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Cadmium, Copper, Lead and Zinc Levels in the Green-Lipped Mussel *Perna viridis* (L.) from the West Coast of Peninsular Malaysia: Safe as Food?

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ABSTRAK

Kupang Perna viridis *telah disampel di antara tahun 2002-2004, daripada 10 lokasi di pantai barat Semenanjung Malaysia. Sampel-sampel tersebut dianalisis untuk kadmium (Cd), kuprum (Cu), plumbum (Pb) dan zink (Zn). Kepekatan logam (µg/g berat kering) adalah di antara 0.11-5.55 bagi Cd, 3.49-31.1 bagi Cu, 1.16-18.62 bagi Pb dan 60.51-119.5 µg/g bagi Zn. Dari segi kesihatan pandangan umum, kepekatan logam adalah di bawah tahap maksimum yang dibenarkan oleh Peraturan-peraturan Makanan Malaysia (1985) dan juga di bawah standard antarabangsa median yang dianggap berbahaya mengikut FAO oleh Bangsa-bangsa Bersatu. 'Non-carcinogenic hazard indices' bagi logam di dalam kupang menunjukkan pemakanan kupang-kupang di dalam kajian ini adalah tidak berbahaya dan risiko pemakanan adalah bergantung pada jumlah kupang yang dimakan dan tempat di mana mereka diambil.*

ABSTRACT

Green-lipped mussels, Perna viridis, *were collected between 2002-2004, from 10 locations on the west coast of Peninsular Malaysia. The samples were analysed for cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn). The metal concentrations (µg/g dry weight) ranged from 0.11 to 5.55 for Cd, 3.49 to 31.1 for Cu, 1.16 to 18.62 for Pb and 60.51 to 119.5 for Zn. From the public health point of view, these metal concentrations were below the maximum permissible levels set by the Malaysian Food Regulations (1985) and were also below the levels regarded as harmful according to the median international standards for metals in mollusks compiled by the FAO of the United Nations. Non-carcinogenic hazard indices of the metals in the mussels in this present study showed that the consumption of the mussels was not risky and any risk is dependent on the amount of mussels consumed and the sites they were collected from.*

INTRODUCTION

The west coast of Peninsular Malaysia has always been an interesting area for investigation especially for chemical pollution since the area is likely to receive impacts from man-induced activities such as urbanisation, industrialisation and other land-based activities (Hamzah 1997; Yap *et al.* 2002). To monitor the sustainable natural resources from the Straits of Malacca, an easy and practical way to fulfill such a purpose is to develop a biomonitoring agent for the west coast of Peninsular Malaysia. Ismail *et al.* (2000) suggested the green-lipped mussel *Perna viridis* as a potential biomonitor of heavy metals for the area. The idea of using marine mussels as a biomonitoring agent for Malaysian coastal waters is prompted by the many studies conducted using the blue mussel *Mytilus edulis.* This is due to the practice of using mussels as biomonitoring agents of heavy metals for coastal waters developed by Goldberg in 1975 and used until today (Nicholson and Szefer 2003; Yap *et al.* 2003; 2004). One of the attributes that has led to the use of marine mussels as a biomonitoring agent for heavy metals is that they are commercially important seafood species

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worldwide (Goldberg 1975; Phillips and Rainbow 1993). Other attributes as to why mussels are often chosen for biomonitoring studies are that they are sedentary organisms, long-living, easily identified and sampled, reasonably abundant and available throughout the year, and tolerant of natural environmental fluctuations and pollution. Besides, they have good net accumulation capacities and they are important ecologically. In this region, reports on the use of the mussel *P. viridis* as a biomonitoring agent for heavy metals had been published from Thailand (Sukasem and Tabucanon 1993), Indonesia (Hutagalung 1989), India (Senthilnathan *et al.* 1998) and Hong Kong (Wong *et al.* 2000). In Malaysia, studies on heavy metals in *P. viridis* have been conducted by Sivalingam and Bhaskaran (1980), Sivalingam (1985), Devi (1986), Liong (1986), Ismail (1993), Ismail *et al.* (2000) and Yap *et al.* (2003; 2004).

This paper reports the concentrations of cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) in the total soft tissues of *P. viridis* collected from 10 sites on the west coast of Peninsular Malaysia in order to determine the safety of the edible mussels from the human health point of view. The determination was based on comparison with the permissible levels set by the Malaysian Food Regulations (1985) and median international standards for metals in mollusks compiled by the Food and Agricultural Organization (FAO) of the United Nations (California EPA 2005) besides other food safety guidelines. In addition, the metal concentrations in the soft tissues of mussels were particularly assessed based on the non-carcinogenic hazard index established by Wong *et al.* (2000), since they were consumed by human.

MATERIALS AND METHODS

The sampling locations are shown in *Fig. 1.* The sampling was conducted between 2002 and 2004, from Bagan Tiang (in the northern part) to Kuala Belungkor (in the southern part) of Peninsular Malaysia. The samples were stored in clean plastic bags and brought back to the laboratory in an ice compartment. In the laboratory, the samples were kept at –10°C until analysis. Before dissection, the mussel samples were thawed at room temperature (27°C) and 20 relatively similar sized mussels from each site were selected and analysed for Cd, Cu, Pb and Zn. The soft tissues from the mussels were dissected by removing the byssus and the shell. Soft tissues were dried in an oven at 105°C until constant dry weight (Mo and Neilson 1994). The dried soft mussel 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 fully digested at high temperature (140°C) for at least 3 hours. The digested samples were then diluted to a certain volume with double distilled water (DDW). After filtration, the prepared samples were determined for heavy metals by an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model 4100. The data are presented in μg/g dry weight. To avoid possible contamination, all glassware and equipment used were acid-washed. Procedural blanks and quality control samples made from the standard solutions for Cd, Cu, Pb and Zn were analysed once for every five samples in order to check for accuracy. Percentages of recoveries for the analysis were 110% for Cd, 96% for Cu, 92.5% for Pb and 92% for Zn. The dry weight basis was converted into the wet weight basis by using a conversion factor of 0.17 (Yap 1999).

Calculation of Non-Carcinogenic Risk

For the public hazard assessment of mussel consumption, the non-carcinogenic hazard index was determined. The non-carcinogenic hazard index is expressed in terms of the ratio of an individual's exposure to the defined maximum level of exposure. The mean concentrations of heavy metals in all mussel populations were determined and were used to calculate the non-carcinogenic hazard index, according to Wong *et al.* (2000). The formula is given as:

$$\text{Non-carcinogenic} = \frac{\text{chronic daily intake}}{\text{reference dose}}$$

Hazard Index

The reference doses for Cd, Cu, Pb and Zn used in this study were 20, 2000, 100 and 10,000 μg, according to Wong *et al.* (2000).

RESULTS AND DISCUSSION

The concentrations of Cd, Cu, Pb and Zn are presented in Table 1. The metal concentrations ranged from 0.11 to 5.55 μg/g dry weight (0.02 to 0.94 μg/g wet weight) for Cd, 3.49 to 31.1

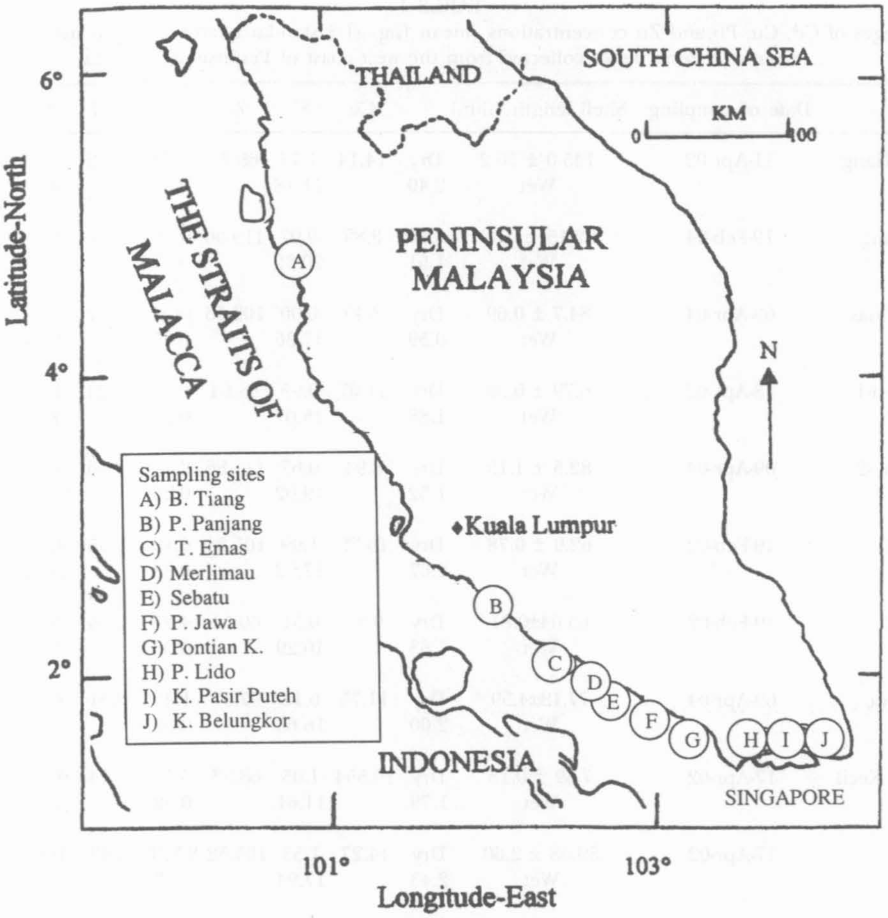

Fig. 1: Sampling sites of Perna viridis *in the coastal waters of Peninsular Malaysia*

μg/g dry weight (0.59 to 5.28 μg/g wet weight) for Cu, 1.16 to 18.62 μg/g dry weight (0.20 to 3.17 μg/g wet weight) for Pb and 60.51 to 119.5 μg/g dry weight (10.29 to 20.32 μg/g wet weight) for Zn (Table 1).

In the present results, the metal concentrations in the mussel samples from the west coast were within the ranges reported by Ismail *et al.* (2000) for mussels. The data in Table 1 were compared to the results from the same locations in the previous collections done in 1998-2001 (Yap *et al.* 2003). It was found that the concentrations of the investigated metals in the mussels from the present study were generally higher than those reported in our previous study. Sukasem and Tabucanon (1993) suggested that the difference in metal levels from two periods of sampling could be explained by the time of sampling. The samples of 1998 were collected during the rainy season (September to December) while samples taken in 2000 were obtained just at the end of the rainy season (January-April). However, little local information on heavy metal variations due to rainy season is available. Therefore, this conclusion can only be confirmed by further studies.

After conversion of the dry weight data into the wet weight basis, the metal levels in *P. viridis* found in this study were lower than those of the maximum permissible levels of heavy metals in food set by the Malaysian Food Regulations (1985) (Table 1). Our mean values (μg/g wet weight) of these metals from all the populations were lower than the limits for Cd (1.00 μg/g wet weight), Cu (30.0 μg/g wet weight), Pb (2.00 μg/g wet weight) and Zn (100 μg/g wet weight).

The metal levels were also lower than the recommended guidelines for Cd, Cu, Pb and Zn set by the Ministry of Public Health of Thailand (MPHT 1986), the Australian Legal Requirement for food safety (NHMRC 1987) and the limits established by the Brazilian Ministry of Health

TABLE 1
Ranges of Cd, Cu, Pb and Zn concentrations (mean [μg/g] ± standard error [SE]) in the total soft tissues of *Perna viridis* collected from the west coast of Peninsular Malaysia

	Date of sampling	Shell length (mm)		Cu	SE	Zn	SE	Cd	SE	Pb	SE
A. Bagan Tiang	11-Apr-02	135.0 ± 10.2	Dry	14.14	1.77	65.75	4.88	0.32	0.09	10.32	3.73
		Wet	2.40		11.18		0.05		1.75		
B. P. Panjang	19-Feb-04	82.15 ± 0.99	Dry	8.89	0.97	119.50	18.45	0.44	0.08	1.16	0.02
		Wet	1.51		20.32		0.07		0.20		
C. Telok Emas	09-Apr-04	84.7 ± 0.69	Dry	3.49	0.00	102.13	14.68	1.47	0.43	3.92	1.20
		Wet	0.59		17.36		0.25		0.67		
D. Merlimau-1	18-Apr-02	6.79 ± 0.09	Dry	11.07	0.95	88.64	8.64	2.24	0.67	13.99	2.80
		Wet	1.88		15.07		0.38		2.38		
Merlimau-2	09-Apr-04	82.5 ± 1.15	Dry	8.94	0.67	111.86	2.14	1.46	0.14	2.53	0.99
		Wet	1.52		19.02		0.25		0.43		
E. Sebatu-1	19-Feb-02	62.9 ± 0.78	Dry	15.72	1.99	105.38	6.19	1.95	0.56	18.62	2.17
		Wet	2.67		17.92		0.33		3.17		
Sebatu-2	19-Feb-02	63.04±0.81	Dry	9.57	0.51	60.51	4.27	0.35	0.04	11.01	0.00
		Wet	1.63		10.29		0.06		1.87		
F. Parit Jawa	09-Apr-04	77.18±4.59	Dry	11.75	0.18	97.97	1.40	2.31	0.11	7.26	4.59
		Wet	2.00		16.65		0.39		1.23		
G. Pontian Kecil	17-Apr-02	7.89 ± 0.13	Dry	10.554	1.05	68.03	3.53	0.14	0.001	6.56	0.85
		Wet	1.79		11.61		0.02		1.12		
H. P. Lido	17-Apr-02	89.08 ± 2.60	Dry	14.27	1.58	105.52	23.21	0.43	0.14	6.09	1.71
		Wet	2.43		17.94		0.07		1.03		
I. K.Pasir Puteh	17-Apr-02	92.84 ± 3.21	Dry	31.09	2.48	69.89	5.07	5.55	1.05	11.85	1.24
		Wet	5.28		11.88		0.94		2.01		
J. K. Belungkor	18-Apr-02	63.4 ± 1.10	Dry	7.96	0.98	86.26	16.77	0.11	0.02	2.78	1.32
		Wet	1.35		14.66		0.02		0.47		

Note: Alphabets follows those indicated in *Fig. 1.*

(ABIA 1991) (Table 2). When compared to median international standards for metals in mollusks compiled by FAO of the United Nations, again our ranges of Cd, Cu, Pb and Zn are lower than the FAO limits and therefore our mussels are safe for human consumption.

However, the potential hazards of metals transferred to humans are probably dependent on the amount (g wet weight) of mussels consumed by an individual. For example, an adult who consumed 2.50 g/day of *P. viridis* daily from Sebatu-1 would take in approximately 7.93 μg (3.17 μg/g × 2.50 g) of Pb per day. If the consumer were to take the mussel for 7 days, then he would have consumed 55.5 μg Pb (7.93 μg × 7 days). This is slightly higher than the recommended limit for the provisional tolerable weekly intake of Pb (50.0 μg/adult) (FAO/WHO 1984). Tukimat *et al.* (2002) reported that the daily intake of Pb in seafood by a population from Kuala Kemaman, Terengganu (east coast of Peninsular Malaysia), was 2.82 μg/day. The estimate of Pb intake from the present study (55.5 μg Pb) is higher than a week's consumption of seafood by a person from Kemaman (2.82 μg/day × 7 days= 20.0 μg Pb).

Similarly, if an adult roughly consumed 2.50 g of mussel per day, then the person who consumed mussels collected from Kg. Pasir Puteh would consume approximately 2.35 μg (0.94 μg/g × 2.50 g) of Cd each day. If the consumer takes mussels for 7 consecutive days, then he

TABLE 2
Guidelines on heavy metals for food safety set by different countries

Location	WB	Cd (μg/g)	Cu (μg/g)	Pb (μg/g)	Zn (μg/g)
Permissible limits set by Malaysian Food Regulations (1985)	Wet	1.00	30.0	2.00	100
Maximum permissible levels established by Brazilian Ministry of Health (ABIA 1991)	Dry	5.00	150	10.0	250
Permissible limit set by Ministry of Public Health, Thailand (MPHT 1986)	Dry	-	133	6.67	667
Australian Legal Requirements (NHMRC 1987)	Dry	10.0	350	-	750
Median international standards for metals in mollusks compiled by the Food and Agricultural Organization of the United Nations (California EPA 2005)	Wet	2.00	10-30	1-6	40-100
Metal levels of *P. viridis* from Peninsular Malaysia (This study)	Wet	0.02-0.94 (0.24 ± 0.08)	0.29-5.28 (2.09 ± 0.33)	0.20-3.17 (1.36 ± 0.26)	10.3-20.3 (15.33 ± 0.98)
	Dry	0.11-5.55 (1.39 ± 0.45)	3.49-31.1 (12.3 ± 1.95)	1.16-18.6 (8.01 ± 1.52)	60.5-119.5 (90.1 ± 5.77)

Note: WB= weight basis.

would have consumed 16.5 mg Cd (2.35 × 7 days). Again, this is higher than the recommended limit for the provisional tolerable weekly intake of Cd (6.70-8.30 μg/adult) (FAO/WHO 1984). Tukimat *et al.* (2002) reported that the daily intake of Cd in seafood by a population from Kuala Kemaman, Terengganu, was 0.74 μg/day. The present estimate (16.5 μg Cd) is also higher than a week's consumption of seafood from Kemaman (0.74 μg/day × 7 days= 5.18 μg Cd). Therefore the above calculations show that the risk of metal toxicity could be dependent on the amount of mussels consumed.

For the calculation of the non-carcinogenic risk of all mussels collected, the mean concentrations of Cd, Cu, Pb and Zn in the mussels are summarized in Table 3. For a person who takes one mussel per day (13.5 g wet weight/day), all the indices are below 1 (Table 3a), indicating that there is no non-carcinogenic risk. However, for a person who takes five mussels per day (67.3 g wet weight /day) (Table 3), the indices for the concentrations of Cd in mussels collected from Merlimau-1, Parit Jawa and Kg. Pasir Puteh are above 1, whereas the indices for Pb concentrations in mussels collected from Bagan Tiang, Merlimau-1, Sebatu-1, Sebatu-2 and Kg. Pasir Puteh are above 1. The same phenomenon of metal risk can be seen if an individual consumed 10 mussels per day in which a greater proportion of the populations is estimated to be at risk of Cd and Pb toxicity since 50% and 58.3% of the population have their non-carcinogenic risk indices higher than 1. According to Wong *et al.* (2000), the non-carcinogenic value of 1 or greater indicates a risk of potential concern (Wong *et al.* 2000). Therefore, the mean concentrations of Cd and Pb indicated a risk if one consumes five or more mussels per day.

CONCLUSION

Although the present data indicate that the possibility of the occurrence of acute toxicities of Cd, Cu, Pb and Zn is unlikely, low-level and chronic toxicities to consumers may still cause health problems in human beings. The latter is poorly understood although this would be expected based on the information found in the literature. By using *P. viridis* as a biomonitoring agent, the contamination of Cd, Cu, Pb and Zn in the west coast of Peninsular Malaysia was found to be not serious. The heavy metal concentrations in the mussels from the west coast of Peninsular Malaysia could be attributed to natural or anthropogenic metal sources affecting their habitats. From the human public health point of view, these results seem to show no possibility of acute toxicities of Cd, Cu, Pb and Zn if edible mussels are consumed. However, the risk is dependent on the amount of mussels consumed and the sites from where the mussels are collected.

TABLE 3
Non-carcinogenic hazard index (CDI/RfD) for oral intake of 1 day, 5 and 10 consecutive days

		Cu			Zn			Cd			Pb		
		Day(s)			Day(s)			Day(s)			Day(s)		
	Sites	1	5	10	1	5	10	1	5	10	1	5	10
1.	Bagan Tiang	0.02	0.08	0.16	0.02	0.08	0.16	0.03	0.17	0.34	0.24	**1.18**	**2.36**
2.	P. Panjang	0.01	0.05	0.10	0.03	0.14	0.24	0.05	0.24	0.48	0.03	0.14	0.28
3.	Telok Emas	0.00	0.02	0.04	0.02	0.12	0.24	0.17	0.84	**1.68**	0.09	0.45	0.90
4.	Merlimau-1	0.01	0.06	0.12	0.02	0.10	0.20	0.26	**1.28**	**2.48**	0.32	**1.61**	**3.22**
	Merlimau-2	0.01	0.05	0.10	0.03	0.13	0.26	0.17	0.84	**1.68**	0.06	0.29	0.58
5.	Sebatu-1	0.02	0.09	0.18	0.02	0.12	0.24	0.22	1.11	**2.22**	0.43	**2.14**	**4.28**
	Sebatu-2	0.01	0.06	0.12	0.01	0.07	0.14	0.04	0.20	0.40	0.25	**1.26**	**2.52**
6.	Parit Jawa	0.01	0.07	0.14	0.02	0.11	0.22	0.26	**1.32**	**2.64**	0.17	0.83	**1.66**
7.	Pontian Kecil	0.01	0.06	0.12	0.02	0.08	0.16	0.01	0.07	0.14	0.15	0.75	**1.50**
8.	P. Lido	0.02	0.08	0.16	0.02	0.12	0.24	0.05	0.24	0.48	0.14	0.70	0.14
9.	K. Pasir Puteh	0.04	0.18	0.36	0.02	0.08	0.16	0.63	**3.17**	**6.34**	0.27	**1.36**	**2.72**
10.	K. Belungkor	0.01	0.05	0.10	0.02	0.10	0.20	0.01	0.07	0.14	0.06	0.32	0.64

Note: Values in bold indicate a risk of potential concern (Wong *et al.* 2000) since they are > 1.0.

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