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Tissue Distribution of Heavy Metals (Cd, Cu, Pb and Zn) in the Green-lipped Mussel *Perna viridis* from Nenasi and Kuala Pontian, East Coast of Peninsular Malaysia

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ABSTRAK

Kajian lepas melaporkan kupang Perna viridis *adalah bertaburan secara meluas in persisiran pantai barat Semenanjung Malaysia tetapi bukan pada persisiran pantai timur Semenanjung. Dalam kajian ini, pemantauan dan pensampelan telah dilakukan dari Tumpat ke Mersing pada April 2004, tetapi kupang hanya boleh dijumpai pada Nenasi dan Kuala Pontian di persisiran pantai Pahang. Oleh itu, kajian ini memastikan bahawa kupang pada persisiran pantai timur adalah tidak bertaburan meluas seperti pada persisiran pantai barat Semenanjung. Kupang yang disampel telah dianalisis untuk kadmium (Cd), kuprum (Cu), plumbum (Pb) dan zink (Zn) dan kepekatan (μg/g berat kering) logam dalam tisu lembut keseluruhan adalah Cd: 1.89-2.13, Cu: 3.84-10.34, Pb: 7.95-8.84 dan Zn: 93.1-119.6. Keputusan menunjukkan bahawa sampel Kuala Pontian menimbun lebih tinggi kepekatan Cu (dalam tinggalan tisu lembut dan mantel) dan Zn (dalam tinggalan tisu lembut, mantel dan otot) apabila dibandingkan dengan Nenasi manakala Cd dan Pb menunjukkan tiada perbezaan yang signifikan ($P > 0.05$) di antara kedua-dua lokasi. Oleh sebab tiada input dan aktiviti antropogenik pada kedua-dua lokasi, perubahan kepekatan logam yang dibiotimbun di dalam tisu lembut berkemungkinan disebabkan oleh perbezaan yang signifikan dari segi saiz (panjang cangkerang dan lebar cangkerang) dan tahap kemasinan bagi kedua-dua lokasi. Faktor-faktor persekitaran yang lain juga mungkin boleh menyebabkan perbezaan dalam 'bioavailability' logam di dalam persisiran pantai yang dicerminkan oleh kepekatan berlainan di tisu lembut yang berlainan bagi kupang tersebut.*

ABSTRACT

Previous studies reported that the green-lipped mussel Perna viridis *were widely found on the west coast of Peninsular Malaysia but not on the east coast of the Peninsula. In this study, surveys and sampling were conducted from Tumpat to Mersing in April 2004, but the mussels were only found at Nenasi and Kuala Pontian in Pahang coastal waters. Therefore, from this study, we confirm that the mussels on the east coast were not as widely found as on the west coast of Peninsular Malaysia. The mussel samples collected were analysed for cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn) and the metal concentrations (μg/g dry weight) were 1.89-2.13, 3.84-10.34, 7.95-8.84 and 93.1-119.6 for Cd, Cu, Pb and Zn, respectively. These results indicate that Kuala Pontian samples accumulated higher concentrations of Cu (in remaining soft tissue and mantle) and Zn (in remaining soft tissue, mantle and muscle) compared to those in Nenasi while Cd and Pb showed no significant difference ($P > 0.05$) between the two sample sites. Since there were no observable anthropogenic inputs or activities at the two sampling sites, the variation of metal concentrations accumulated in the soft tissues could be mostly attributable to the significant difference in size (shell length, and shell width) and salinity of the two sites. The other environmental factors could also cause differences in the metal bioavailabilities in the coastal waters that were reflected in different concentrations accumulated in the different soft tissues of the mussels.*

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INTRODUCTION

The *Perna viridis* mussels are widely distributed in the Asian Pacific coastal waters (Tanabe 2000) and they are an ecologically and economically important species. The mussel species has been proposed as a biomonitor of heavy metals (like its temperate counterpart *Mytilus*) in Hong Kong (Phillips 1985), Thailand (Sukasem and Tabucanon 1993) and in Peninsular Malaysia (Yap *et al.* 2003a). The wide distribution of *P. viridis* in coastal waters, sedentary life style, long life, easy identification and sampling (reasonably abundant and available throughout the year), tolerance of natural environmental fluctuations and pollution and enough tissue for metal analysis (Yap *et al.* 2003a) make it a good biomonitoring agent. *Perna viridis* are abundant on the west coast of Peninsular Malaysia since they are widely found in the west coastal waters (Yap *et al.* 2002; 2003b). Yap *et al.* (2003a) reported that the mussel species could only be found at Kg. Tg. Batu (east coast of Peninsular Malaysia). However, it is still not sure if *P. viridis* could be found in other sites on the east coast of the Peninsula. Therefore, in this study, surveys and sampling were conducted from Tumpat to Mersing along the east coast of Peninsular Malaysia. The objectives for this study are (1) to survey and to sample any available *P. viridis* along the coastal waters from Tumpat to Mersing of the east coast of Peninsular Malaysia, and (2) to measure the background concentrations of heavy metals in mussels collected in the east coast (Nenasi and Kuala Pontian).

METHOD AND MATERIALS

Sampling and Storage of Samples

The samples were collected during a sampling trip from Kuala Pontian and Nenasi in April 2004 (*Fig. 1*) on the east coast of Peninsular Malaysia. These two sites are known to be fishing and aquacultural sites and there were no signs of direct pollution. The collected mussels were immediately put into an ice compartment and transported to the laboratory for further analysis.

Sample Preparation

In each sampling site, twenty individual mussels were separated by gender and carefully dissected into different soft tissues (remainder, mantle, muscle, gills, gonad, foot, byssus and crystalline style). They were placed in aluminium foil and later dried in the oven for 72 hours at 60°C to a constant dry weight. Dried samples were then stored in clean plastic bags.

Metal Analysis

About 0.5g of dried soft tissues were digested in concentrated nitric acid (AnalaR grade, BDH 69%) and placed in a digestion block at 40°C for 1 hour and then fully digested at 140°C for 3 hours (Yap *et al.* 2002; 2003a). After cooling, it was diluted to a certain volume with double de-ionised water. The digested samples were then filtered through Whatman No. 1 (filter speed: medium) filter paper into acid-washed pillbox. All samples stored in acid-washed pillboxes were then analysed using an air-acetylene Perkin-Elmer™ flame atomic absorption spectrophotometer (AAS) model Analyst 800 for four heavy metals (Cd, Cu, Pb and Zn). Blank determination was carried out for calibration of the instrument. Standard solutions were prepared from 1000 mg/L stock solution provided by MERCK Titrisol for metals such as Cu, Zn, Cd and Pb (Yap *et al.* 2002) and data obtained from the AAS were presented in μg/g dry weight basis. Recoveries were done by using standard solutions as quality control samples. The percentage of recoveries for all metals was in the range of 90-110%.

RESULTS

Distributions of Cd, Cu, Pb, and Zn in the different soft tissues of *P. viridis* are shown in *Fig. 2(a)* and *2(b)*. For Cu, it was found that the crystalline style accumulated the highest level (Nenasi=33.5 μg/g, Kuala Pontian=68.4 μg/g) among all the different soft tissues in both sites followed by the byssus (Nenasi=18.3 μg/g, Kuala Pontian=22.2 μg/g) and other soft tissues.

For Zn, remainder of Nenasi accumulated the highest level (140 μg/g) followed by crystalline style (106 μg/g), muscle (80.7 μg/g), gills (79.3 μg/g), gonad (70.1 μg/g), mantle (65.7 μg/g), byssus (65.5 μg/g) and foot (62.4 μg/g). In Kuala Pontian, gills accumulated the highest level of Zn (167 μg/g) followed by remainder (140 μg/g), gonad (115 μg/g), muscle (113 μg/g), mantle (103 μg/g), byssus (94.9 μg/g), foot (72.1 μg/g) and crystalline style (59.9 μg/g).

For Cd, crystalline style for both sites accumulated the highest levels, which were 3.60 μg/g (Nenasi) and 4.14 μg/g (Kuala Pontian),

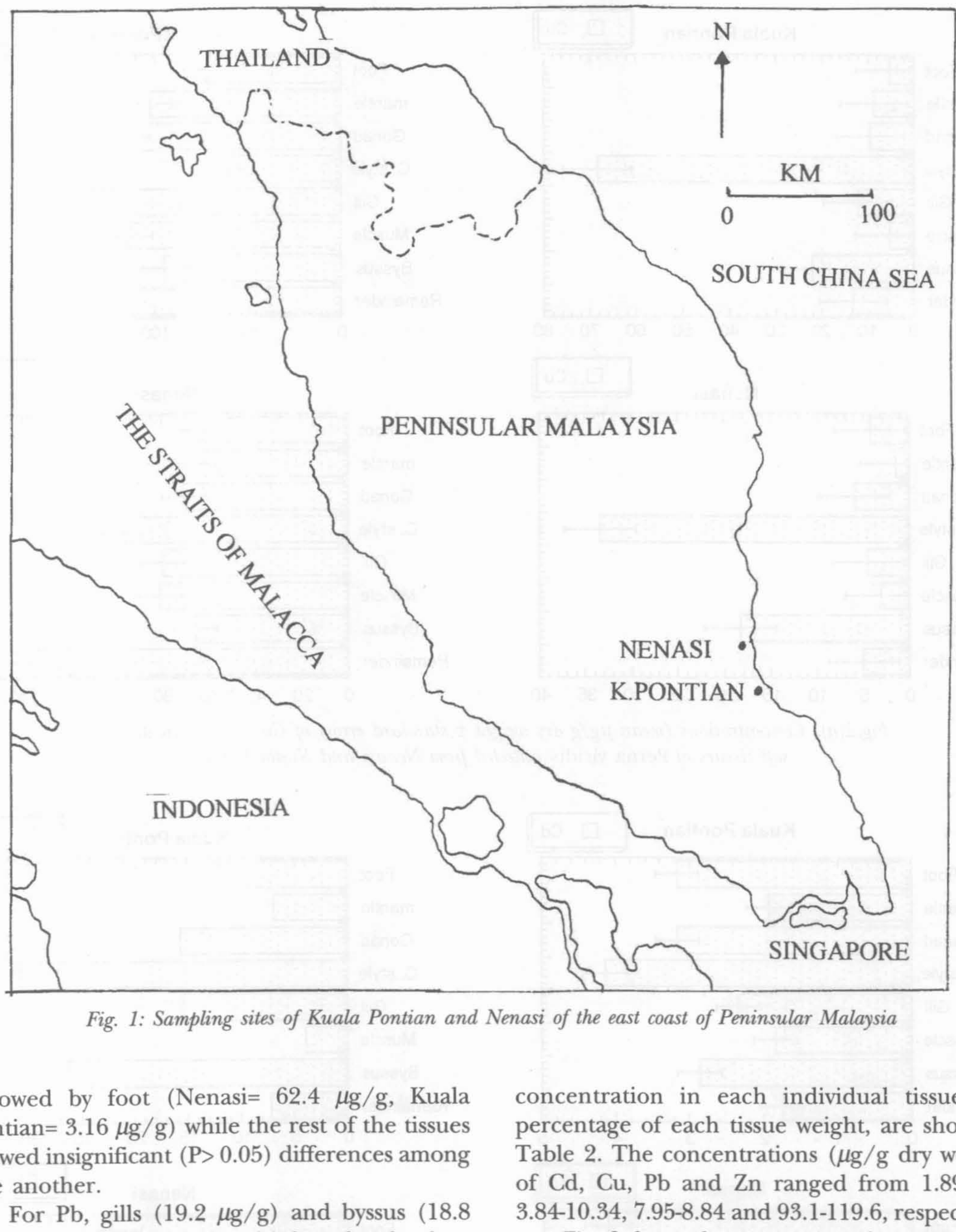


Fig. 1: Sampling sites of Kuala Pontian and Nenasi of the east coast of Peninsular Malaysia

followed by foot (Nenasi= 62.4 μg/g, Kuala Pontian= 3.16 μg/g) while the rest of the tissues showed insignificant (P> 0.05) differences among one another.

For Pb, gills (19.2 μg/g) and byssus (18.8 μg/g) accumulated the higher level when compared to the other tissues for Nenasi. In Kuala Pontian, crystalline style (30.9 μg/g) and byssus (26.3 μg/g) accumulated the highest level of Pb.

As shown in Table 1, shell length and shell width of the mussels collected from the two sampling sites show significant differences (P< 0.05) while shell height showed no significant difference (P> 0.05). The metal concentrations in total soft tissues, calculated based on metal concentration in each individual tissue and percentage of each tissue weight, are shown in Table 2. The concentrations (μg/g dry weight) of Cd, Cu, Pb and Zn ranged from 1.89-2.13, 3.84-10.34, 7.95-8.84 and 93.1-119.6, respectively.

Fig. 3 shows the comparison between metal concentrations of male and female *P. viridis*, based on T-tests carried out on the metal concentrations of remainder, mantle, muscle, gonad and foot. Most obviously, in both sites, female gonad accumulated significantly (P< 0.05) higher concentrations of Cu, Zn and Cd than in the male. However, for Pb, the male accumulated significantly (P< 0.05) higher level than the female. The rest showed no significant (P> 0.05) differences between the two genders.

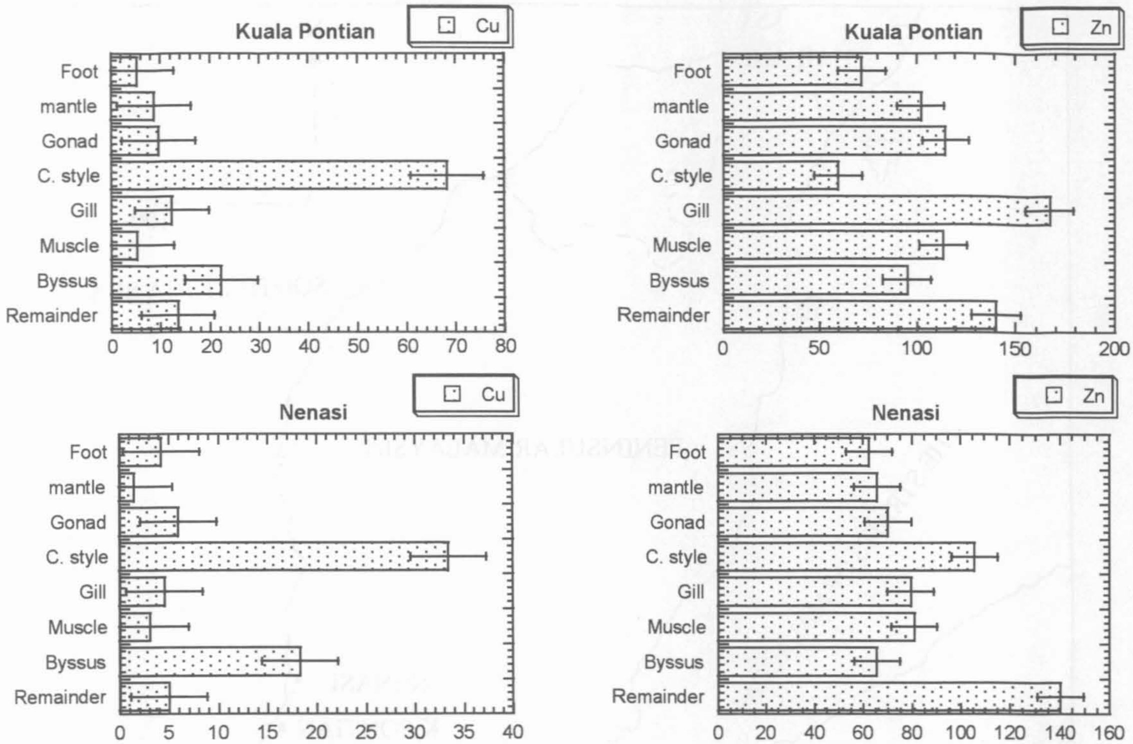


Fig.2(a): Concentrations (mean µg/g dry weight ± standard error) of Cu and Zn in different soft tissues of Perna viridis *collected from Nenasi and Kuala Pontian*

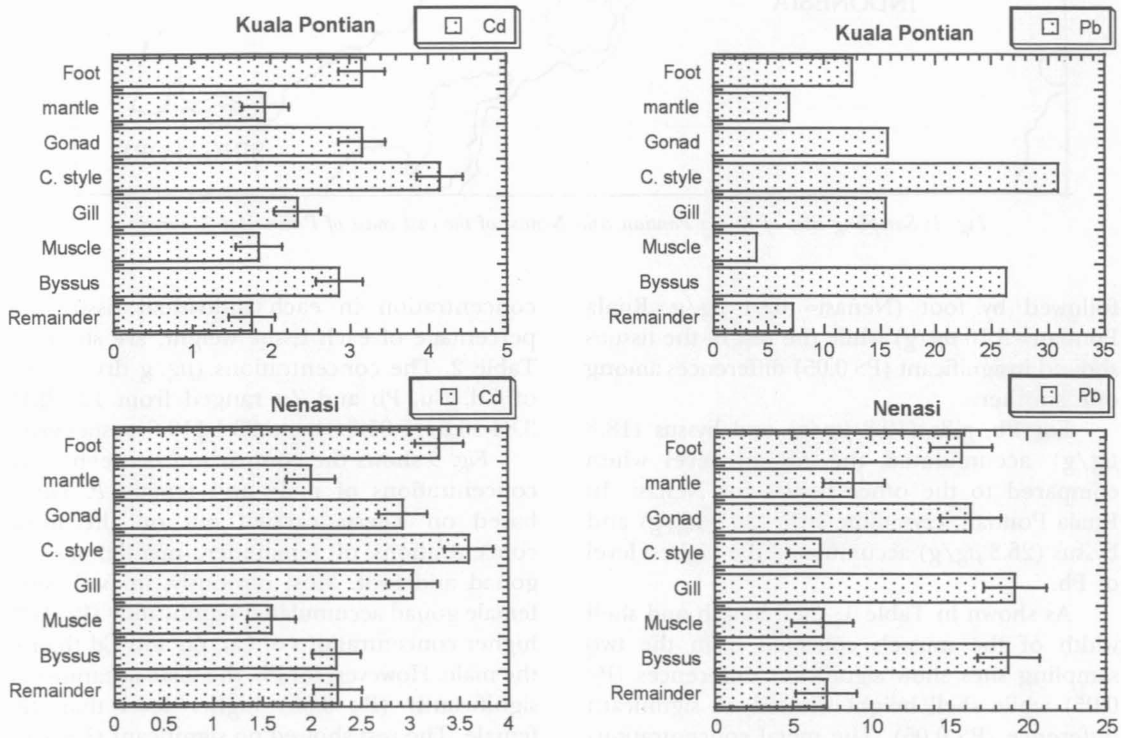


Fig. 2(b): Concentrations (mean µg/g dry weight ± standard error) of Cd and Pb in different soft tissues of Perna viridis *collected from Nenasi and Kuala Pontian*

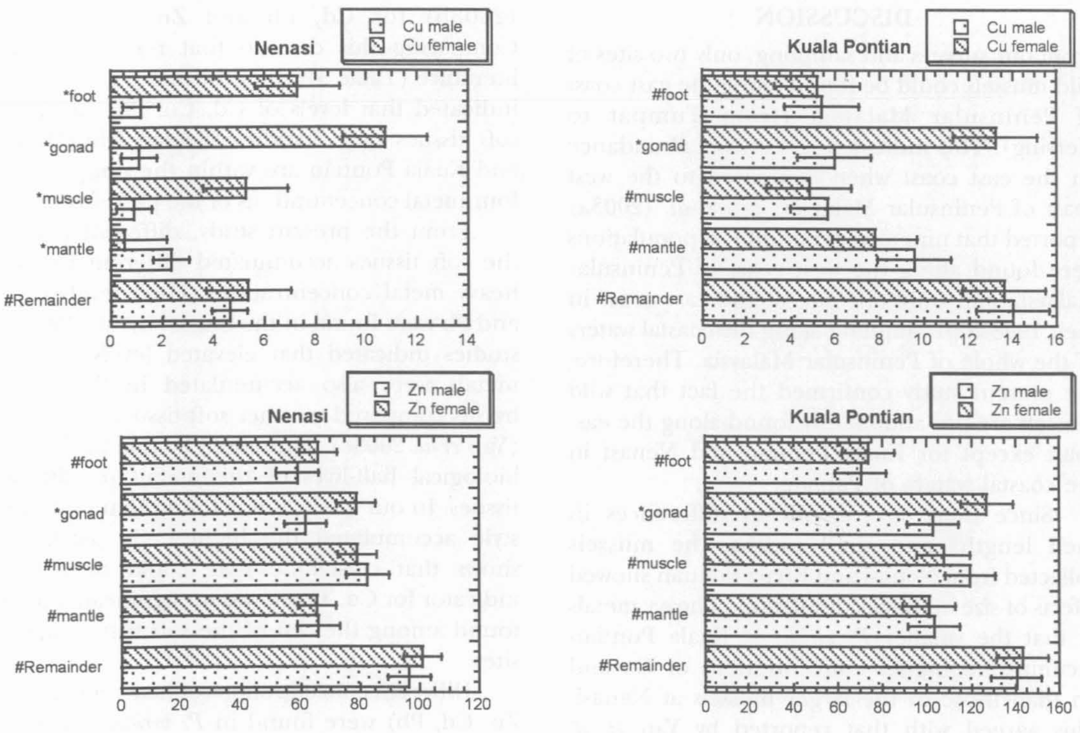
TABLE 1
Date of sampling, physico-chemical parameters and allometric data (mean ± standard error) of *Perna viridis* collected from Nenasi and Kuala (K.) Pontian

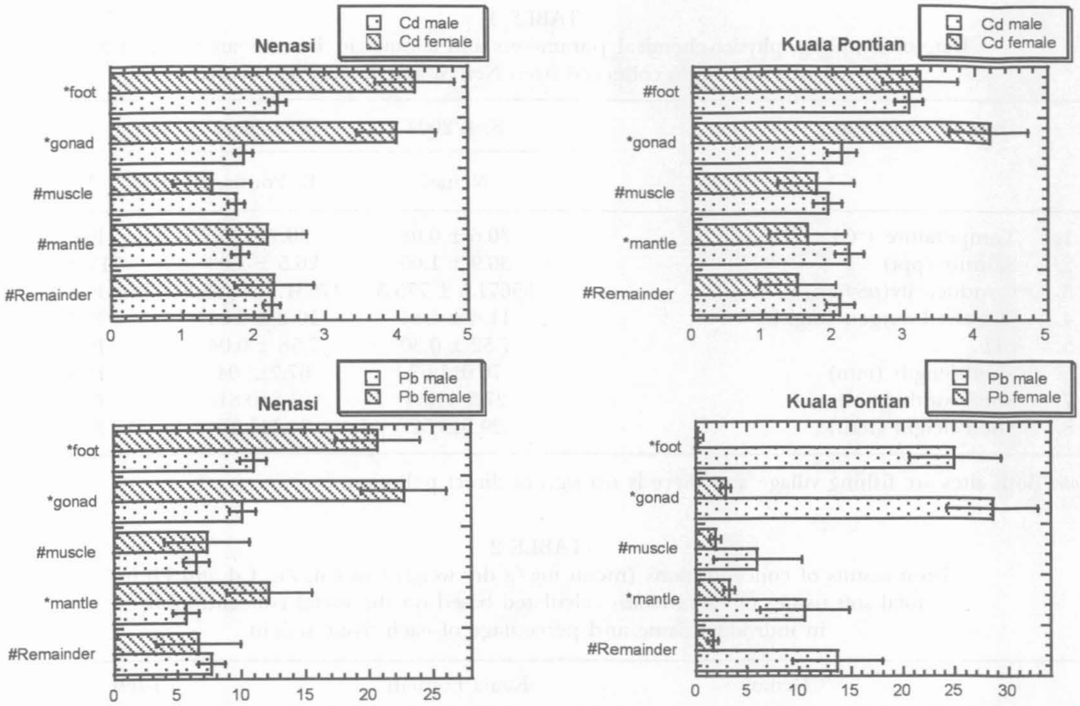
	Date of sampling	8/4/2004	8/4/2004	
		Nenasi	K. Pontian	T-test
1.	Temperature (°C)	30.6 ± 0.08	30.7±0.12	P>0.05
2.	Salinity (ppt)	30.9 ± 1.00	26.5 ± 1.00	P< 0.05
3.	Conductivity(μS/cm)	55671.5 ± 775.5	47831.5 ± 848.5	P<0.05
4.	Dissolved oxygen (mg/L)	11.4 ± 1.84	10.2 ± 0.04	P>0.05
5.	pH	7.52 ± 0.30	7.58 ± 0.04	P>0.05
6.	Shell length (mm)	76.6±1.67	67.7±3.04	P<0.05
7.	Shell width (mm)	27.3 ±1.64	21.8±0.81	P<0.05
8.	Shell height (mm)	29.5±3.52	34.1±1.16	P>0.05

Note: Both sites are fishing village and there is no sign of direct pollution.

TABLE 2
T-test results of concentrations (mean mg/g dry weight) of Cu, Zn, Cd and Pb in total soft tissues of *Perna viridis* calculated based on the metal concentration in individual tissue and percentage of each tissue weight

	Nenasi	Kuala Pontian	T-test
Cu	3.84	10.34	P<0.05
Zn	93.1	119.6	P>0.05
Cd	2.13	1.89	P>0.05
Pb	8.84	7.95	P>0.05





* Significant difference (P<0.05)
\# No significant difference (P>0.05)

Fig. 3: Heavy metal concentrations (mean µg/g dry weight ± standard error) in different soft tissues of male and female Perna viridis *in Nenasi and Kuala Pontian*

DISCUSSION

From our surveys and sampling, only two sites of wild mussels could be found along the east coast of Peninsular Malaysia (from Tumpat to Mersing). This indicated less mussel abundance on the east coast when compared to the west coast of Peninsular Malaysia. Yap *et al.* (2003a) reported that nineteen geographical populations were found along the west coast of Peninsular Malaysia and only one site at the east coast in their 1998-2001 sampling along the coastal waters of the whole of Peninsular Malaysia. Therefore, the present study confirmed the fact that wild mussels are not abundantly found along the east coast except for Kuala Pontian and Nenasi in the coastal waters of Pahang.

Since there are significant differences in shell lengths and shell widths, the mussels collected from Nenasi and Kuala Pontian showed effects of size on bioaccumulation of heavy metals in that the smaller *P. viridis* at Kuala Pontian accumulated higher concentrations of Cu and Zn than those in the larger mussels at Nenasi. This agreed with that reported by Yap *et al.* (2003b) for Cd, Pb and Zn in *P. viridis*. Comparing this data to that reported in the literature (Table 3), all of the above comparisons indicated that levels of Cd, Cu, Pb, and Zn in soft tissues of *P. viridis* collected from Nenasi and Kuala Pontian are within the ranges of the four metal concentrations of the previous studies.

From the present study, different parts of the soft tissues accumulated different levels of heavy metal concentrations. High levels of Cu and Pb were found in the mussel byssus. Previous studies indicated that elevated levels of heavy metals were also accumulated in the mussel byssus compared to other soft tissues of *P. viridis* (Yap *et al.* 2003c). This could be due to different biological half-lives of the metals in different tissues. In our study, it was found that crystalline style accumulated the highest Cu level. This shows that crystalline style could be a good indicator for Cu. There was no significant pattern found among the rest of the soft tissues at both sites.

Different concentrations of heavy metals (Cu, Zn, Cd, Pb) were found in *P. viridis* of the two

TABLE 3
A comparison of reported concentrations (μg/g) of Cd, Cu, Pb and Zn in the soft tissues of *Perna viridis* with the present results (WB: weight basis)

Location	WB	Cd	Cu	Pb	Zn	References
Regional studies						
The Gulf of Thailand	Dry	<0.02-19.1	1.50-11.3	-	25.7-79.0	Sukasem and Tabucanon (1993)
Putai coastal of Taiwan	Dry	-	1.78-5.41	-	14.4-25.7	Han *et al.* (1997)
South east coastal of India	Dry	1.59-4.40	33.6-49.2	2.48-6.92	60.4-94.1	Senthilnathan *et al.* (1998)
Tolo Harbour, Hong Kong	Dry	0.45-1.44	6.02-24	2.02-4.36	90.0-135	Wong *et al.* (2000)
Guang Dong market, China	Wet	0.38	2.05	0.18	9.9	Fang *et al.* (2001)
Fish cultured sites at Hong Kong	Dry	0.31-0.87	19.0-20.1	4.34-25.9	96.7-201	Wong *et al.* (2001)
Venezuala/Trinidad coastal waters	Dry	0.81 (0.1-3.05)	8.7 (5.1-17.2)	-	124.5 (446)	Astudillo *et al.* (2002)
Singapore coastal waters	Dry	< 0.20	28 (23-35)	5.6	280 (185-446)	Bayen *et al.* (2004)
Hong Kong (1998-2003)	Dry	0.66 (0.17-2.90)	37.15 (8.9-130)	7.65 (2.0-20.0)	116.4 (67-170)	Liu and Kueh (2005)
East coast of China	Dry	0.48-5.31	1.45-54.17	0.81-2.93	66.1-137.7	Fung *et al.* (2004)
Uncontaminated site at Kat O, Hong Kong	Dry	3.13-5.4	10.1-15.8	3.10-5.01	104-115	Nicholson and Szefer (2003)
Contaminated site at Kennedy Town, Hong Kong	Dry	1.02-1.30	16.4-18	3.76-6.98	126-152	Nicholson and Szefer (2003)
Malaysia's studies						
Penang, Malaysia	Dry	BDL	8	7	76	Sivalingam and Bhaskaran (1980)
Bau Merbok, Perak	Wet	0.05	1.93	0.24	13.8	Liong (1986)
Lekir, Perak	Wet	0.18	2.7	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 (20 sites; 1998-2001)	Dry	0.68-1.25	7.76-20.1	2.51-8.76	75.1-129	Yap *et al.* (2003a)
Peninsular Malaysia (10 sites)	Dry	0.10-2.88	2.09-8.55	0.20-1.69	52.12-95.43	Yusof *et al.* (2004)
Nenasi and Pontian, east coast of Peninsular Malaysia	Dry	1.89.-2.13	3.84-10.34	7.95-8.84	93.1-119.6	This study

WB=weight basis. BDL=below detection limit sites between genders. This could be due to the gonadal condition (Lobel and Wright 1982; Karaseva 1993). Female *P. viridis* were found to accumulate more heavy metals than male and this could be due to the presence of eggs, which are not released. The weight contribution of the eggs or ovum is also larger than the sperms of the male *P. viridis* and therefore was able to store more heavy metals (Yap *et al.* 2005 In press).

Since there were no observable pollution activities found between Nenasi and Kuala Pontian, the difference in metal concentrations between the two sites could be due to the significant ($P < 0.05$) differences in shell length, shell width and salinity. The salinity of both sites showed significant difference ($P < 0.05$) while temperature, dissolved oxygen and pH showed no significant difference ($P > 0.05$). It has been reported by Mo and Neilson (1993) that salinity could affect metal availability and changes in salinity may affect several physiological processes that influence the accumulation of trace metals by bivalves.

CONCLUSION

This study confirmed that *P. viridis* is not abundantly found on the east coast of Malaysia compared to the west coast. Different soft tissues have different binding affinities to heavy metals (Cu, Zn, Cd, and Pb). The crystalline style was found to accumulate high levels of Cu and therefore could be a good indicator of Cu contamination compared to other soft tissues. Differences in heavy metal levels between genders were also found and could be due to gonadal conditions (differences of weight contribution of sperms and eggs). The different levels of heavy metals found in the soft tissues of *P. viridis* collected from Nenasi and Kuala Pontian could be due to the significant ($P < 0.05$) differences in mussels' shell length and shell width and salinity of seawater.

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