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Heavy metal (Cd, Cu, Pb and Zn) concentrations in the green-lipped mussel *Perna viridis* (Linnaeus) collected from some wild and aquacultural sites in the west coast of Peninsular Malaysia

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Abstract

The green-lipped mussel *Perna viridis* (L.), collected from nine (four wild and five aquacultural) sites between 1999 and 2001, off the west coast of Peninsular Malaysia, were analysed for cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn). The concentrations (μg/g dry weight) of these heavy metals ranged from 0.68 to 1.25 for Cd, 7.76 to 20.1 for Cu, 2.51 to 8.76 for Pb and 75.1 to 129 for Zn. These levels should result in no acute toxicities of the metals since they are lower than the permissible limits for human consumption. In addition, these metal concentrations are also considered to be low when compared with regional data using *P. viridis* as a biomonitoring agent.

Keywords: Heavy metals; *Perna viridis*; West coast of Peninsular Malasia

1. Introduction

The green-lipped mussel *Perna viridis* (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. One of the attributes, that had led to the use of marine mussels as a biomonitoring agent for heavy metals is that they are commercially important seafood species worldwide (Farrington, Davis, Tripp, Phelps, & Galloway, 1987; Phillips, 1980; Widdows, 1985). Other attributes, due to which mussels are often chosen for biomonitoring studies, are that 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. The importance of bivalves in pollution impact studies is shown by the concept of the International Mussel Watch programme (Goldberg, 1975) that has continued to maintain its momentum until today (Kavun, Shulkin, & Khristoforova, 2002). Since it is edible and marketed commercially, the determination of contaminant levels in mussel species provides a means of assessing the possible toxicant risk to public health.

Marine mussels provide a cheap source of protein for human consumption. For *P. viridis*, it had been reported that there was about 60% protein in every 100 g (dry weight) of mussel soft tissues (Choo & Ng, 1990). From the nutritional point of view, the mussel is an important food source for supplying essential trace metals (e.g. Ca, Fe) and certain vitamins such as niacin, thiamin and riboflavin (Cheong & Lee, 1984). Moreover, fish and shellfish may also contain the polyunsaturated *n*-3 fatty acids which are biologically important and have been associated with a decreased risk of cardiovascular disease (Kromhout, Bosschieter, & Lezenne, 1985). However, from the toxicological point of view, excessive consumption of metal-contaminated seafood may cause toxicity to humans. Since heavy metals are inorganic chemicals that are non-biodegradable, cannot be metabolized and will not break down into harmless forms (Kromhout et al., 1985), the measurement of levels of metals in the soft tissues of *P. viridis* is becoming more significant. They could simply accumulate through time, becoming more and more of a toxic threat as their concentrations increase. Levels

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of metals above the permissible limits would certainly create a notorious food image from the public health point of view. Chronic exposure to heavy metals such as Cu, Pb and Zn is associated with Parkinson's disease and the metals might act alone or together over time to cause the disease (Gorell et al., 1997).

Some metals, such as Cd and Pb, have long been known to accumulate within the aquatic food chain. Since Cd, Cu, Pb and Zn are widely distributed in the coastal environment, both from natural geological processes and anthropogenic activities, this is of much interest to public health, since the metals are readily accumulated in the soft tissues of *P. viridis* (Ismail, Yap, & Tanabe, 1999). Mussels are well known to accumulate a wide range of contaminants in their soft tissues (Goldberg et al., 1978).

The intertidal areas are the natural habitats of marine mussels and they are usually close to estuaries. Therefore, the chance of exposure to many contaminants from land-based activities through the riverine system as well as sea-based sources, is high.

Although *P. viridis* has been aquacultured in Malaysia since the 1970s (Sivalingam, 1977), only few reports concerning heavy metal concentrations in it could be found. These reports include data, mainly, from the west coast of Peninsular Malaysia (Sivalingam & Bhaskaran, 1980; Liong, 1986; Ismail, 1993; Din & Jamaliah, 1994). In particular, *P. viridis* had been suggested as a potential biomonitoring agent of heavy metals in the west coast of Peninsular Malaysia (Ismail, Yap, Zakaria, Tanabe, Takada, & Rahim Ismail, 2000). The present study aimed at determining the concentrations of Cd, Cu, Pb and Zn in the soft tissues of *P. viridis* collected from the wild (scattered mussels found around the sampling sites) and from mussel aquacultural farms (in clusters) in the west coast of Peninsular Malaysia, to investigate whether these metals are within the permissible limits for human consumption.

2. Materials and methods

2.1. Sampling, storage and sample preparation

The sampling sites are shown in Fig. 1. The samplings were conducted between 1999 and 2001. Four sites were collected from the wild and the rest were from mussel aquacultural farms. Since the mussels are processed commercially straight away without depuration, the collected mussels were immediately put into an ice compartment and transported to the laboratory for further analysis. In the laboratory, the samples were kept at −10 °C until metal analysis. Before dissection, the mussel samples were thawed at room temperature (27 °C) with the posterior margin facing downwards in order to allow excess water to drain away.

About 17–25 mussels from each sampling site were selected and analysed for heavy metals (Table 1). The soft tissues of mussels were dissected by removing the byssus and the shell. The total soft tissues were dried in an oven at 105 °C to constant dry weight (Mo & Neilson, 1994).

2.2. Analysis of heavy metals

All samples were digested in concentrated HNO_3 (AnalaR grade, BDH 69%). They were placed in a hot-block digester first at low temperature for 1 h and then they were fully digested at high temperature (140 °C) for at least 3 h. The digested samples were then diluted to a certain volume with double-distilled water. After filtration, the prepared samples were determined for Cd, Cu, Pb and Zn by using an air-acetylene flame atomic absorption spectrophotometer (AAS) Perkin-Elmer Model 4100. The data were presented in μg/g of sample dry weight (dw). The dw was converted into wet weight (ww) by using a conversion factor of 0.17 ± 0.04 (Yap, 1999). The latter was then used for comparative purposes. To avoid possible contamination, all glassware and equipment used were acid-washed. To check for contamination, procedural blanks were analysed in every five samples. Quality control samples, made from standard solutions of Cd, Cu, Pb and Zn, were analysed in every five samples to check for the metal recoveries. The percentages of recoveries were 105% for Cd, 96% for Cu, 92.5% for Pb and 92% for Zn.

3. Results and discussion

The metal concentrations in the soft tissues of *P. viridis* ranged from 0.68 to 1.25 μg/g dw (0.12–0.22 μg/g ww) for Cd, 7.76 to 20.1 μg/g dw (1.32–3.42 μg/g ww) for Cu, 2.51 to 8.76 μg/g dw (0.43–1.49 μg/g ww) for Pb and 75.1 to 129 μg/g dw (12.8–21.9 μg/g ww) for Zn (Table 2). In comparison with the permissible limits set by the Malaysian Food Regulation (1985) for Cd (1.00 μg/g ww), Cu (30.0 μg/g ww), Pb (2.00 μg/g ww) and Zn (100 μg/g ww), all the mean values (μg/g ww) of these metals from all populations were lower than the limits. The metal levels were also lower than the recommended guidelines for Cd, Pb, Cu and Zn set by the USFDA (1990), the Hong Kong government (HKEPD, 1997), 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 (ABIA, 1991) (Table 3). As for the status of the 'increased contamination' reported by ICES (1988), the Cd levels of the present study were lower than the 'increased contamination' level (1.80 μg/g dw) for Cd but higher than the status for Pb (3.00 μg/g dw) (Table 3).

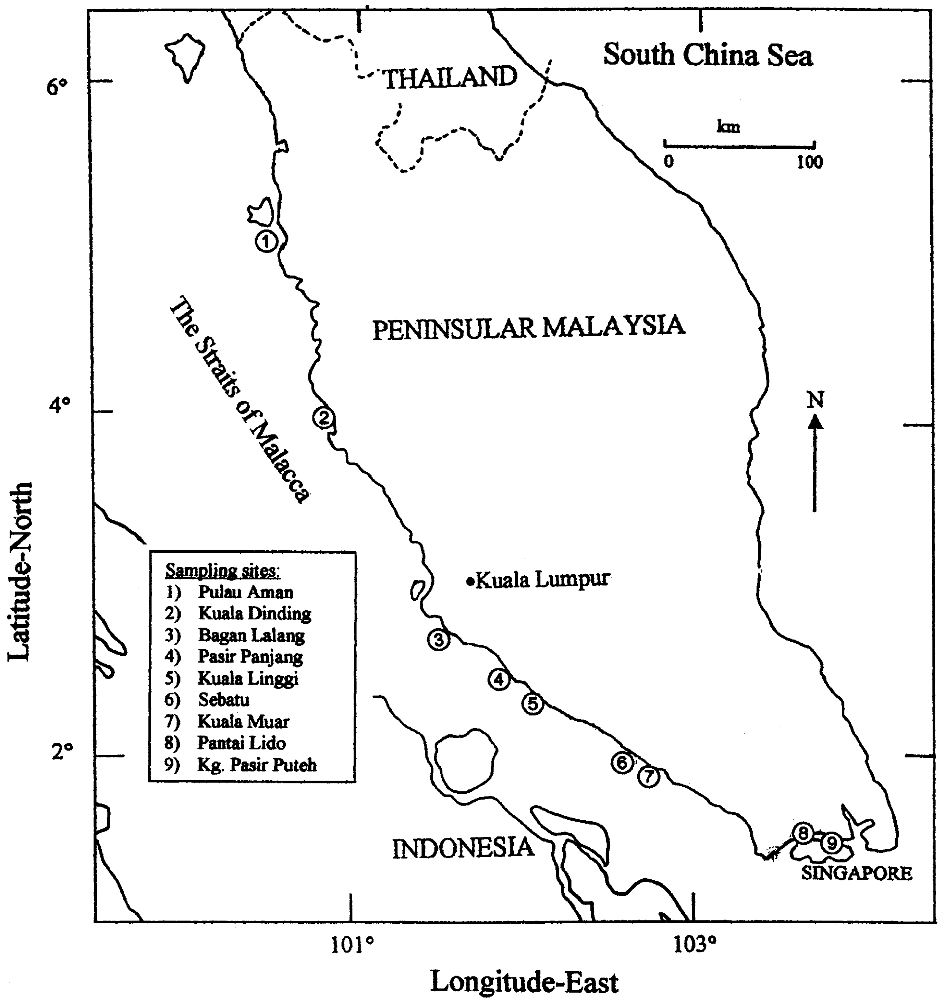

Fig. 1. Sampling sites of *Perna viridis* from the west coast of Peninsular Malaysia.

Table 1
Sampling dates and shell lengths of green-lipped mussel *Perna viridis* collected from the west coast of Peninsular Malaysia

No.	Location	Sampling Date		*N*	Shell length (mm)
1	Pulau Aman, Penang	11 Sep 1999	Wild	25	86.3 (70.0–96.8)
2	Kuala Dinding, Perak	18 May 2001	Cultured	20	87.1 (67.2–98.8)
3	Bagan Lalang, Selangor	04 April 2000	Cultured	25	88.9 (73.5–97.1)
4	Pasir Panjang, Negeri Sembilan	07 Oct 2000	Cultured	17	8.78 (7.41–10.78)
5	Kuala Linggi. Negeri Sembilan	21 Nov 2000	Cultured	20	8.00 (7.50–9.86)
6	Sebatu, Malacca	12 Aug 2000	Cultured	25	85.4 (75.2–88.0)
7	Muar Estuary, Malacca	21 Jan 2000	Wild	25	84.4 (68.2–99.2)
8	Pantai Lido, Johore	20 Jan 2000	Wild	25	64.6 (53.4–82.4)
9	Kg. Pasir Puteh, Johore	19 Jan 2000	Wild	25	61.1 (51.8–91.3)

Nos. follow those indicated in Fig. 1. *N* is number of samples analysed for Cd, Cu, Pb and Zn.

According to Hutton (1987), the major health problems of Pb are manifested in three organ systems namely the haematological, nervous and renal systems. In the haematological system, Pb interferes with the last stage of haem synthesis, the incorporation of Fe into protoporphyrin, catalyzed by haem synthetase. Acute effects of Pb on the central nervous system are generally seen in children and are manifested by severe encephalopathy that can culminate in coma and death (Hutton, 1987). 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 2.5 g/day of *P. viridis* daily from Kg. Pasir Puteh would take in approximately 3.73 μg (1.49 μg/g×2.50 g) of Pb each day. If the consumer were to take the mussel for 7 days, then he would

Table 2
Concentrations of cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) in the soft tissues of *Perna viridis* collected from the west coast of Peninsular Malaysia

No.	Location	Weight basis	Cd (μg/g)	Cu (μg/g)	Pb (μg/g)	Zn (μg/g)
1	Pulau Aman, Penang	Dry	0.87 (0.60–1.42)	10.8 (8.88–13.3)	4.76 (2.50–5.99)	110 (61.7–173)
		Wet	0.15 (0.10–0.24)	1.84 (1.51–2.26)	0.81 (0.43–1.02)	18.6 (10.5–29.4)
2	Kuala Dinding, Perak	Dry	1.06 (0.74–1.80)	7.76 (4.96–25.1)	2.51 (6.22–0.53)	90.0 (65.2–119)
		Wet	0.18 (0.13–0.31)	1.32 (0.84–3.58)	0.43 (0.09–1.06)	15.3 (11.1–20.3)
3	Bagan Lalang, Selangor	Dry	1.12 (0.71–2.14)	8.20 (6.46–10.7)	3.41 (0.68–8.96)	96.4 (75.4–139)
		Wet	0.19 (0.12–0.36)	1.39 (1.10–1.82)	0.58 (0.12–1.52)	16.38 (12.8–23.6)
4	Pasir Panjang, Negeri Sembilan	Dry	1.08 (0.63–1.61)	10.87 (8.85–13)	7.89 (4.82–11.2)	98.9 (74.13–135)
		Wet	0.18 (0.11–0.27)	1.85 (1.50–2.21)	1.34 (0.82–1.91)	16.82 (12.6–22.9)
5	Kuala Linggi, Negeri Sembilan	Dry	1.25 (0.77–2.11)	9.14 (4.31–12.8)	7.98 (4.95–9.74)	101 (66.8–145)
		Wet	0.22 (0.13–0.36)	1.55 (0.73–2.18)	1.36 (0.84–1.66)	17.2 (11.4–24.7)
6	Sebatu, Malacca	Dry	1.04 (0.61–1.65)	11.2 (8.89–14.8)	7.59 (4.77–12.3)	75.1 (63.1–89.7)
		Wet	0.18 (0.10–0.28)	1.90 (1.51–2.51)	1.29 (0.81–2.08)	12.8 (10.7–15.3)
7	Muar Estuary, Malacca	Dry	0.83 (0.26–1.73)	7.93 (4.74–11.9)	3.05 (1.45–12.4)	79.0 (53.3–97.0)
		Wet	0.14 (0.04–0.29)	1.35 (0.81–2.02)	0.52 (0.25–2.11)	13.4 (9.06–16.5)
8	Pantai Lido, Johore	Dry	0.68 (0.36–1.53)	9.39 (5.19–18.40)	4.03 (1.83–8.98)	117 (68.1–165)
		Wet	0.12 (0.06–0.26)	1.60 (0.88–3.13)	0.69 (0.31–1.53)	19.9 (11.6–28.0)
9	Kg. Pasir Puteh, Johore	Dry	0.82 (0.51–1.36)	20.10 (11.0–34.9)	8.76 (3.61–12.8)	129 (90.5–158)
		Wet	0.14 (0.09–0.23)	3.42 (1.88–5.93)	1.49 (0.61–2.18)	21.9 (15.4–26.9)
	Peninsular Malaysia	Wet	0.12–0.22	1.32–3.42	0.43–1.49	12.8–21.9
		Dry	0.68–1.25	7.76–20.1	2.51–8.76	75.1–129

Nos. follow those indicated in Fig. 1.

Table 3
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 Regulation (1985)	Wet	1.00	30.0	2.00	100
International Council for the Exploration of the Sea (ICES, 1988) for status: 'increased contamination'	Dry	1.80	–	3.00	–
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
Food and Drug Administration of the United States (USFDA, 1990)	Dry	25.0	–	11.5	–
	Wet	3.70	–	1.70	–
Australian Legal Requirements (NHMRC, 1987)	Dry	10.0	350	–	750
Permissible limit set by the Hong Kong Environmental Protection Department (HKEPD, 1997)	Wet	2.00	–	6.00	–
Metal levels of *P. viridis* from the west coast of Peninsular Malaysia (this study)	Wet	0.12–0.22	1.32–3.42	0.43–1.49	12.8–21.9
	Dry	0.68–1.25	7.76–20.1	2.51–8.76	75.1–12.9

WB = weight basis.

Table 4
A comparison of reported concentrations (μg/g) of cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) in *Perna viridis* from regional studies with the present results (WB: weight basis) and those from other studies done in Malaysia

Location	WB	Cd	Cu	Pb	Zn	References
Regional studies						
Coastal waters of Hong Kong	Dry	0.29–1.43	16.0–2790	7.50–60.50	89.0–164	Phillips (1985)
The Gulf of Thailand	Dry	<0.02–19.1	1.50–11.3	–	25.7–79.0	Sukasem and Tabucanon (1993)
Putai coast of Taiwan	Dry	–	1.78–5.41	–	14.4–25.7	Han et al. (1997)
Southeast coast of India	Dry	1.59–4.40	33.6–49.2	2.48–6.92	60.4–94.1	Senthilnathan, Balasubramian, & Venugopalan, (1998)
The Gulf of Thailand	Dry	0.17–3.25	2.94–15.0	0.19–3.75	24.9–213	Ruangwises and Ruangwises (1998)
Tolo Harbour, Hong Kong	Dry	0.45–1.44	6.02–24	2.02–4.36	90.0–135	Wong, Cheung, & Wong (2000)
Guangdong market, China	Wet	0.38	2.05	0.18	9.90	Fang, Cheung, & Wong (2001)
Fish cultured sites at Hong Kong waters	Dry	0.31–0.87	19.0–20.1	4.34–25.9	96.7–201	Wong, Wong, &Chu (2001)
Malaysia						
Penang, Malaysia	Dry	BDL	8.00	7.00	76.0	Sivalingam and Bhaskaran (1980)
Ban Merbok, Perak	Wet	0.05	1.93	0.24	13.8	Liong (1986)
Lekir, Perak	Wet	0.18	2.70	0.52	22.8	Devi (1986)
West coast of Peninsular Malaysia (8 sites)	Wet	0.10–1.80	1.00–3.00	0.50–5.90	10.8–30.0	Ismail (1993)
Penang waters	Wet	0.12–0.22	1.32–3.42	0.43–1.49	12.8–21.9	Din and Jamaliah (1994)
Peninsular Malaysia (9 sites)	Dry	0.68–1.25	7.76–20.1	2.51–8.76	75.1–129	This study

WB = weight basis. BDL = below detection limit.

consume 26.1 μg Pb (3.73 μg×7 days). This is lower than the recommended limit for the provisional tolerable weekly intake of Pb (50.0 μg/adult) (FAO/WHO, 1984). Tukimat, Abu Bakar, Zaidi, and Sahibin (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 (26.1 μg Pb) is close to a week's consumption of seafood by a person from Kemaman (2.82 μg/day×7 days = 20.0 μg Pb). Also, if the person consumes more *P. viridis* (>4.8 g/day) the value will exceed the recommended limit for the provisional tolerable weekly intake.

Similarly, if an adult consumes approximately 2.50 g of mussels per day, then a person who consumes mussels collected from K. Linggi would consume approximately 0.55 μg (0.22 μg/g×2.50 g) of Cd each day. If the consumer takes the mussel for 7 consecutive days, then he will consume 3.85 μg Cd (0.55×7 days). Again, this is lower than the recommended limit for the provisional tolerable weekly intake of Cd (6.70–8.30 μg/adult) (FAO/WHO, 1984) unless the consumer takes more than 5.4 g/day of *P. viridis* from Kuala Linggi. 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 (3.85 μg Cd) is also lower than a week's consumption of seafood from Kemaman (0.74 μg/day×7 days = 5.18 μg Cd).

Nevertheless, since the elimination rate of Cd is so slow (an average 2.00 μg/day), prolonged excessive Cd ingestion will cause Cd accumulation inside the human body (Filov, Ivin, & Bandman, 1993). The acute toxic symptoms of Cd are nausea, vomiting, diarrhea, headache, abdominal pain, muscular ache, salivation and shock (Patnaik, 1992) whereas chronic Cd poisoning induces renal tubular dysfunction, indicated by low molecular proteinuria followed by abnormal blood findings and lastly reaching the 'Itai-itai' disease state. However, based on the present range of Cd found in the soft tissues of *P. viridis*, the occurrence of 'Itai-itai' disease by consuming *P. viridis* from Peninsular Malaysia is unlikely. 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 pose an irreversible hazard. The latter is poorly understood although this could be expected, based on information found in the literature.

Finally, when compared with the metal levels found in *P. viridis* from other areas of this region, our metal levels in the soft tissues of *P. viridis* are comparable to most of those reported from India, Thailand, Hong Kong and previous studies from Malaysia (Table 4).

4. Conclusion

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 not to be serious. Since *P. viridis* accumulates heavy metals in the soft tissues and constitutes one of the important food-chains in the coastal environment, this information is therefore useful for predicting any metal contamination in the coastal communities. 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. The observation that the wild and aquacultured populations of *P. viridis* collected from the west coast of the peninsula were not seriously contaminated by Cd, Cu, Pb and Zn suggests that the mussels spend most of their time and obtain much of their food in non-contaminated areas of the coastal waters. Future studies should concentrate on the relative importance of water, sediment and food in the accumulation of metals by the mussels. 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 the edible mussels are consumed.

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