

Concentrations of Heavy Metal in Different Parts of the Gastropod, *Faunus ater* (Linnaeus), Collected from Intertidal Areas of Peninsular Malaysia

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ABSTRACT

Marine gastropods, *Faunus ater* (Linnaeus), were collected from Pantai Sri Tujoh (Kelantan), Pantai Bisikan Bayu (Kelantan), Kg. Telaga Nenas (Perak) and Kesang Laut (Johor). Soft tissues of gastropods were dissected into digestive caecum (DC), foot, remainder, muscle, and operculum. The shell and dissected parts were analyzed for Cd, Cu, Ni, and Pb. It was found that the DC and the remainder accumulated high concentrations of Cu ranging between 159.1 and 290.2 µg/g dw. The shell was shown to highly accumulate non-essential Pb, Ni, and Cd compared to the soft tissues. Meanwhile, higher bioavailabilities of Cd and Cu were found in Pantai Sri Tujoh, whereas higher bioavailabilities of Ni and Pb were found in Pantai Bisikan Bayu compared to other sampling sites. The present results suggested that *F. ater* could be used as a potential biomonitor of heavy metal contamination. However, further studies are still needed in order to validate the use of *F. ater* as a good biomonitor of heavy metal pollution.

Keywords: Different parts, *Faunus ater*, heavy metals, Peninsular Malaysia

INTRODUCTION

Peninsular Malaysia is known to have a high diversity of marine molluscs. This advantage allows the advancement of biomonitoring studies, especially for heavy metal contamination in coastal areas. In its 2005-06 Malaysia Fisheries Directory, the Department of Fisheries Malaysia (2005) indicated that there are about 18 species of marine gastropods in Malaysian coastal areas. Apart from that, it is crucial to maintain the marine environment at pristine levels since extensive industrialization and urbanization have led to a strong risk of heavy metal contamination in many coastal environments around the world

(Tam and Yao, 1998), including Peninsular Malaysia. Malaysia's economic growth is rapidly increasing and this leads to the increment in the production and usage of toxic chemicals such as trace elements (Yap *et al.*, 2002) to the marine system of Malaysia. The west coast of Peninsular Malaysia is a principle repository for agricultural, industrial, and domestic wastes originating from land-based and sea-based activities (Shazili *et al.*, 2006). In order to obtain sustainable resources from coastal areas, the ecological distributions and the background densities of intertidal molluscs should first be determined. Besides, the well-established green-lipped mussel as a biomonitor of heavy

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metal pollution (Yap *et al.*, 2002: 2003: 2004: 2006), other intertidal gastropods should also be utilized for biomonitoring studies since a suite of intertidal biomonitors could reflect metal bioavailabilities and contaminations better (Rainbow *et al.*, 2002). In Malaysia, there is a line of different gastropods species which have been attempted to be used as good biomonitoring agents such as *Nerita lineata* (Amin, 2006b; Yap *et al.*, 2009a), *Telescopium telescopium* (Yap *et al.*, 2008a) and *Pomacea insularum* (Yap *et al.*, 2008b).

Locally known "Siput belitung", *Faunus ater* (Potamididae), is a brackish-water snail inhabiting mangrove areas. Studies on the distributions of *F. ater* in this region have only been reported in Thailand (Sri-aroon *et al.*, 2005; Sri-aroon *et al.*, 2006). The likely habitats of this mangrove snail may include water plants, leaf-filled surface depressions, log-mud interfaces, log and stone crevices, soil, sand or mud around roots and on leaves, stones and trunks of mangrove trees (Sri-aroon *et al.*, 2006). They are filter-feeders which use their gills to extract organic matter from the water in which they live (Yap *et al.*, 2009b).

The use of gastropods as biomonitor organisms offer several advantages (Goldberg, 1975). Firstly, gastropods have reasonable sizes for analysis and repeatable samplings. Secondly, they are sedentary or less mobile than any other organisms such as fishes, and thus accumulate contaminants more efficiently than that of the surrounding waters. Thirdly, gastropods exhibit low or undetectable enzyme activities which metabolize pollutants. Finally, some gastropods are important seafood or source of protein; thus, studying them has significant human health implications.

It is known that the ability of aquatic molluscs to accumulate heavy metals, in their different parts to elevated levels reaching concentrations which are much higher than those of the ambient water concentrations, makes these molluscs useful for heavy metal biomonitoring purposes (Phillips and Rainbow, 1994; Rainbow, 2002; Saha *et al.*, 2006). Besides, Rainbow

(1995) stated that the use of particular organisms as biomonitors of heavy metal bioavailabilities in coastal waters allows comparisons to be made over space and time for biomonitors provide integrated measures of the ecotoxicologically significant fraction of ambient metal in those waters. On the other hand, Rainbow *et al.* (2002) mentioned that a biomonitor could provide information on heavy metal bioavailabilities specific to that particular biomonitor. However, it is usually considered valid to extrapolate from that biomonitor to draw conclusions about heavy metal bioavailabilities of the site in general. *F. ater* could probably act as a potential biomonitor of heavy metal bioavailabilities in the sites undertaken in this study.

The reliability of gastropods as a biomonitor of heavy metal contaminations has been revealed by a number of researchers. Among other, Liang *et al.* (2004), who conducted a study in the Chinese Bohai Sea, found that *Rapana venosa* accumulated a high level of Cd. The ability of *Patella caerulea* to accumulate heavy metals was revealed in the study conducted by Hamed and Emara (2006) in the Gulf of Suez, Red Sea. The studies of heavy metals in *Patella caerulea* and *Mullus barbatus* in the Ionian Sea, Italy were done by Storelli and Marcotrigiano (2005). These studies strongly supported the use of gastropods as biomonitors of heavy metal pollution in the marine environments. Meanwhile, suggestions on the monitoring of heavy metal contaminations and bioavailabilities, using the different parts of gastropods, were also very interesting. The strategy may overcome the inaccuracy caused by determining the heavy metal level in the total soft tissues. In addition, the spawning season of the gastropods and environmental factors may contribute to the wide variability of heavy metal concentrations in the total soft tissues of molluscs (Yap *et al.*, 2006).

Since there have been no reported studies on heavy metals in *F. ater* from Malaysia, the present study focused on the work done using four geographical populations of *F. ater* in Peninsular Malaysia and the heavy metal distributions in the different parts of *F. ater*.

MATERIALS AND METHODS

Samplings were conducted from June to September 2007 in Pantai Seri Tujoh and Pantai Bisikan Bayu in the eastern part of Peninsular Malaysia and in Kg. Telaga Nenas and Kesang Laut in the western coast (Fig. 1). Further details of the sampling sites are given in Table 1. The samples of *F. ater* (Linnaeus) were identified for their family, genus, and species with the aid of the identification keys proposed by Uptham *et al.* (1983), Brandt (1974) and Van Benthem Jutting (1956).

Approximately 20 snails, with sizes ranging from 3.82 - 6.79 cm, were collected from each sampling site. The samples were brought back to the laboratory and were stored at -10 °C until further analysis. Prior to the analyses, the soft tissues of the snails (besides the shells) were carefully dissected and pooled into five different parts, namely remainder, operculum, muscle, foot, and digestive caecum (DC). The samples were then dried in an oven for 72 hours at 105 °C to constant dry weights (Mo and Neilson, 1994).

The dried shell and soft tissue parts were weighed and placed in acid-washed digestion tubes. 10 ml of concentrated nitric acid (AnalaR grade, BHD 69%) was added into the digestion tube for digestion. The samples were placed in

a digestion block at 40 °C for 1 hour and they were fully digested at 140 °C for 3 hours after that (Yap *et al.*, 2006). The cooled samples were diluted to 40 ml with double deionised water (DDW). The digested samples were then filtered through Whatman No. 1 (filter speed: medium) filter paper into acid-washed pill boxes. The samples were analyzed for Cd, Cu, Ni, and Pb using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkim-Elmer™ Model 800. Standard solutions were prepared from 1000 ppm stock solutions provided by MERCK Titrisol for Cd, Cu, Ni, and Pb.

In order to avoid possible contamination, all the glassware and equipment used were acid-washed. Procedural blanks and quality control samples made from standard solutions for Cd, Cu, Ni, and Pb were analyzed after every 5 - 10 sample in order to check for the sample accuracy. The percentages of recoveries for the heavy metal analyses were acceptable at 80 - 110 %. The analytical procedures for the gastropod were checked with the Certified Reference Material (CRM) for dogfish liver (DOLT-3, National Research Council Canada). The recoveries of all the metals were satisfactory (Table 2).

One-way ANOVA-Student-Newman-Keuls (S-N-K) was applied to detect significant differences among the mean values using the statistical software, SPSS version 12.

TABLE 1
Locations, sampling dates, number of samples analyzed (N), longitude, latitude and descriptions of sampling sites of *Faunus ater* collected from Peninsular Malaysia

No	Location	Sampling date	N	Longitude	Latitude	Description of sampling site	Individual size, (cm)
1	Pantai Sri Tujoh	29 July 2007	20	5°52'N	102°30'E	Aquacultural area and recreational beach	4.83-6.79
2	Pantai Bisikan Bayu	03 July 2007	20	06°13'N	102°07'E	Aquaculture area	3.82-5.20
3	Kg. Telaga Nenas	25 August 2007	20	4°27'N	100°37'E	A fishing village	3.98-6.78
4	Kesang Laut	15 September 2007	20	2°10'N	102°34'E	A fishing village	4.33-6.71

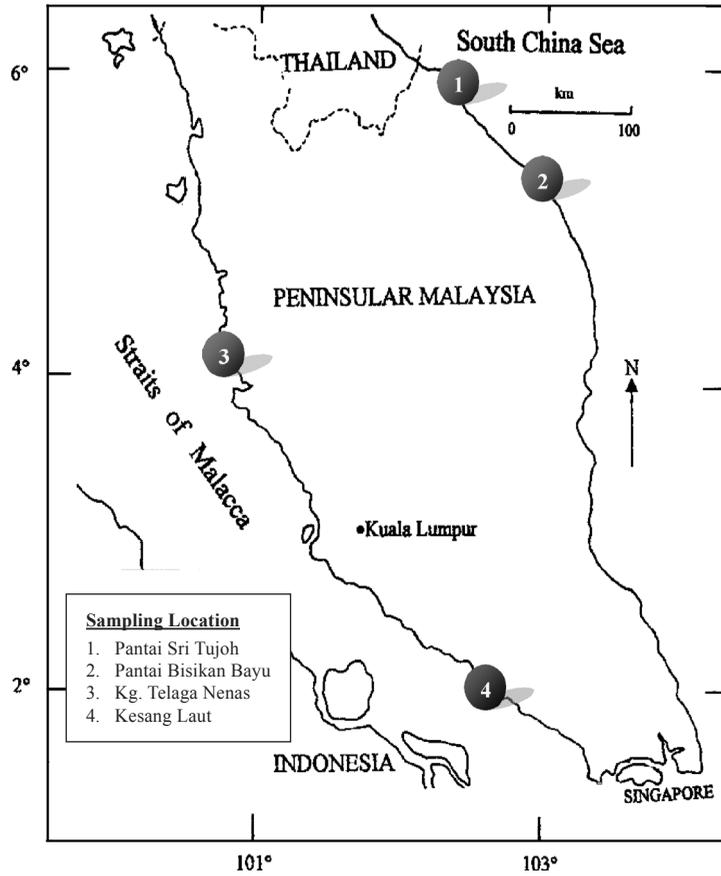


Fig. 1: Map showing sampling sites of *Faunus ater* in the East and West Coast of Peninsular Malaysia

TABLE 2
Analytical results for the Certified Reference Material (CRM) and the certified values for each metal (All values are presented $\mu\text{g/g}$ dry weight)

Metal	Sample	CRM values	Measured values	Percentage of recovery
Cd	DOLT-3 Dogfish-liver	19.4 ± 0.600	20.5 ± 0.439	106 ± 2.26
Cu	DOLT-3 Dogfish-liver	31.2 ± 1.00	26.5 ± 2.58	85.0 ± 8.28
Ni	DOLT-3 Dogfish-liver	2.72 ± 0.350	2.77 ± 0.741	102 ± 27.2

NA: Pb value is not available

RESULTS AND DISCUSSION

Heavy Metals Distribution in the Tissues of F. ater

The heavy metal concentrations in the different parts of *F. ater* from the four sampling sites are shown in Table 3. It was found that the concentrations of Cu in DC and the remainder are in higher range (159.1-290.2 µg/g dw) than the shell of *F. ater*, (9.28-18.5 µg/g dw). On the other hand, the shell was the main tissue for the accumulation of non-essential heavy metals such as Pb, Ni, and Cd. The heavy metal concentrations found in the shell were in the range of 54.2-65.7, 24.5-31.5, and 4.97-5.52 µg/g dw for Pb, Ni and Cd, respectively.

The differences in the affinities of the metals to the binding sites of the metallothioneins in the different tissues (Roesijadi, 1980; Viarengo *et al.*, 1985; Rainbow, 2002; Yap *et al.*, 2009b) could cause the different heavy metal levels found in the gastropods. Besides, the function or the location of a specific organ in the gastropod could also be associated with the heavy metal accumulations in the different tissues (Rainbow, 2002). The heavy metals found in the shell could be explained on the basis of calcification in molluscs occurring within the extrapallial fluid, which is secreted by the mantle. The composition of the extrapallial fluid might be significantly altered with respect to seawater due to the influence of mantle metabolic activity on the transport of metals through the mantle (Klein *et al.*, 1996), to the contributions of metals and carbon from metabolic source (Tanaka *et al.*, 1986; Klein *et al.*, 1996) or to organic complexation (Crenshaw, 1972). Additionally, heavy metals are not necessarily incorporated into the calcite crystal structure but they can also be adsorbed onto the skeletal organic matrix (Lingard *et al.*, 1992) or entrapped as a separate mineral phase (Fritz *et al.*, 1990). Consequently, shells may provide a more realistic indication of the degree of contamination for shell to exhibit less variability (unlike soft tissues due to seasonal changes), integrate elemental concentrations over the life of the animal (Foster and Cravo, 2003; Cravo *et al.*, 2002) and the

shells also act as a biodeposition site of unwanted chemical species such as heavy metals (Yap *et al.*, 2003).

Heavy Metal Bioavailability in the Sampling Sites

Heavy metal bioavailability of the sampling locations was estimated using the concentrations in the different parts of *F. ater* (Figs. 2-7). It was assumed that high bioavailabilities of Cd and Cu were found in Pantai Sri Tujoh based on the elevated concentrations of these metals exhibited in the different parts such as the foot, muscle, remainder, DC, and shell. High bioavailabilities of Ni and Pb were found in Pantai Bisikan Bayu as exhibited in the similar tissues. According to Rainbow *et al.* (2002), the accumulated concentrations in a biomonitor are a direct reflection of the total integrated bioavailability and contamination of the sampling sites. This is because biomonitors such as molluscs bioaccumulate metals in their tissues in proportion to the degree of environmental contamination from seawater, suspended particles, and sediments and through food chains (Louma, 1983; Blackmore, 2001). Therefore, the comparisons of such accumulated concentrations in a biomonitor among sites are measurements of the bioavailabilities and contaminations of heavy metals of the sampling sites (Phillips and Rainbow, 1994).

In addition, the characteristics (anthropogenic activities) of the sampling locations could also contribute to the heavy metal bioavailabilities and contaminations of these areas. Based on the data presented in Table 1, it is known that all the four places are well-known as aquacultural areas and fishing villages. Therefore, it is suggested that the heavy metal bioavailabilities of these four sampling locations could be due to the anthropogenic activities found in these areas besides natural sources. There was a study reported in the literature which mentioned that heavy metal such as Pb contaminations originated from aquacultural activities and fishing villages (Yap *et al.*, 2002). Moreover, the organic wastes discharged from fish/or mussel

TABLE 3
Decreasing order of heavy metal ($\mu\text{g/g}$) concentrations in the different parts of *Faunus ater*

Metal	Site	Metal distribution in the different parts																																		
Cu	Pantai Sri Tujoh	226.43 \pm 4.38 DC	177.20 \pm 10.37 Foot	153.08 \pm 5.47 Remainder	122.97 \pm 1.18 Muscle	37.83 \pm 9.74 Operculum	18.47 \pm 3.18 Shell	Pantai Bisikan Bayu	193.86 \pm 10.52 Remainder	138.14 \pm 9.35 Foot	127.01 \pm 2.58 DC	104.85 \pm 6.16 Muscle	94.64 \pm 15.09 Operculum	9.28 \pm 1.54 Shell	Kg. Telaga Nenas	159.09 \pm 3.16 DC	144.38 \pm 4.88 Remainder	139.03 \pm 0.87 Foot	115.48 \pm 10.67 Muscle	51.84 \pm 3.54 Operculum	11.66 \pm 1.80 Shell	Kesang Laut	290.16 \pm 9.17 Remainder	211.79 \pm 3.18 DC	163.98 \pm 2.41 Foot	146.91 \pm 13.68 Muscle	32.61 \pm 0.68 Operculum	9.65 \pm 0.82 Shell								
	Pb	Pantai Sri Tujoh	61.31 \pm 1.57 Shell	27.30 \pm 8.55 Operculum	24.78 \pm 1.23 Remainder	15.50 \pm 2.07 DC	7.59 \pm 1.34 Foot		8.88 \pm 1.23 Muscle	Pantai Bisikan Bayu	65.66 \pm 3.68 Shell	26.60 \pm 4.75 DC	15.62 \pm 4.95 Remainder	11.52 \pm 2.62 Muscle		5.36 \pm 0.45 Foot	4.80 \pm 1.79 Operculum	Kg. Telaga Nenas	54.22 \pm 3.37 Shell	18.08 \pm 0.23 Operculum	14.69 \pm 2.20 Remainder		3.57 \pm 1.46 DC	3.39 \pm 0.48 Muscle	1.04 \pm 0.02 Foot	Kesang Laut	57.80 \pm 1.50 Shell	31.64 \pm 0.97 Operculum	18.87 \pm 2.80 Remainder	6.68 \pm 0.48 DC	2.89 \pm 0.84 Foot	2.75 \pm 1.35 Muscle				
		Ni	Pantai Sri Tujoh	26.42 \pm 1.42 Shell	7.19 \pm 1.25 DC	7.01 \pm 0.82 Remainder	3.41 \pm 0.45 Operculum		2.26 \pm 0.73 Muscle		0.34 \pm 2.00 Foot	Pantai Bisikan Bayu	31.49 \pm 2.21 Shell	10.29 \pm 1.31 DC		9.77 \pm 1.10 Remainder	6.77 \pm 0.37 Foot		5.35 \pm 0.29 Muscle	1.62 \pm 0.55 Operculum	Kg. Telaga Nenas		26.53 \pm 1.94 Shell	11.05 \pm 2.85 Operculum	9.86 \pm 1.64 Remainder		8.59 \pm 0.33 DC	1.70 \pm 0.66 Muscle	0.63 \pm 0.20 Foot	Kesang Laut	24.53 \pm 1.23 Shell	4.82 \pm 1.75 Remainder	2.62 \pm 0.13 Operculum	1.81 \pm 0.62 Muscle	0.64 \pm 1.69 DC	0.11 \pm 0.05 Foot

Concentrations of Heavy Metal in Different Parts of the Gastropod

Cd	Pantai Sri Tujoh	5.52 ± 0.13	2.58 ± 0.25	1.57 ± 0.11	1.40 ± 0.14	0.86 ± 0.11	0.74 ± 0.24
		Shell	Remainder	DC	Muscle	Foot	Operculum
	Pantai Bisikan Bayu	5.11 ± 0.08	2.09 ± 0.05	1.20 ± 0.16	0.77 ± 0.11	0.72 ± 0.23	0.11 ± 0.03
		Shell	Remainder	DC	Muscle	Foot	Operculum
	Kg. Telaga Nenas	4.99 ± 0.19	2.26 ± 0.15	1.40 ± 0.12	1.29 ± .19	0.69 ± 0.09	0.55 ± 0.10
		Shell	Remainder	DC	Operculum	Muscle	Foot
	Kesang Laut	4.97 ± 0.19	1.53 ± 0.14	1.11 ± 0.06	0.77 ± 0.23	0.57 ± 0.06	0.32 ± 0.06
		Shell	Remainder	Operculum	DC	Muscle	Foot

Note: DC = Digestive caecum

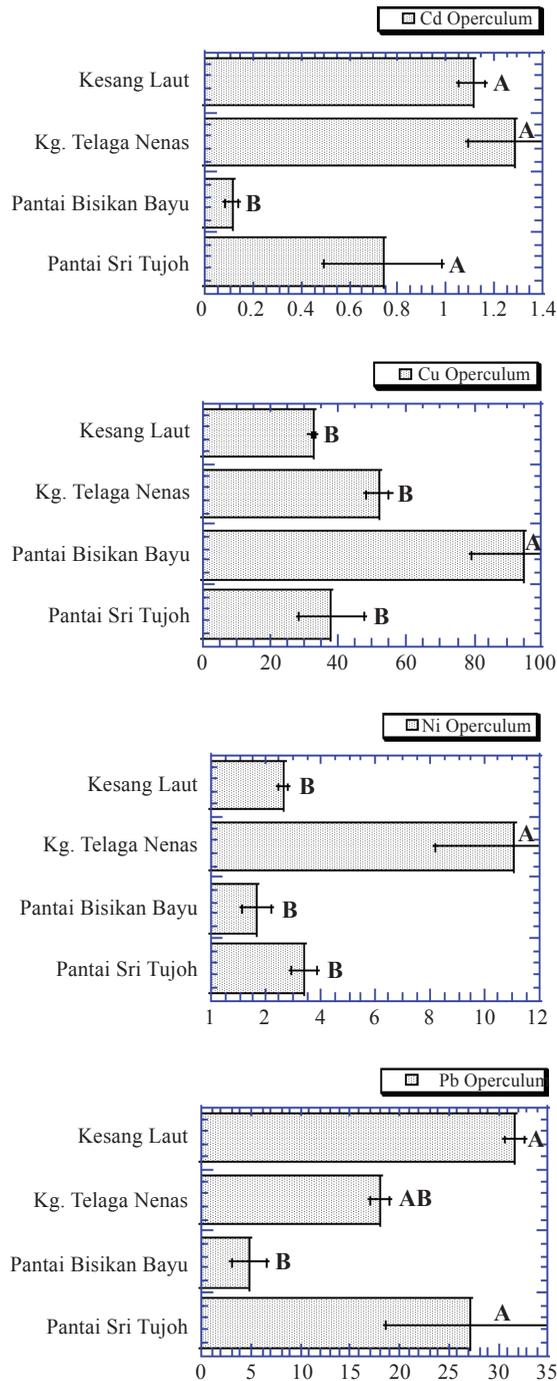


Fig. 2: Heavy metal concentration (mean $\mu\text{g/g dw} \pm \text{S.E.}$ (n=3)) in the operculum of *Faunus ater* collected from the East and West Coasts of Peninsular Malaysia

Note: Students-Newman-Keuls (SNK) comparisons of metal levels in different soft tissues and shell of *F. ater*; Means with different letters are significantly different, $P \leq 0.05$

Concentrations of Heavy Metal in Different Parts of the Gastropod

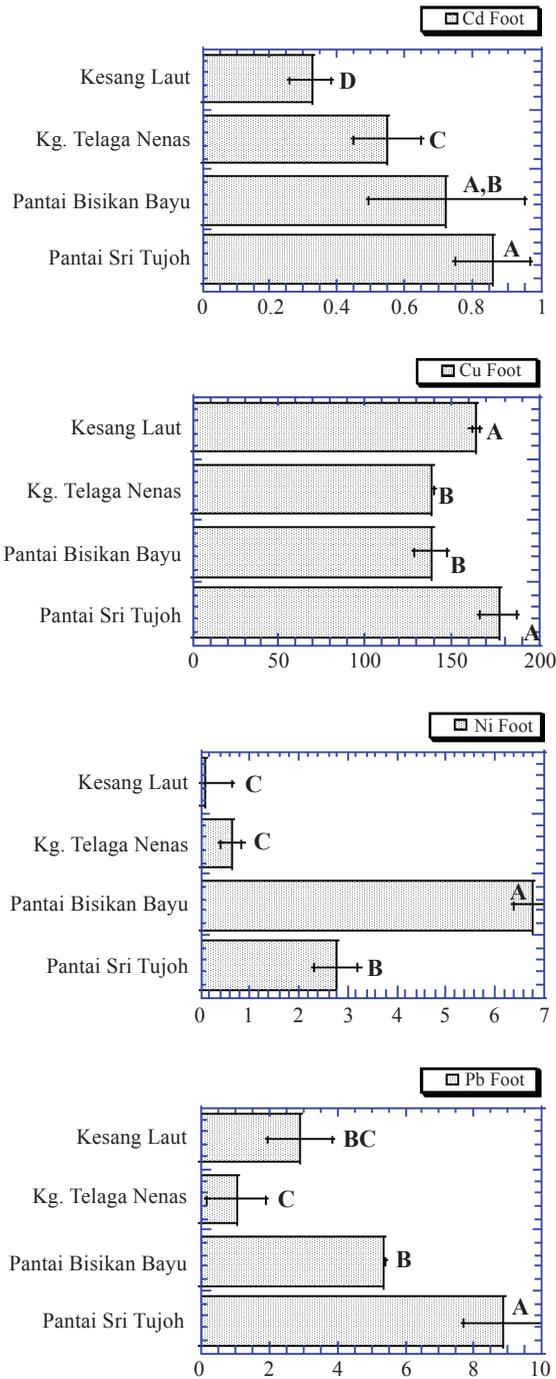


Fig. 3: Heavy metal concentration (mean $\mu\text{g/g dw} \pm \text{S.E.}$ (n=3)) in the foot of *Faunus ater* collected from the East and West Coasts of Peninsular Malaysia

Note: Students-Newman-Keuls (SNK) comparisons of metal levels in different soft tissues and shell of *F. ater*; Means with different letters are significantly different, $P \leq 0.05$

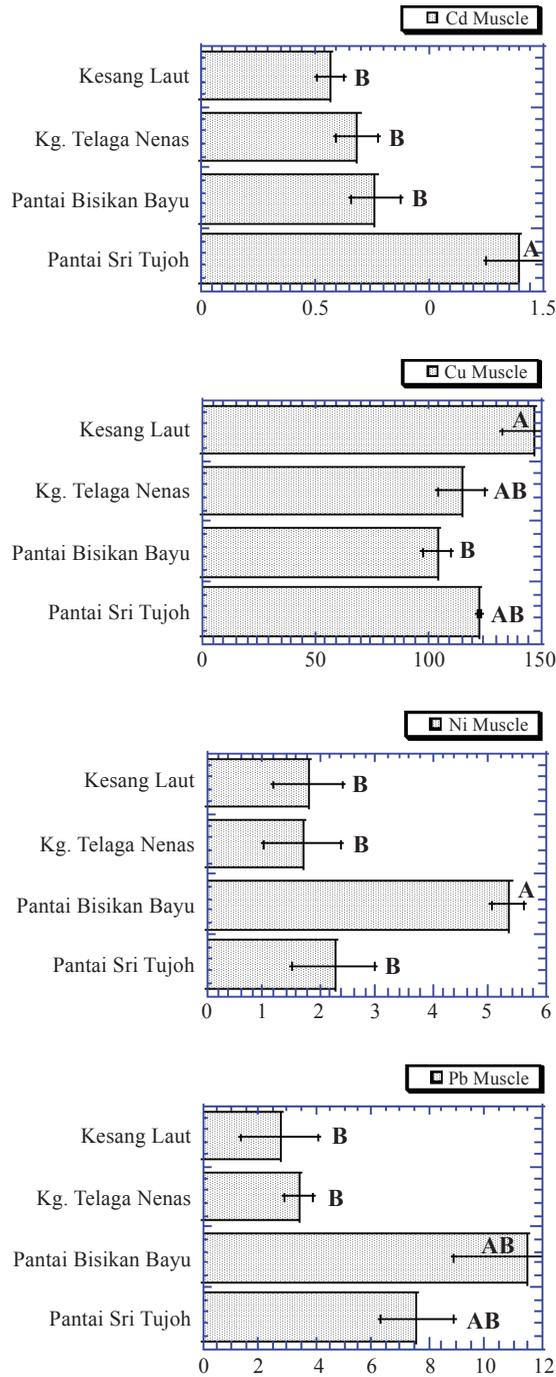


Fig. 4: Heavy metal concentration (mean $\mu\text{g/g dw} \pm \text{S.E.}$ (n=3)) in the muscle of *Faunus ater* collected from the East and West Coasts of Peninsular Malaysia

Note: Students-Newman-Keuls (SNK) comparisons of metal levels in different soft tissues and shell of *F. ater*; Means with different letters are significantly different, $P \leq 0.05$

Concentrations of Heavy Metal in Different Parts of the Gastropod

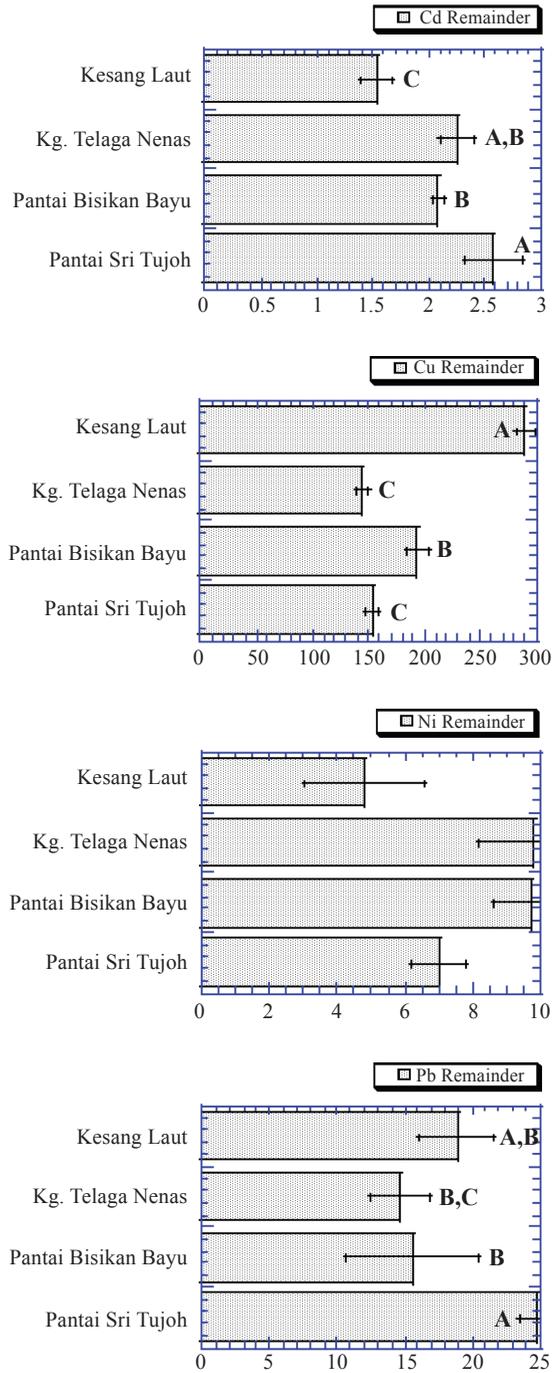


Fig. 5: Heavy metal concentration (mean $\mu\text{g/g dw} \pm \text{S.E.}$ ($n=3$)) in the remainder of *Faunus ater* collected from the East and West Coasts of Peninsular Malaysia

Note: Students-Newman-Keuls (SNK) comparisons of metal levels in different soft tissues and shell of *F. ater*; Means with different letters are significantly different, $P \leq 0.05$

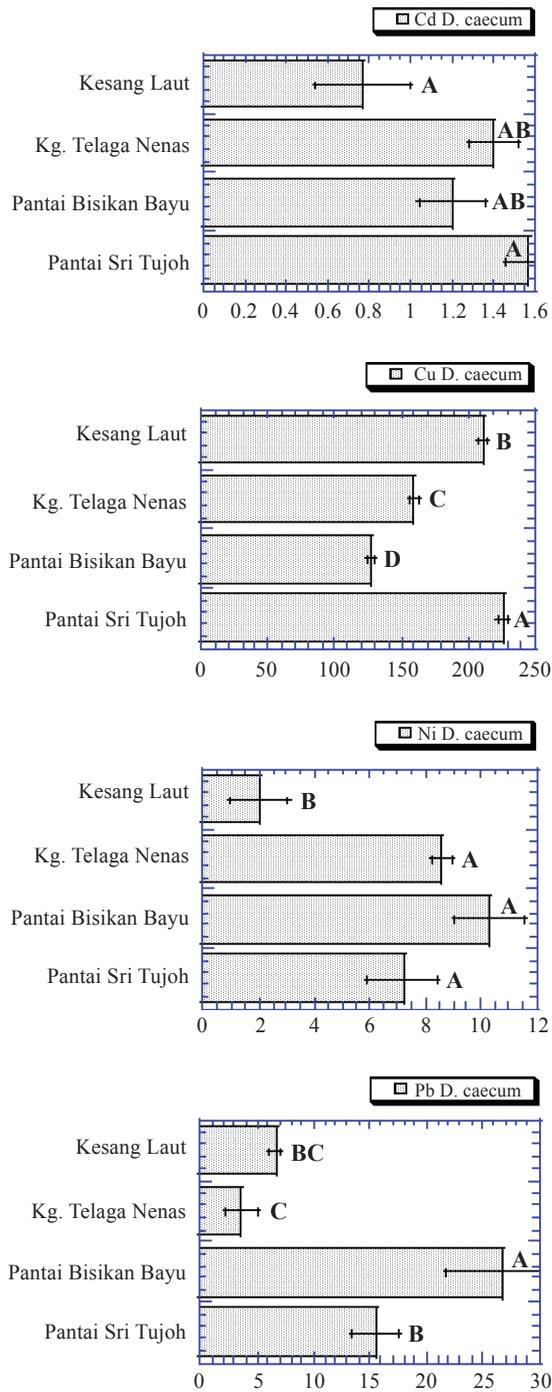


Fig. 6: Heavy metal concentration (mean µg/g dw ± S.E. (n=3)) in the digestive caecum of *Faunus ater* collected from the East and West Coasts of Peninsular Malaysia

Note: Students-Newman-Keuls (SNK) comparisons of metal levels in different soft tissues and shell of *F. ater*; Means with different letters are significantly different, $P \leq 0.05$

Concentrations of Heavy Metal in Different Parts of the Gastropod

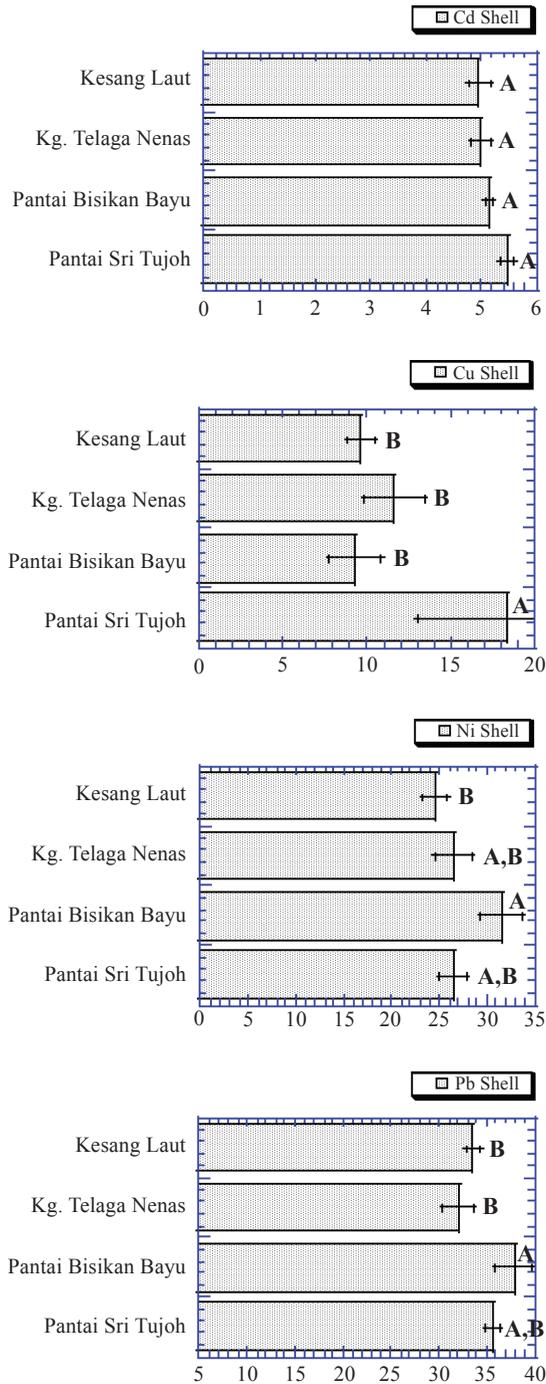


Fig. 7: Heavy metal concentration (mean $\mu\text{g/g dw} \pm \text{S.E.}$ ($n=3$)) in the shell of *Faunus ater* collected from the East and West Coasts of Peninsular Malaysia

Note: Students-Newman-Keuls (SNK) comparisons of metal levels in different soft tissues and shell of *F. ater*; Means with same letters are not significantly different, $P \geq 0.05$

farms were found to have impacts on the water quality around fish culture zones (Wu *et al.*, 1994; Yap *et al.*, 2003) which could contribute to the bioavailabilities and contaminations of the metals. The heavy metal concentrations in *F. ater* were slightly higher for Cd, Cu, and Ni and within the range for Pb as compared to other species in Malaysia (Amin, 2006b; Yap *et al.*, 2008a; Yap *et al.*, 2008b; Yap *et al.*, 2009a).

CONCLUSIONS

From the present findings, it was concluded that the DC and remainder of *F. ater* accumulated high concentrations of Cu. The shell was accumulative of the non-essential metals Pb, Ni and Cd. On the other hand, bioavailabilities of Cd and Cu were found to be high in Pantai Sri Tujoh, whereas high bioavailabilities of Ni and Pb were found in Pantai Bisikan Bayu. The results gathered in the present study suggested that *F. ater* could be used as a potential biomonitor of heavy metal contamination in the intertidal area of Peninsular Malaysia and as an alternative biomonitor mollusc to the well-established green-lipped mussel *P. viridis*.

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