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DISTRIBUTION OF HEAVY METAL CONCENTRATIONS (Cd, Cu, Ni, Fe and Zn) IN THE DIFFERENT SOFT TISSUES AND SHELLS OF WILD MUSSELS *Perna viridis* COLLECTED FROM BAGAN TIANG AND KUALA KEDAH

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ABSTRACT

Concentrations of Cd, Cu, Ni, Fe and Zn were determined in the *Perna viridis* populations collected in April 2005 from Bagan Tiang and Kuala Kedah. The ranges of Cd, Cu and Zn found in this study were lower than the maximum permissible limits set by Malaysian Food Regulations 1985 and other established guidelines. Therefore, the consumption of wild mussels collected from Bagan Tiang and Kuala Kedah should pose no acute toxicological risks of Cd, Cu and Zn to humans. Despite the apparently low concentrations of Cd, Cu, Fe, Ni and Zn in the different soft tissues of *P. viridis* collected from Bagan Tiang and Kuala Kedah, from a public health risk perspective, the data suggested the need for continued monitoring of heavy metal pollution in the mussels.

ABSTRAK

Kepekatan Cd, Cu, Ni, Fe dan Zn dalam populasi *Perna viridis* yang disampel pada April 2005 di Bagan Tiang dan Kuala Kedah telah ditentukan. Julat bagi Cd, Cu dan Zn yang dikaji didapati adalah lebih rendah berbanding had maksimum yang dibenarkan sepertimana yang telah ditetapkan dalam Peraturan-Peraturan Makanan Malaysia 1985 dan garis panduan lain. Oleh itu, pengambilan kupang liar yang dikutip dari Bagan Tiang dan Kuala Kedah seharusnya tidak memberikan risiko toksikologi Cd, Cu dan Zn yang merbahaya terhadap manusia. Meskipun kandungan Cd, Cu, Fe, Ni dan Zn dalam pelbagai bahagian tisu lembut *P. viridis* dari Bagan Tiang adalah rendah, namun dari sudut perspektif risiko kesihatan orang ramai, data menunjukkan bahawa pemonitoran berterusan terhadap pencemaran logam berat dalam kupang amat diperlukan.

Keywords: Heavy metals, *Perna viridis*, Bagan Tiang, Kuala Kedah

INTRODUCTION

In 2002, Bagan Tiang was one of the mussel aquacultural sites in the northern part of Peninsular Malaysia. Most of the collected green-lipped mussel *Perna viridis* from the offshore of Bagan Tiang were put in gunny bags and transported to the Bagan Tiang Jetty. These mussels were known to be grown in the wild.

When the background of heavy metal concentrations in *P. viridis* from Peninsular Malaysia was reported by Yap *et al.* (2003a), this has signified the importance of Mussel Watch Approach, as suggested by Goldberg (1985), for biomonitoring the heavy metal pollution in the coastal waters in Malaysia. This is because of coastal pollution has become a problem for local management authority where estuaries and coastal marine waters are ultimately the repositories of contaminants from anthropogenic

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activities (Ong and Din, 2001). Therefore, a continued study on the similar biomonitoring work should be done regularly.

Concentrations of metals in sediments may be difficult to interpret, as only those portion of metals (e.g. adsorbed on surfaces or associated with soluble phases) which are potentially bioavailable (Campbell *et al.*, 1988) are of ecotoxicological significance. Therefore, when considering the impact of metals on biota, measurement of total metal concentrations in sediment can be misleading because total concentrations will include non-reactive metal species. To overcome the above problems, the use of marine mussels are preferred since the concentrations found in the soft tissues of mussels can provide a time-integrated measurement of metal pollution apart from contamination and bioavailability of heavy metals in the coastal water (Nelson *et al.*, 1995; Yap *et al.*, 2006d). Mussels can readily accumulate metals in coastal ecosystems by direct transport of water across gills, and from ingestion of suspended particles and bottom sediments. The effectiveness of this mussel species has also been tested positively by looking at the recommended criteria for a good biomonitor for the Malaysian coastal waters (Yap *et al.*, 2002, 2003, 2004, 2005, 2006a, b, c, d). They are sedentary, long-lived, widely distributed and tolerant of high trace metal concentrations. As a result, their metal body burdens can reflect the contamination history of a certain coastal environment (Phillips, 1977).

The use of *P. viridis* as a biomonitor of heavy metals is well established in Malaysia (Yap *et al.*, 2002, 2003, 2004, 2005, 2006a, b, c, d), apart from Asia-Pacific coastal waters such as Thailand and Hong Kong (Tanabe, 2000; Nicholson and Lam, 2005). The objective of this study was to determine the concentrations of Cd, Cu, Fe, Ni, and Zn in the different tissues of *P. viridis* collected from Bagan Tiang and Kuala Kedah.

MATERIALS AND METHODS

Mussels were collected in April 2005 from Bagan Tiang [Perak] and Kuala Kedah on the west coast of Peninsular Malaysia (Fig. 1). Sites and sampling dates for each mussel population are given in Table 1. About 25 similar sized mussels of male and female from Bagan Tiang and Kuala Kedah (Table 1) were selected for analysis. The soft tissues from the mussels were dissected by removing the byssus and the shell. Apart from analyzing the total soft tissues, the mussel population from Bagan Tiang were carefully dissected by gender into different parts namely mantle, muscle, gonad, foot, gills, crystalline style, byssus and remainder (visceral mass) (Fig. 2).

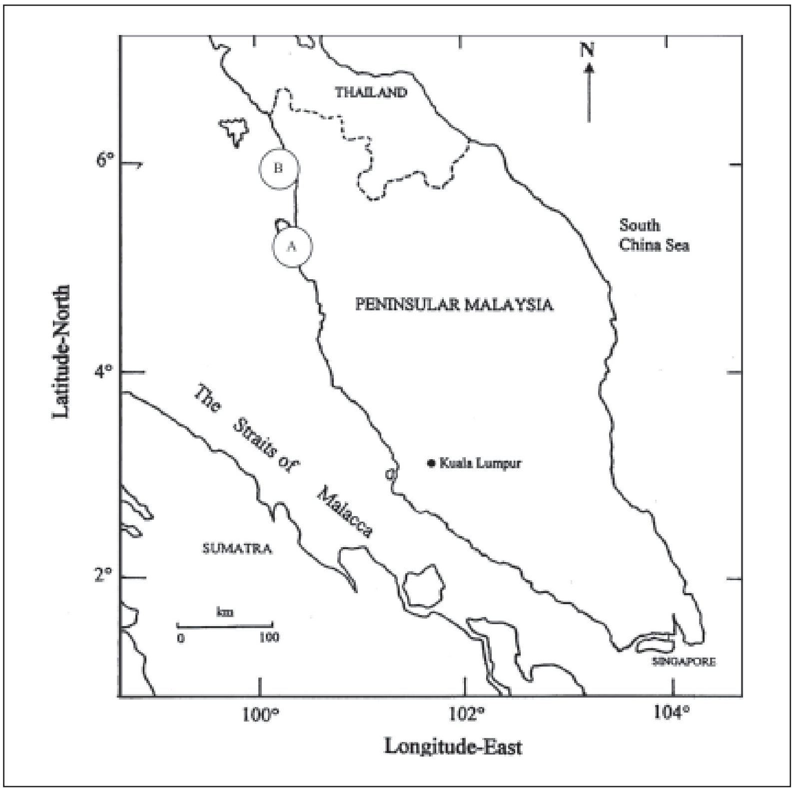

Fig. 1. Sampling sites of *Perna viridis* at Bagan Tiang (A) and Kuala Kedah (B) on the west coast of Peninsular Malaysia.

Table 1. Sites and sampling dates of *Perna viridis*

No.	Location	Sampling date	Longitude-N	Latitude-E	Description of sampling site
1	Bagan Tiang (offshore), Perak	19 April 2005	05°08.517'	100°22.459'	An offshore, fishes and mussels aquacultural area
2	Kuala Kedah, Kedah	20 April 2005	06°06.333'	100°17.149'	A jetty and fishing village A fish landing site.

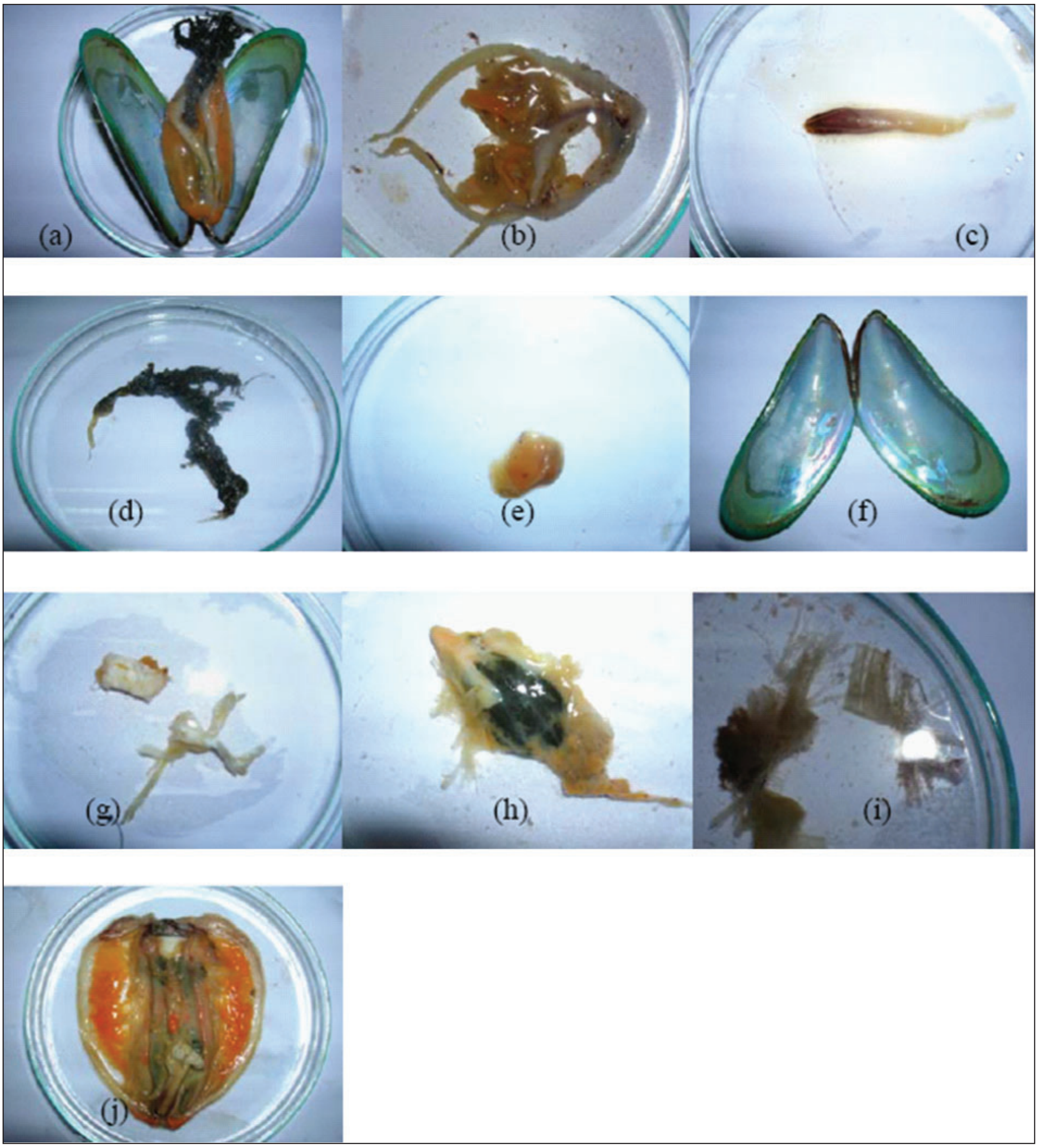

Fig. 2. Different parts in an individual of *Perna viridis* showing the muscle scar (a), mantle (b) foot (c), byssus (d) gonad (e), shell and periostracum (f), muscle (g), remainder (h), gills (i) and total soft tissue (j).

However, for the Kuala Kedah population, since the mussel size was too small to be dissected into different parts, only total soft tissues [combining male and female individuals] of the mussels were analysed for metal concentrations.

All samples were placed into aluminum foil and were oven-dried at 60ºC until constant dry weights (Mo and Neilson, 1994). The dried soft tissues were digested in concentrated HNO_3 (AnalaR grade, BDH 69%). They were placed in a hot-block digester first at low temperature for one hour and then were fully digested at high temperature (140ºC) for at least 3 hours (Yap *et al.*, 2003a). The digested samples were then diluted to 40 mL with double distilled water (DDW). After filtration, heavy metals were determined by an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model AAnalyst 800. The data were presented in mg/g dry weight. The dry weight was converted into wet weight (ww) by using a conversion factor of 0.17 ± 0.04 (Yap *et al.*, 2003a). The latter was then used for comparative purposes. To avoid possible contamination, all glassware and equipment used were acid-washed and the accuracy of the analysis was checked against blanks. Procedural blanks and quality control samples made from the standard solutions for Cd, Cu, Fe, Ni, and Zn were analysed in every five samples in order to check for sample accuracy. Percentage recoveries for heavy metal analyses were between 80-110%.

For the statistical analysis, T-test between any two variables was conducted by using the STATISTICA software package.

RESULTS AND DISCUSSION

Table 2 shows the allometric parameters of *P. viridis* analysed in the present study. The water content found in the present study [78.7-81.3%] and shell thickness [0.50-5.67g/cm^2] were comparable to those reported by Yap *et al.* (2003d) for Peninsular Malaysia mussels collected in 1998-2002 [water content: 81.8-85.4%; shell thickness: 0.41-0.70g/cm]. However, it should be noted that the size [indicated by shell length, shell width, shell height and shell thickness] was significantly higher (P <0.05) in the Bagan Tiang mussels than those in the Kuala Kedah population.

Table 3 shows concentrations of Cu and Zn in the total soft tissues and shells of *P. viridis* are not significantly different (P <0.05) between Kuala Kedah and Bagan Tiang populations. They ranged from 81.7 to 106.6 µg/g dw [soft tissue] and 3.29 to 5.04 µg/g dw [shells] for Zn, and 12.61 to 15.0 µg/g dw [soft tissue] and 7.26 to 8.64 µg/g dw [shells] for Cu. These Cu and Zn concentrations are within those reported by Yap et al. (2000a) [Cu: 6.31-20.10 µg/g dw; Zn: 53.82-128.90 µg/g dw] for the total soft tissues of *P. viridis* collected from 20 sites in the west coast of Peninsular Malaysia, and comparable those to reported by Nicholson and Szefer (2003) [Cu: 10.1-18.0 µg/g dw; Zn: 104-152 µg/g dw] for the total soft tissues of *P. viridis* collected from 2 sites in Hong Kong coastal waters.

For Cd, the concentration was significantly (P< 0.05) higher in the total soft tissues of Kuala Kedah mussel [2.90 µg/g dw] than in the Bagan Tiang mussels [0.547 µg/g dw] but the Cd level in the shells from Bagan Tiang mussels [10.4 µg/g dw] was higher than in the Kuala Kedah mussels [8.13 µg/g dw]. The Cd levels from the two populations are lower than those reported by Nicholson and Szefer (2003) [Cd: 1.02-5.42 µg/g dw] for the total soft tissues of *P. viridis* collected from 2 sites in Hong Kong coastal waters while comparable to those reported by Yap *et al.* (2003a) [Cd: 0.25-1.25 µg/g dw] for the total soft tissues of *P. viridis* collected from 20 sites in the west coast of Peninsular Malaysia.

For Ni and Fe, the soft tissues of Bagan Tiang mussels recorded significantly (P< 0.05) higher levels of Ni [11.42 µg/g dw] and Fe [1271.2 µg/g dw] than those [Ni: 4.34 µg/g dw; Fe: 358.2 µg/g dw]) in the Kuala Kedah mussels but the in the shells, the concentrations of Ni and Fe were higher in the Kuala Kedah mussels than in the Bagan Tiang mussels. The Ni levels from the two populations are within those reported by Nicholson and Szefer (2003) [Ni: 2.84-13.4 µg/g dw]. For Fe, the level from Kuala Kedah is comparable to those reported by Nicholson and Szefer (2003) [Fe: 97.2-354 µg/g dw] but the Fe level from Bagan Tiang is higher than that reported by Nicholson and Szefer (2003).

The distributions of heavy metal concentrations in the different soft tissues, periostracum and shells of *P. viridis* are shown in Fig. 3 and 4. Between the soft and hard tissues, higher concentrations of Cd and Ni are found in the shells of the mussels. The higher levels of Cd found in the mussel shells than in the soft tissues confirmed the previous study done by Yap *et al.*, (2003b). These non-essential metals had more binding sites in the crystalline lattices of the hard carbonate tissues (Yap *et al.*, 2003b) while the metals in the soft tissues were bound to metallothionein (Variego *et al.*, 1985). However, the high level of Ni found in the shells is unknown and difficult to explain to our knowledge.

Elevated levels of Cu and Fe were found in the byssus while the crystalline style (CS) recorded the highest level of Cu. This agreed with those

Table 2. Allometric parameters of *Perna viridis* analysed in this study. N= 20

Kuala Kedah (Female)	Mean	Std error	Minimum	Maximum
Shell length (cm)	0.08	0.01	0.03	0.22
Shell height (cm)	1.00	0.05	0.54	1.35
Shell width (cm)	3.28	0.07	2.64	3.65
Dry total soft tissue weight (g)	1.69	0.03	1.35	2.00
Dry shell weight (g)	1.12	0.03	0.89	1.28
Water content (%)	78.69	2.35	34.67	86.27
Shell thickness (g/cm^2)	0.51	0.03	0.28	0.70
Condition index (g/cm^3)	8.74	0.95	4.18	23.74
Kuala Kedah (Male)	**Mean**	**Std Error**	**Minimum**	**Maximum**
Shell length (cm)	0.08	0.00	0.05	0.13
Shell height (cm)	0.98	0.04	0.51	1.37
Shell width (cm)	3.23	0.05	2.67	3.60
Dry total soft tissue weight (g)	1.66	0.03	1.35	1.91
Dry shell weight (g)	1.12	0.03	0.83	1.34
Water content (%)	81.04	0.48	76.22	87.18
Shell thickness (g/cm^2)	0.50	0.02	0.26	0.74
Condition index (g/cm^3)	9.04	0.52	4.46	13.30
Bagan Tiang (Female)	**Mean**	**Std Error**	**Minimum**	**Maximum**
Shell length (cm)	8.76	0.26	7.04	10.57
Shell height (cm)	3.75	0.08	3.20	4.60
Shell width (cm)	2.56	0.08	2.10	3.62
Dry total soft tissue weight (g)	1.45	0.09	0.94	2.44
Dry shell weight (g)	19.15	1.50	10.86	37.04
Water content (%)	81.32	0.53	75.75	84.48
Shell thickness (g/cm^2)	5.67	0.54	3.14	12.67
Condition index (g/cm^3)	17.32	0.58	12.91	22.74
Bagan Tiang (Male)	**Mean**	**Std Error**	**Minimum**	**Maximum**
Shell length (cm)	8.51	0.27	6.49	10.22
Shell height (cm)	2.58	0.10	2.00	3.69
Shell width (cm)	3.74	0.11	2.97	4.66
Dry total soft tissue weight (g)	NA	NA	NA	NA
Dry shell weight (g)	19.01	1.76	9.88	42.16
Water content (%)	NA	NA	NA	NA
Shell thickness (g/cm^2)	NA	NA	NA	NA
Condition index (g/cm^3)	NA	NA	NA	NA

Note: NA= data not available

Table 3. The concentrations of Cd, Cu, Fe, Ni and Zn in the *Perna viridis* collected from Kuala Kedah and Bagan Tiang

	K. Kedah	Bagan Tiang	t-test	K. Kedah	Bagan Tiang	t-test
	Total soft tissues			Shells		
Cd	2.90 ± 0.40	0.51 ± 0.12	P< 0.05	8.13 ± 0.34	10.39 ± 1.84	P> 0.05
Fe	358.2 ± 1.59	1271.2 ± 43.52	P< 0.05	50.66 ± 1.67	42.76 ± 0.75	P> 0.05
Cu	15.01 ± 0.14	12.61 ± 0.41	P> 0.05	8.64 ± 0.16	7.26 ± 0.02	P> 0.05
Ni	4.34 ± 0.45	11.42 ± 0.88	P< 0.05	39.92 ± 0.85	36.91 ± 1.95	P> 0.05
Zn	81.74 ± 0.28	106.6 ± 8.80	P> 0.05	5.04 ± 0.39	3.29 ± 0.12	P> 0.05

reported by Yap *et al.* (2006a) in which byssus could act as an excretion route for Cu and Fe. The Cu in the CS ranged from 34.72 – 43.04 µg/g dw and they were within the Cu concentration ranges from previous study as reported by Yap et al. (2005) which ranged from 22.15 to 99.12 µg/g dw. However, the pattern of Cu accumulation in the soft tissues were similar to the pattern reported by

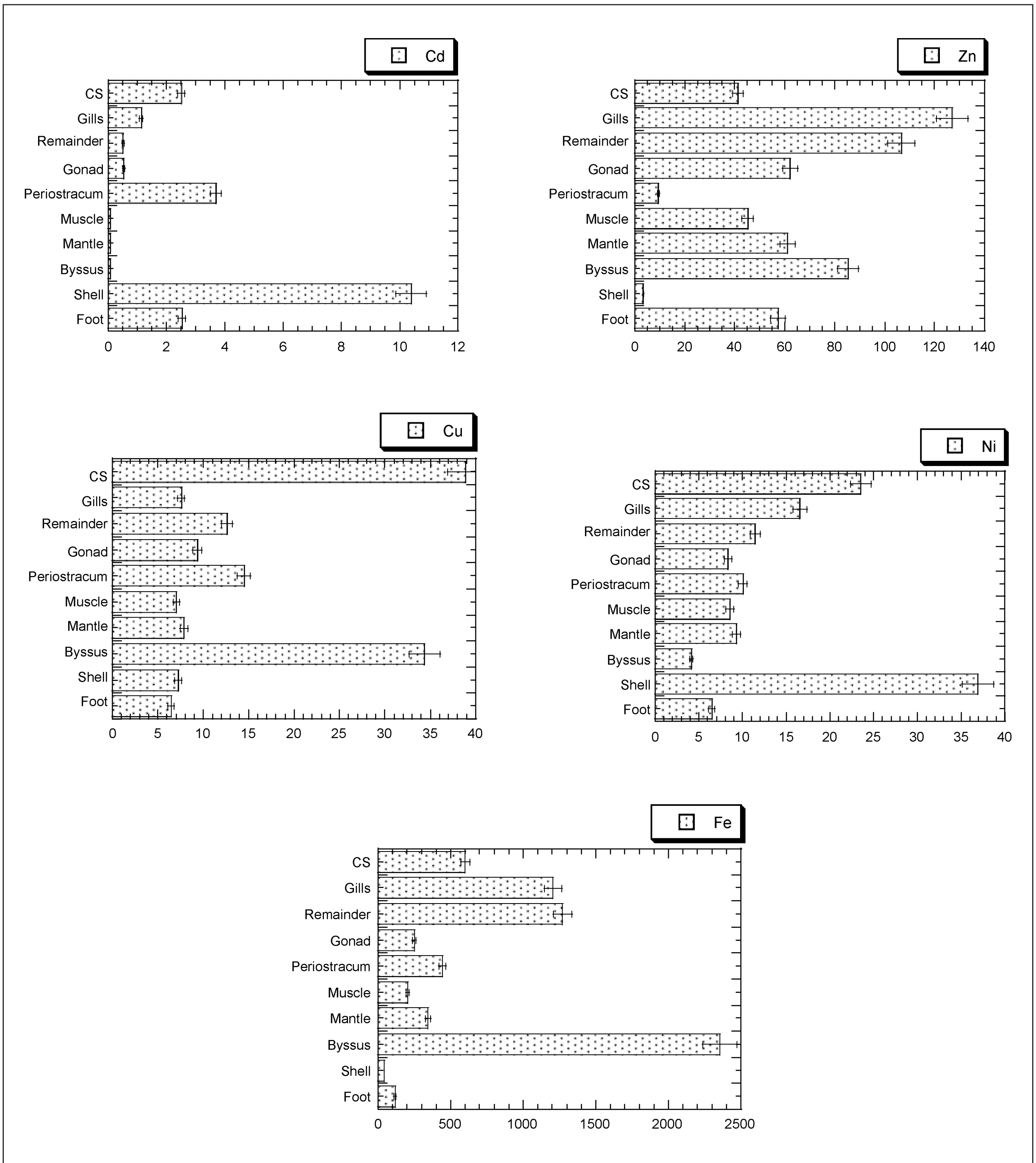

Fig. 3. Distributions of concentrations (μg/g dry weight) of Cd, Cu, Fe, Ni, and Zn in the males of shells and different soft tissues of *Perna viridis* collected from Bagan Tiang, Perak. Note: CS= crystalline style.

Yap *et al.* (2006a) where the CS always had higher Cu concentrations than the remaining soft tissues. The high level of Cu found in the CS could be related to its function as digestive tracts with enzymatic activities that Cu is much needed for the physiological function (Yap *et al.*, 2006b). Yap *et al.* (2006b) suggested that crystalline style is a good biomonitoring organ of Cu contamination in the coastal waters due to high Cu level and positive pattern with the sediment samples. Crystalline style (CS) which plays role in extracellular digestion where it secretes digestive organs (Yap *et al.*, 2005) was found to accumulate highest level of Cu. The high level of Cu in crystalline style may be due to the function in detoxifications of metals and other enzymatic activities (Morton, 1983).

The highest level of Zn was found in the mussel gills, followed by remaining soft tissue, byssus and the rest of the tissues. This indicated

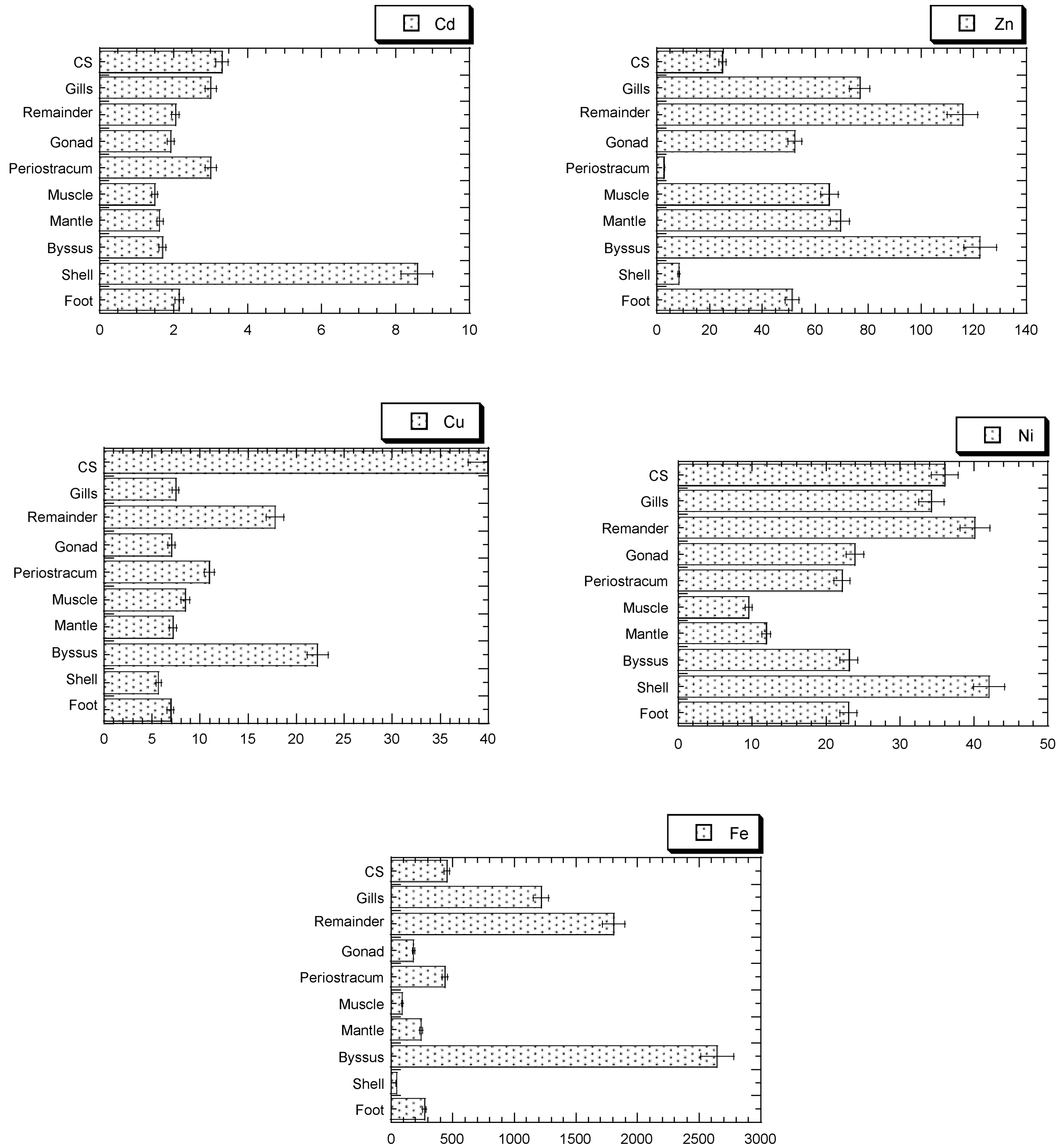

Fig. 4. Distributions of concentrations (μg/g dry weight) of Cd, Cu, Fe, Ni, and Zn in the females of shells and different soft tissues of *Perna viridis* collected from Bagan Tiang, Perak. Note: CS= crystalline style.

that the mussel gills can be used as an indicator of ambient level of surrounding seawater in the coastal area (Yap *et al.*, 2006b). High Zn level found in the byssus of *P. viridis* indicated that the byssus could be used as a biomonitoring organ of Zn pollution in the coastal waters (Yap *et al.*, 2003e).

Comparing of metals between male and female of *P. viridis*, the female generally had higher levels of metals than in the males. This again confirmed the previous finding by Yap et al. (2003d, 2006c). The difference of metals between the sexes is due to gonadal condition (Lobel and Wright, 1982; Karaseva, 1993). This is because female always carried the eggs which are larger than the sperm therefore stored more heavy metals (Karaseva, 1993).

From this study, the results showed different soft and hard tissues of *P. viridis* accumulated different levels of metals. The results of the shells

Table 4. Comparison of metal concentrations (mg/g) in the total soft tissues of *P. viridis* with the guidelines on heavy metals for food safety set by different countries

Location	WB	Cd	Cu	Zn
Permissible limits set by Malaysian Food Regulations (1985)	Wet	1.00	30.0	100
International Standards in mollusks/shellfish compiled by the Food and Agricultural Organization (FAO) of the United Nations (CEP, 2002)	Wet	2.00	10-30	40-100
International Council for the Exploration of the Sea (ICES, 1988) for status: 'increased contamination'	Dry	1.80	–	–
Maximum permissible levels established by Brazilian Ministry of Health (ABIA, 1991)	Dry	5.00	150.0	250
Permissible limit set by Ministry of Public Health, Thailand (MPHT, 1986)	Dry	–	133.0	667
Food and Drug Administration of the United States (USFDA, 1990)	Dry Wet	25.00 3.70	– –	– –
Australian Legal Requirements (NHMRC, 1987)	Dry	10.00	350.0	750
Permissible limit set by the Hong Kong Environmental Protection Department (HKEPD, 1997)	Wet	2.00	–	–
Metal levels of *P. viridis* from Kuala Kedah (this study)	Dry	2.90	15.0	81.70
Metal levels of *P. viridis* from Kuala Kedah (this study)	Wet	0.49	2.55	13.89
Metal levels of *P. viridis* from Bagan Tiang (this study)	Dry	0.547	9.69	83.70
Metal levels of *P. viridis* from Bagan Tiang (this study)	Wet	0.093	1.65	14.23

Note: WB= weight basis. The dry weight was converted into wet weight by using a conversion factor of 0.17 (Yap, 1999).

showed the following order of abundance Fe > Ni > Cd > Cu >Zn (Table 2). This order is in contrast to that in the soft tissues in which the order of abundance was: Fe> Zn> Cu> Ni> Cd (Table 2). The pattern were similar to the order from previous study done by Yap *et al.* (2003b) for the mussel shell where the order of abundance were Cd > Zn except for Ni, Fe and Cu which were not included in previous study.

This inter-tissue distribution of heavy metals showed that some tissues (organs) were responsible for the elevated levels of metals analyzed in *P. viridis*. There are three points that can be presented to explain why there was a distribution of metals in the different soft tissues of *P. viridis*. Firstly, differences in surface of contact in different soft tissues; secondly, differences in affinities of metals to binding sites at metallothionein (Roesijadi, 1982; Viarengo *et al.*, 1985) in different soft tissues. In a review by Roesijadi (1980), he mentioned that the binding of toxic metals to metallothionein could fix the metals within the different tissues and this would result in a slow turnover time (slow depuration) for these metals. Thirdly, different rates of accumulation and excretion of metals in different soft tissues. The differences in the rates of accumulation and depuration indicated that they were the results of internal metal treatment and regulation (Gundacker, 1999). Excretion through depuration was shown to be mainly from the byssus. Probably, it was an effective strategy to prevent toxic injury in *P. viridis*.

From the human health point of view, the ranges of Cd, Cu and Zn found in this study were lower than the (since guidelines for Ni and Fe are unavailable) maximum permissible limits set by Malaysian Food Regulations (1985) and other food safety limits as shown in Table 4. Therefore, the consumption of mussels collected from the Kuala Kedah and Bagan Tiang should pose no acute toxicological risks of Cd, Cu and Zn to humans.

CONCLUSION

Since there are no observable man-induced activities in the surrounding of Bagan Tiang, the data obtained from this study reflect the background bioavailability of Cd, Cu, Ni, Fe and Zn, less related to anthropogenic sources. In contrast, the metal levels found in the Kuala Kedah population should be contributed by both the anthropogenic and natural origins. From the human health point of view, most of the metal levels found in this study were lower than those of maximum permissible limits and other food safety limits as shown in Table 4. Therefore, the consumption of mussels collected from both locations should pose no acute toxicological risks of Cd, Cu and Zn to humans but it is recommended that regular monitoring of heavy metal concentrations in the west coast of Peninsular Malaysia should be done.

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