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Bioaccumulation of Some Metals by Green Mussel *Perna viridis* (Linnaeus 1758) from Pekan, Pahang, Malaysia

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ABSTRACT

A study was conducted to determine the bioaccumulation of some essential and non-essential heavy metals by the soft tissue of *Perna viridis* (Green mussel) collected from Pekan, Pahang during November 2009. A total of fifty individuals of Green lipped mussel *P. viridis* (Family: Mytilidae) were sampled and metals such as Iron (Fe), Zinc (Zn), Copper (Cu), Cobalt (Co), Lead (Pb) and Cadmium (Cd) concentration were determined using Inductively Coupled Plasma Mass Spectrometry (ICP-MS). The observed mean concentration of Fe, Zn, Cu, Co, Pb and Cd in the soft edible tissue of *P. viridis* was 576.45±87.78, 45.54±8.75, 19.05±4.12, 17.85±2.28, 0.47±0.14 and 0.3±0.06 $\mu g\ g^{-1}$, respectively. The bioaccumulation of metals in the soft tissue flowed in Fe>Zn>Cu >Co>Pb>Cd order indicating that the essential heavy metal accumulates in faster rate than non-essential metals. Comparison with earlier studies showed that Fe and Cu bioaccumulation level was higher in samples from Pekan area with average of 576.45 and 19.05 $\mu g\ g^{-1}$, respectively. All the other metals concentration was lower in the soft tissue of green mussel indicating comparatively lower heavy metal contamination in the Pekan, Pahang compared with other coasts.

Key words: *Perna viridis*, bioaccumulation, Mytilidae, heavy metals, Pekan

INTRODUCTION

Knowledge on the accumulation and distribution of metals in the soft tissues may help us to understand the processes involved in the uptake and excretion of metals by the different parts of mollusks. Numerous factors influence the bioconcentration of heavy metal in mollusks tissues. It was observed that the concentrations of heavy metal accumulated by marine organisms are not only depending on the water quality but also seasonal factor, temperature, salinity, diet or food intake, spawning and individual variation (Hamed and Emara, 2006). The bioaccumulation of heavy metals by marine mollusks and other marine organisms may reach many orders of magnitude above background concentration of certain locality. This phenomenon may demonstrate the potential of these species as a biomonitor of heavy metal pollution (Hamed and Emara, 2006). Many studies have shown that intertidal mollusks can be good biomonitoring organisms (Ismail, 2006).

It was observed that different tissues of any organism accumulate metals at different Concentration and at different rates and the biological half-lives of metals at each type of soft tissues also differ from one another (Lakshmanan and Nambisan, 1989; Yap *et al.*, 2007b). This phenomenon is due to the role played by the different parts of the bioindicator organisms, their metabolic rate and the physiological conditions of the internal body parts. This directly influences the distribution of metals in the different soft body parts. It is also to be noted that metals contamination are very localized and closed to discharge point sources and mollusks inhabit in different microhabitat of intertidal areas where metal discharge is high due to various anthropogenic sources, a detail studies on bioaccumulation of heavy metals by different species from these habitats are essential. This will help in proposing the best indicator organisms of the particular environment through biomonitoring process (Yap *et al.*, 2007a).

The Asian green mussel (*Perna viridis*), also known as the philippine green mussel, is an economically important mussel, abivalve belonging to the family Mytilidae. It is harvested for food but is also known to harbor toxins and cause damage to submerged structures such as drainage pipes. The Asian green mussel is found in the coastal waters of the Indo-Pacific region (Benson *et al.*, 2002). Due to its stress tolerant capacity, present research work focused on determination of essential and non-essential heavy metals such as Iron (Fe), Copper (Cu), Zinc (Zn), Cadmium (Cd), Lead (Pb), Cobalt (Co) and Chromium (Cr) concentrations in the soft tissue of common bivalve (*Perna viridis*) which were collected from the coastal waters of Pekan, Pahang Malaysia.

MATERIALS AND METHODS

Study area: Pahang river is the longest waterway in Peninsular Malaysia which terminates into the South China Sea through Pekan estuary (Fig. 1) (N 03° 30' 0.00" E103° 25' 1.20"). This area

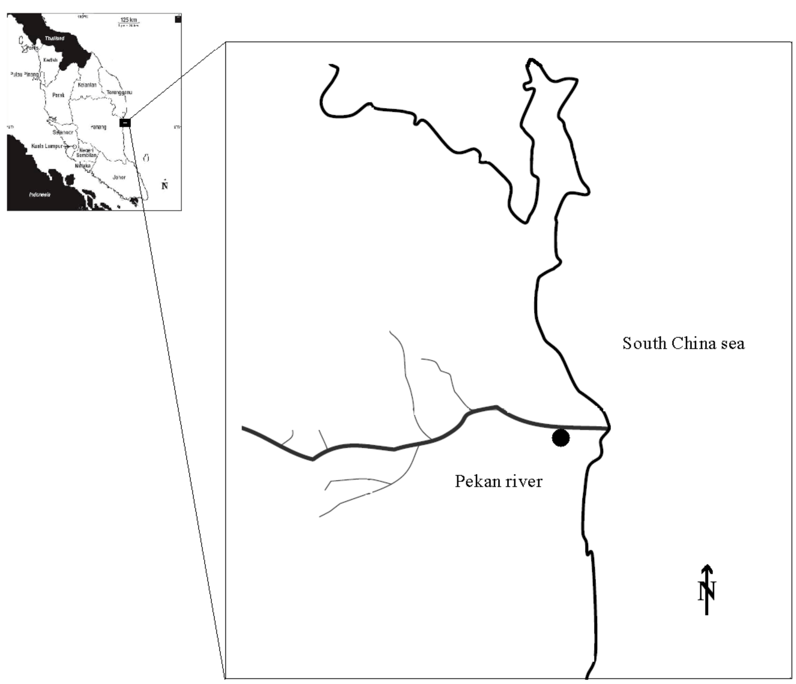

Fig. 1: Location of sampling area Pekan Pahang, Malaysia

Table 1: Location of sampling sites and date of sampling is represented with Global Positioning System (GPS) information

Sites	GPS	Sampling date
Present study (Pekan, Pahang)	N 03° 30' 0.00" E103° 25' 1.20"	Nov 2009
Senggarang	N 01° 45.150' E 103° 03.080'	Aug 2004
Gelang patah	N 01° 25.714' E 103° 40.159'	Aug 2004
Senibong	N 01°28.993' E 103°48.748'	Aug 2004
Telok jawa	N 01° 28.560' E 103° 50.414'	Aug 2004
Kampung masai	N 01° 27.910' E 103° 51.772'	Aug 2004

is highly influenced by human inhabitation, automobile industries, agriculture and recreational activities which could possibly introduce considerable level of contaminants into the water body.

Sample collection and preparation: A total of 50 green Mussels *(Perna viridis)* were collected randomly by hand picking during low tide from Pekan in the state of Pahang, Malaysia on November 2009 to examine essential and non-essential heavy metal accumulation in the soft tissue part (Amiard *et al.*, 1986). Collected mussels were placed in plastic bags and iced prior to laboratory analysis. The samples were cleansed to remove the mud or any attachments and then washed with double distilled water. Soft tissue (edible portion) from the shells were excised with a plastic knife and wrapped with aluminum foil and dried in oven at 65°C for 72 h.

Tissue digestion: Acid digestion method was performed to digest the samples which involved heating of 0.5 g of dried tissues of green mussel in Teflon beaker with mixed concentrated acids (Hydrogen Peroxide (H_2O_2), Nitric acid (HNO_3), hydrochloric acid (HCl)) and sulphuric acid (H_2SO_4) in the ratio of 1:1 (Kamaruzzaman *et al.*, 2007). After the digestion process hundred times dilution was performed using Mili-Q water then the samples were analyzed using Inductively Coupled Plasma Spectrophotometer (ICP-MS). The values of the heavy metal concentrations in the tissues were calculated based on dry weights as this discounts the variability due to inner parts differences in the moisture content of organisms. International certified standards (DORM-2) by National Research Council of Canada and a blank in replicates were used to control the accurateness of this procedure. One-way Analysis of Variance (ANOVA) statistical test was performed to check the significance of p value. Table 1 shows location of sampling sites and date of sampling is represented with Global Positioning System (GPS) information.

The determined concentration of various heavy metals were compared with previous studies on the heavy metal accumulation in the soft tissues of *Perna viridis* collected from different sampling sites of Malaysia (Yap *et al.*, 2007a). Hence, present study also gives insight on the current status of heavy metal accumulation level by the green mussel collected from Pekan, Pahang area.

RESULTS

Present study revealed that, Iron (Fe) concentration in the edible soft tissues of *Perna viridis* collected from study area (Pekan river, Pahang) ranged between 259.21-1121.43 $\mu g\ g^{-1}$ with the mean concentration of 576.45±87.78 $\mu g\ g^{-1}$. It was also observed that the detected amount of Fe in the green mussel collected from the sampled area was higher than the observed concentration of Fe in same samples from other sampling locations. The concentration of Zn in the mussel ranged between 2.18-94.28 $\mu g\ g^{-1}$ with the mean concentration of 45.54±8.75 $\mu g\ g^{-1}$. It was apparent from this result that the Zn concentration in samples from Pekan river was lower than the same samples from other sampling locations. There was no much fluctuation in the mean concentration of Cu and

Table 2: Comparison of different heavy metals (Fe, Zn, Cu, Cd and Pb excluding Co) concentrations (in µg g^{-1} dry wt.) in soft tissue of *Perna viridis* collected from Pekan, Pahang with other sampling locations from previous studies

	Metals (µg g^{-1} dry wt.)				
Sampling area	Cu	Cd	Zn	Pb	Fe
Senggarang	4.6[a]	2.35[a]	79.92[a]	59.46[a]	66.27[a]
Gelang patah	6.15[a]	1.88[a]	57.55[b]	6.71[a]	98.76[a]
Senibong	8.97[a]	1.26[a]	70.72[a]	6.1[a]	34.79[a]
Telok jawa	8.2[a]	1.58[a]	87.64[b]	14.85[a]	59.35[a]
Kampung masai	9.2[a]	1.37[a]	76.82[a]	7.46[a]	74.88[a]
Present study (pekan, pahang)	19.05±4.12[a]	0.3±0.06[a]	45.54±8.75[a]	0.47±0.14[a]	576.45±87.78[a]
Certified Reference material (CRM)	2.34±0.16	0.043±0.008	25.6±2.3	0.065±0.007	142±10
Maximum permissible levels (MPL) (WHO, 1982)	10	2	100	5	-
Maximum permissible levels (MPL) (FDA, 2001)	100	0.2	150	1.5	-
Maximum permissible levels (MPL) (MFR, 1985)	30	1	100	2	-

Significant value a indicates $p<0.05$;a indicates $p>0.05$

Co in the soft tissues of green mussel with the mean concentration of 19.05±4.12 and 17.85±2.28, respectively. The observed concentration of Cu in the sample was within permissible level but higher than other sampling sites indicating the source of Cu in the Pekan was substantially higher than other sampling locations.

Lead (Pb) and Cadmium (Cd) were accumulated in lower concentration in the soft edible tissue of *Perna viridis* with the mean concentration range of 0.47±0.14 and 0.3±0.06, respectively. When compared with previous study, Pb and Cd concentration in the soft tissue of *P. viridis* from Pekan river was lower and within the Maximum Permissible Limit (MPL). It was evident from the present study that the concentration of essential metals was higher in the soft tissue than the non-essential metals. The order of metal accumulation in the soft tissue of *P. viridis* collected from Pekan, Pahang was Fe >Zn >Cu >Co >Pb >Cd in this present study (Table 2).

DISCUSSION

The green-lipped mussel, *Perna viridis,* is an established biomonitor of heavy metal contamination in the coastal waters of Asia-Pacific (Tanabe, 2000) and particularly in Malaysia (Yap *et al.*, 2003, 2006). So, the knowledge on heavy metal contamination in green lipped mussel would provide better understanding on how the environment gets polluted by various heavy metals. Heavy metals are also among the major environmental hazards due to their affinity for metal sensitive groups, such as thiol groups. It blocks the functional groups of proteins, displace and/or substitute essential metals, induce conformational changes, denature enzymes and disrupt cells and organelle integrity (Hall, 2002). The observed high concentration of Fe in the soft tissue of green mussel was clearly indicating the tendency of organism in accumulating higher concentration of Fe in soft tissue. This might also be due to the major role played by this essential metal in catalyzing various enzymatic activities. Higher concentration of Fe in the samples collected from Pekan indirectly signifies the source of Fe in Pekan was higher than other sampling locations. This might be probably due to the various anthropogenic sources plays a major role in increasing the concentration of Fe in Pekan river.

The uses of Zn as an anti corrosion agent and its ability to get speedy oxidation might enhanced the level of Zn in the Pekan river. Since, it is an essential precursor for various enzymatic activities, the organisms tend to accumulate high amount of Zn in its soft tissues. The low fluctuation level of Cu and Co in the soft tissues of green mussel clearly showed that the need of same level of Cu and Co to maintain the normal body functions of *Perna viridis*. Cu in the different soft tissues of the bivalve might be due to various mechanisms which included homoeostatic processes in the body in response to varying metabolic demands and entrapment of Cu under certain conditions by additional mucilage production/extrusion by the animal (Pyatt *et al*., 2003).

Cadmium is widely distributed at low level in the environment and most foods have an inherently low level of Cd which has been shown to bind to the protein and accumulate significantly in higher level (FDA, 2001). Ololade *et al*. (2008) reported that Cd level is almost 10 times higher in shell fishes than in finfishes. This could also be due to fertilizer application in nearby area are transported to the estuaries by leaching and erosion as agriculture is also an important activity of the village folks besides fishing. According to Li *et al*. (2006) bivalves do not regulate Cd usually accumulate this element. Hence, green mussel might not be able to regulate Cd in their body. When compared with previous study, Pb and Cd concentration in the soft tissue of *P. viridis* from Pekan river was lower and within the Maximum Permissible Limit (MPL).

The order of metal accumulation in the soft tissue of *P. viridis* collected from Pekan, Pahang was Fe >Zn >Cu >Co >Pb >Cd. Consumption of these heavy metal contaminated bivalves might result in bioaccumulation of toxic metals in the human system and may lead to adverse health effects (Bergback *et al*., 1992; Koller *et al*., 2004). It is also to be noted that the knowledge on the accumulation of heavy metal concentrations in the tissues of mussels is affected by a number of intrinsic and extrinsic factors. The extrinsic factors include spawning season (Lobel *et al*., 1991) and mussel size (Boyden, 1977; Williamson, 1980; Lobel *et al*., 1991; Riget *et al*., 1996). In previous studies, many authors reported negative relationships between body size and the accumulation of aquatic contaminants in suspension-feeding bivalves (Williamson, 1980; Amiard *et al*., 1986; Martincic *et al*., 1992). For instance, Williamson (1980) reported that higher levels of Cd, Pb and Zn were found in smaller individual and suggested that this might be due to the variations in the metabolic activities at different ages of the organisms. He also suggested that the increase in metabolic rates, in relation to different body sizes, might affect the uptake and elimination of metal. The same inverse relationships between the body size and heavy metal accumulation were also observed in *Penaeus stylirostris* (Paez-Osuna and Ruiz-Fernandez, 1995) and *Corbicula fluminea* (Bilos *et al*., 1998). The decrease in metal concentrations with body size was observed in various studies which indicated that a significant proportion of the metal content was surface-adsorbed as smaller mussels have a larger surface area to volume ratio (Jones *et al*., 2001).

CONCLUSION

As a conclusion, soft mussel tissue of *Perna viridis* is suitable to be used as a biomonitoring tool for heavy metal contamination due to its natural capacity to regulate and accumulate elevated concentration of various metals. This allows its use as a potential biomonitor of metal bioavailability in the coastal area since biomonitor facilitates comparison of metal bioavailability (Rainbow and Blackmore, 2001). The finding of the present study also revealed that the concentration of different heavy metals in the Pekan river was comparatively lower than other sampling locations (Yap *et al*., 2007a) except the concentration of Fe and Cu. This clearly indicates that various anthropogenic sources influence the concentration of various heavy metals in Pekan river. Detailed study on the bioaccumulation level of heavy metals by other bivalve species from Pekan river would substantially provide comprehensive information on their utility in bio-monitoring process.

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