meanwhile the concentration of Pb in muscle of juvenile and adult mussel was 1.39 μg g^{-1} dry weight and 1.72 μg g^{-1} dry weight, respectively. Similar with other metals, the Pb concentration in adult mussel feather was lower (2.32 μg g^{-1} dry weight) compared to the juvenile mussel (3.70 μg g^{-1} dry weight). Zn was found higher in the adult size of *P. veridis* mussel which indicating the average concentration of 85.49 μg g^{-1} dry weight followed by the juvenile size which is 42.13 μg g^{-1} dry weight. However, the concentration Zn is even higher in the feather of juvenile *P. veridis* which as high as 118.94 and 100.36 μg g^{-1} dry weight for adult mussel feather.

Generally, the concentration of all study metal in mussel feather was slightly higher compared to the muscle in both juvenile and adult mussel. Meanwhile, the metal concentration in juvenile mussel feather was higher than the adult mussel. This may due to the *P. veridis* has the greatest growth rate of the mussels. Maximum growth of the green mussel occurs 2 m below the surface because of the increased productivity of the water at that depth and a narrow area of temperature and salinity fluctuation (Yap *et al*., 2003).

The well relationship formed between the study metal concentration and *P. veridis* suggesting that metals bioaccumulation has occurred and many results for different species in other studies indicating the occurrence of bioaccumulation. In this study, a statistically significant correlation was also observed between metal concentrations in *P. veridis* with the length of the mussel (Fig. 3). The correlation of the Cu, Cd, Pb and Zn concentration in *P. veridis* were fairly significantly with r = 0.8099, r = 0.6619, r = 0.7170 and r = 0.6769, respectively.

Present study shows that the level of Cu in the *P. veridis* collected from Muar estuary were lower than other studies done in Malaysia but higher than Kuala Dinding, Perak and Bagan Lalang, Selangor. Meanwhile, the concentration of Cd and Pb of present study were lower than other study by Yap *et al.* (2004). For Zn, *P. veridis* collected from Sebatu, Malacca has the lower concentration compared to this study (Table 1). Finally, when this study compared with metal levels found in *P. veridis* from other area of this region, our concentration level are lower and comparable to the study by other scientist (Table 2). The metal levels were also lower than the recommended guidelines for Cu, Cd, Pb and Zn set by different countries (Table 3). Thus, the

Table 1: A comparison of mean concentration (μg g^{-1} dry weight) of heavy metals in *Perna veridis* from other studies done in Malaysia with present study

Location	Cu	Cd	Pb	Zn	Reference
Pulau Aman, Penang	10.80	0.87	4.76	110.00	Yap *et al.* (2004)
Bagan Lalang, Selangor	8.20	1.12	3.41	96.40	
Kuala Linggi, N. Sembilan	9.14	1.25	7.98	101.00	
Pantai Lido, Johor	9.39	0.68	4.03	117.00	
Sebatu, Malacca	11.20	1.04	7.59	75.10	
Kuala Dinding, Perak	7.76	1.06	2.51	90.00	
Muar Estuary	8.96	0.58	2.28	86.73	Present study

Table 2: A comparison of mean concentration (μg g^{-1} dry weight) of heavy metals in *Perna veridis* from regional studies with present study

Location	Cu	Cd	Pb	Zn	Reference
Cochin, India	11.7	-	3.70	84.6	Sivaperumal *et al.* (2007)
Morocco coast	7.0	0.41	-	231.0	Banaoui *et al.* (2004)
Dakar (Senegal)	7.2	2.37	-	121.0	Sidoumou *et al.* (2006)
Hong Kong	37.15	0.66	7.65	116.4	Liu and Kueh (2005)
Taiwan	1.78-5.41	-	-	14.4-25.7	Han *et al.* (1997)
Gulf of Thailand	1.50-11.3	<0.02-19.10	0.19 -3.75	24.9-213	Sukasem and Tabucanon (1993)
Muar Estuary	8.96	0.58	2.28	86.73	Present study

Table 3: Guidelines on heavy metals concentration (μg g^{-1} dry weight) for food safety set by different countries (Yap *et al*., 2004)

Guidelines	Cu	Cd	Pb	Zn
Malaysian food regulation	30.00	1.00	2.00	100.00
International council for the exploration of the sea	-	1.80	3.00	-
Food and Drug Administration of the United State	-	3.70	1.70	-
Australian legal requirement	350.00	10.00	-	750.00
Hong Kong environment protection department	-	2.00	6.00	-
Muar estuary	8.96	0.58	2.28	86.73

average concentration of heavy metal of *P. veridis* at Muar estuary was considered safe to be consume but however there was still an obvious bioaccumulation of pollutant in the muscle content and feather content.

CONCLUSION

The concentration of Cu, Cd, Pb and Zn in the *P. veridis* of the Muar estuary was found not to be serious. In this study, *P. veridis* accumulates these metals in their soft tissues and constitutes one of the important food-chains in the coastal environment. From the data obtained, these metal concentrations could be contributed to natural and anthropogenic metal sources that affecting their habitats. From the human public health point of view, these results seem to show no possibility of acute toxicities of Cu, Cd, Pb and Zn if the edible mussels are consumed by human.

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