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Ni, Pb AND Zn CONCENTRATIONS IN THE GREEN-LIPPED MUSSEL, *Perna viridis* COLLECTED FROM THE NORTHERN COASTAL WATERS OF PENINSULAR MALAYSIA

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Abstract: Continuous monitoring of heavy metal bioavailabilities and contamination in the marine environment by the Mussel Watch Program has been proposed in order to maintain the coastal environment at a sustainable level. This study focused on the heavy metal concentrations in different parts of *P. viridis* collected from Kuala Perlis (Perlis), Kg. Pulau Sayak (Kedah), Pantai Sri Tujoh and Pantai Bisikan Bayu (both in Kelantan). The results indicated that the accumulation of Ni, Pb and Zn by the byssus were significantly different from the rest of the tissues. In particular, the accumulation of Cu by the crystalline style was significantly higher ($P < 0.05$) from the other tissues. The above two findings were similar to those reported by Yap et al. (2005) and Yap et al. (2006a). However, the concentrations of Ni, Pb and Zn found in different parts of *P. viridis* were generally lower than those reported by Yap et al. (2006a) for the polluted eastern part of the semi-enclosed ecosystem in the Straits of Johore. The present findings are important in providing metal baseline information prior to the start of the newly launch Economic Development Corridor (NEDC) project.

KEYWORDS: Mussel Watch Program, northern part, Peninsular Malaysia, *Perna viridis*

Introduction

Yap et al. (2003b) reported heavy metal concentrations in *P. viridis* collected from Kuala Perlis, Tg. Dawai and Penang Bridge. Although all the three previously studied populations were collected from the northern coastal waters of Peninsular Malaysia, data on three out of the four populations in the present study had not yet been reported in the literature. Moreover, the present study sites are situated in the new Economic Development Corridors (NEDC) namely the Northern Corridor Economic Region (NCER) and the East Coast Economic Region (ECER) recently launched in 2006. Kuala Perlis (Perlis) and Pulau Sayak (Kedah) are situated in the NCER while Pantai Sri Tujoh and Pantai Bisikan Bayu (both in Kelantan) are located in ECER. Biomonitoring of metal bioavailabilities in the northern region by using *P. viridis* are again studied to provide baseline information on the current metal contamination status of the NEDC.

Mussels have been used in many parts of the world as biomonitors because of their sessile behaviour, long life, accumulation of heavy metals and the positive relationships of metal contents between mussels and their environment (Rainbow, 1995; Yap et al. 2002a). Programs such as the National Oceanic and Atmospheric Administration's National Status and Trends program, which began in the early 1980's (Sericano et al. 1995; Fung et al. 2004) and the California Mussel Watch, which was first implemented in 1977 (Martin, 1992; Fung et al. 2004) exemplified the use of this approach in pollutant biomonitoring. The Mussel Watch's role and suitability for biomonitoring programmes in the Indo-Pacific had been reviewed by Rainbow (1995). Recent mussel-based monitoring of heavy metal concentrations in Malaysian coastal waters had also proven to be effective (Yusof et al. 1994; Yap et al. 2002a, 2003b, 2004a, b, 2006a, 2007).

Since reports on the use of *P. viridis* to monitor heavy metal contamination in the northern regions of Peninsular Malaysia are scarce, the objective of the present study was to determine the heavy metal concentrations in different parts of *P. viridis* collected from the northern coastal waters of Peninsular Malaysia.

Materials and Methods

The samples were collected during trips to the northern region of Peninsular Malaysia (Figure 1) between June to July 2007. The description of the sampling locations is shown in Table 1. The collected mussels were immediately placed in an ice compartment and transported to the laboratory for analysis. Prior to the metal analyses, 20 individual mussels were carefully dissected into types of soft tissues (remainder, mantle, muscle, gills, gonad, foot, byssus and crystalline style-CS). Each category of tissues was then placed in separate aluminium foils. They were dried in the oven for 72 hours at 60 ºC to constant dry weights. Dried samples were then stored in clean plastic bags.

Triplicates of each dried category of bivalve tissues were digested in ten mL concentrated HNO_3 (Analar grade, BDH 69 %). They were first placed in a hot-block digester first at a low temperature for 1 hour and were then fully digested at a high temperature (140 °C) for 3 hours (Yap et al. 2003a). The digested samples were diluted to 40 mL with double-distilled water (DDW). After filtration, heavy metal concentrations were determined using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin-Elmer Model Aanalyst 800. The detection limits of the AAS for Ni, Pb and Zn were 0.009, 0.080 and 0.010 mg L^{-1}, respectively. The data are presented in µg/g dry weight.

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

A one-way ANOVA-Student-Newman-Keuls (S-N-K), was applied to detect significant differences among the mean values using a statistical software, SPSS version 12. All the data were log10 (X + 1) transformed prior to cluster analysis using STATISTICA 99 edition.

Results and Discussion

Distribution of heavy metals in different parts of P. viridis

The distributions of Ni, Pb ad Zn concentrations in different parts of *P. viridis* are shown in Table 3. As shown in Table 3, the accumulations of Pb, Ni and Zn were elevated in the byssus of *P. viridis*. The distributions of heavy metal in the different parts of *P. viridis* were further explained by the cluster analysis (Figure 2). In general, it was found that the byssus was clustered differently from the rest of the soft tissues.

The distributions of heavy metals in different soft tissues of the *P. viridis* can be explained as follows. The differences in the affinities of the metals to the binding sites of the metallothioneins in different soft tissues (Roesijadi, 1980; Viarengo et al. 1985) could influence the accumulation levels of metals found in the mussel. Besides, the function or the location of a specific organ in the *P. viridis* could also be associated with the metal accumulation in different tissues of the mussel.

Varying levels of Pb, Ni and Zn found in the byssus of the mussel could be due to the excretion of the metals mainly through the byssus (Goldberg et al. 1978; Yap et al. 2003a; Yap et al. 2005a; Yap et al. 2007). This is an indication of the bioavailability of metals for the production of the byssal thread.

The metal concentrations found on the gill surfaces as exhibited when comparing the metal concentrations between different parts of the tissues in the four locations (Table 3), might be due to their direct contact with the seawater (Phillips and Rainbow, 1993; Yap et al. 2006a, b). The large surface areas of mussel gills increase their metal uptake through facilitated diffusion (Phillips and Rainbow, 1993; Yap et al. 2003a). Besides, metal concentrations in the mantle and foot which were due to their contacts with the external medium, were also considered to be responsible for the transfer of the metals into the organisms. Yap et al. (2005b) also mentioned that the variations of the metal concentrations accumulated in the soft tissues could be due to the salinity of the water at the sampling sites. Other environmental factors could also cause differences in the metal bioavailabilities in coastal waters that were reflected in the different metal concentrations accumulated in different soft tissues of the mussels (Yap et al. 2005b).

Comparisons of metal bioavailabilities in the sampling locations

Table 3 shows the concentrations of Ni, Pb and Zn in different tissues of *P. viridis* based on the four geographical populations. 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 site. Therefore, comparisons of such accumulated concentrations in a biomonitor among sites are measurements of the bioavailabilities and contamination of heavy metals of the sampling sites (Phillips and Rainbow, 1994). Heavy metal concentrations in different parts of *P. viridis* indicated by the present study are compared with those of previously reported regional studies (Table 4). It was found that metal concentrations in most parts of different tissues were higher than the metal concentrations found in the total soft tissues reported by other studies. Although the different soft tissues were compared with the total tissues, it is important to know which tissue(s) accumulate heavy metal(s). However, when these results were compared to studies from the Johore Straits and Pantai Pasir Panjang, it was found that the metal concentrations found in different tissues obtained in this study were within or lower than the ranges of those which had been reported based on similar tissues of *P. viridis*.

Anthropogenic activities at the sampling locations could have contributed to the metal bioavailabilities and contaminations of these areas. From Table 1, it is known that Kuala Perlis and Pantai Bisikan Bayu are fishing villages while Pantai Sri Tujoh and Kg. Pulau Sayak are fish and mussel aquaculture sites. Therefore, it is suggested that the metal bioavailabilities of these four sampling locations could mainly be due to aquacultural activities conducted in the areas besides natural sources. Yap et al. (2002b) reported that heavy metals such as Pb contamination originated from aquacultural activities. Organic waste discharged from fish/or mussel farms effect the water quality around fish culture zones (Wu et al. 1994; Yap et al. 2003b) which could contribute to the bioavailabilities and contamination of the metals.

Conclusion

The concentrations of Ni, Pb and Zn in the byssus were significantly different from the rest of the soft tissues. High bioavailabilities of Ni, Pb and Zn were found in Pantai Sri Tujoh, Pantai Bisikan Bayu and Kg. Pulau Sayak, respectively. Since the effectiveness of using of *P. viridis* to monitor heavy metal bioavailabilities and contaminations is proven, continuous monitoring studies in Malaysian coastal waters especially in the northern and eastern coastal waters of the Peninsular Malaysia is proposed due to the recently launching of the New Economic Development Corridor-NEDC. It is important to understand the fluctuations of metal levels in the northern coastal waters of Peninsular Malaysia, as part of concerted efforts to maintain our sustainable marine resources.

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Table 1: Site descriptions for the mussel populations

No.	Location	Sampling date	Longitude	Latitude	Description of sampling site
1.	Kuala Perlis	09.06.2007	6°23'34.''N	100°07'48.''E	A fishing village
2.	Kg Pulau Sayak	10.06.2007	5°40'46.''N	100°22'20.''E	An offshore, fish and mussel farming area
3.	Pantai Sri Tujoh	29.06.2007	06°13'05."N	102°07'43."E	Aquacultural area, A beach
4.	Pantai Bisikan Bayu	03.07.2007	5°51'56.'' N	102°29'38."E	A fishing village

Table 2: Analytical results for the Certified Reference Material (CRM) and the certified value for each metal (All values are presented as µg/g dry weight).

Metal	Sample	CRM values	Measured values	Percentage of recovery
Ni	DOLT-3 Dogfish-liver	2.72 ± 0.350	2.77 ± 0.741	102 ± 27.2
Zn	DOLT-3 Dogfish-liver	86.6 ± 2.40	80.9 ± 1.94	93.4 ± 2.24

Table 3: Distributions and comparisons of heavy metal concentrations (µg/g dry weight) in the different soft tissues of *Perna viridis* collected from four sampling locations.

Tissue	Site	Ni	S-N-K	Pb	S-N-K	Zn	S-N-K
Byssus	PST	21.37±0.24	A	22.37±0.70	A	93.22±0.06	B
	PBB	21.10±0.17	A	21.06±0.86	A	92.88±0.35	B
	KP	10.92±0.09	C	15.81±0.17	B	145.24±0.12	A
	KPS	12.88±0.78	B	16.42±0.73	B	202.93±0.58	A
CS	PST	10.22±0.81	A	6.24±0.79	B	77.88±0.63	B
	PBB	5.32±0.10	B	9.95±0.12	A	63.89±0.33	C
	KP	1.73±0.05	C	6.78±0.24	B	44.32±0.08	D
	KPS	6.11±1.49	B	2.20±0.35	C	101.28±1.62	A
Foot	PST	6.48±0.06	A	0.55±0.18	C	58.90±0.21	A
	PBB	5.05±0.11	A, B	6.02±0.14	A	73.10±0.34	A
	KP	3.18±1.15	B	5.60±0.18	B	52.38±1.22	A
	KPS	3.01±0.51	B	5.08±0.54	B	71.88±15.79	A
Gill	PST	12.06±1.35	A	7.24±1.30	A	69.88±1.48	C
	PBB	8.31±1.92	A, B	10.29±0.73	A	97.45±1.87	B
	KP	6.07±0.84	B, C	7.39±0.11	A	65.64±2.32	C
	KPS	2.98±0.45	C	9.03±0.44	A	146.53±8.74	A
Gonad	PST	9.09±0.20	A	7.70±0.55	B	67.25±0.22	B
	PBB	3.23±0.16	C	10.08±0.16	A	117.38±0.26	A
	KP	6.44±0.22	B	6.46±0.68	B	49.63±0.92	B
	KPS	2.53±0.33	C	2.61±0.45	C	99.01±14.20	A
Mantle	PST	7.50±2.00	A	2.92±0.13	C	53.55±2.09	C
	PBB	6.76±0.88	A	8.13±0.70	A	89.85±1.27	B
	KP	3.30±0.92	A	7.26±0.72	A	39.44±1.64	D
	KPS	2.81±0.38	A	5.53±0.09	B	128.61±7.11	A
Muscle	PST	14.11±0.12	A	3.13±0.08	B	49.28±2.17	C
	PBB	6.87±0.85	B	7.11±0.73	A	77.39±5.59	B
	KP	6.70±0.59	B	6.17±0.04	A	41.46±1.46	C
	KPS	1.44±0.63	C	6.54±0.62	A	148.33±15.99	A
Remainder	PST	9.14±1.65	A	5.38±0.62	B	81.08±0.98	C
	PBB	9.58±1.34	A	12.99±2.09	A	123.56±4.41	B
	KP	6.28±0.33	A	5.90±0.13	B	61.25±0.68	C
	KPS	4.44±1.47	A	6.57±1.52	B	166.05±15.08	A

Note: CS = Crystalline style; S-N-K= Student-Newman-Kuels; PST= Pantai Sri Tujoh; PBB= Pantai Bisikan Bayu; KP=Kuala Perlis; KPS= Kg. Pulau Sayak. Same alphabets indicate that they are not significantly different ($P > 0.05$).

Table 4: Comparisons of heavy metals concentrations (µg/g dry weight) in the tissues of *Perna viridis* from previous regional studies with those from the different soft tissues obtained in this study.

Study locations	Tissue	Ni	Pb	Zn	Reference
Tolo Harbour, Hong Kong	Total soft tissues	3.25-6.87	2.02-4.36	90-135	Wong et al. (2000)
The Gulf of Thailand	Total soft tissue	0.41-3.22	0.19-3.75	24.9-213	Ruangwises and Ruangwises (1998)
Singapore coastal water	Total soft tissue	3.80-13.0	3.40-7.90	185-446	Bayen et al. (2004)
Langkawi, Malaysia	Total soft tissue	NA	4.02-4.38	94.52-100.28	Yap et al. (2003b)
Tanjung Dawai, Kedah Malaysia	Total tissue	NA	0.86-8.41	84.38-122.6	Yap et al. (2003b)
Penang coastal area, Malaysia	Total tissue	NA	3.43-4.76	108.7-109.6	Yap et al. (2003b)
The Johore Straits, Malaysia (2004) (8 sites)	Gill	4.64-127	5.90-24.9	61.7-167	Yap et al. (2006a)
	Gonad	3.04-121	4.69-20.9	49.5-133.5	Yap et al. (2006a)
	Foot	2.49-87.5	2.68-22.9	40.7-89.7	Yap et al. (2006a)
	Muscle	0.08-91.6	5.51-20.4	47.9-105	Yap et al. (2006a)
	Mantle	2.30-132	5.50-38.9	39.1-108	Yap et al. (2006a)
	Remainder	3.30-108	6.98-26.2	62.7-145	Yap et al. (2006a)
Uncontaminated Pasir Panjang, Peninsular Malaysia	CS	NA	0.79±0.09	3.92±0.32	Yap et al. (2007)
	Byssus	NA	2.70±0.13	19.56±2.11	Yap et al. (2007)
	Gonad	NA	0.59-1.10	16.01-21.79	Yap et al. (2007)
	Remainder	NA	1.07-1.38	17.25-23.98	Yap et al. (2007)
	Foot	NA	0.93±0.13	14.39±0.76	Yap et al. (2007)
	Muscle	NA	0.85±0.24	16.59±0.60	Yap et al. (2007)
Northern coast of Peninsular Malaysia (4 Sites)	Gill	2.98-12.06	7.39-9.03	65.64-146.53	Present Study
	Muscle	1.44-14.11	3.13-7.11	41.46-148.33	Present Study
	CS	1.73-10.22	2.20-9.95	44.32-101.28	Present Study
	Foot	3.01-6.48	0.55-6.02	52.38-73.10	Present Study
	Mantle	3.30-7.50	2.92-8.13	39.44-128.61	Present Study
	Remainder	4.44-9.58	5.38-12.99	61.25-166.05	Present Study
	Gonad	2.53-9.09	2.61-10.08	49.63-117.38	Present Study
	Byssus	10.92-21.37	15.81-22.37	92.88-202.93	Present Study

Note: CS = Crystalline style; NA = Not available

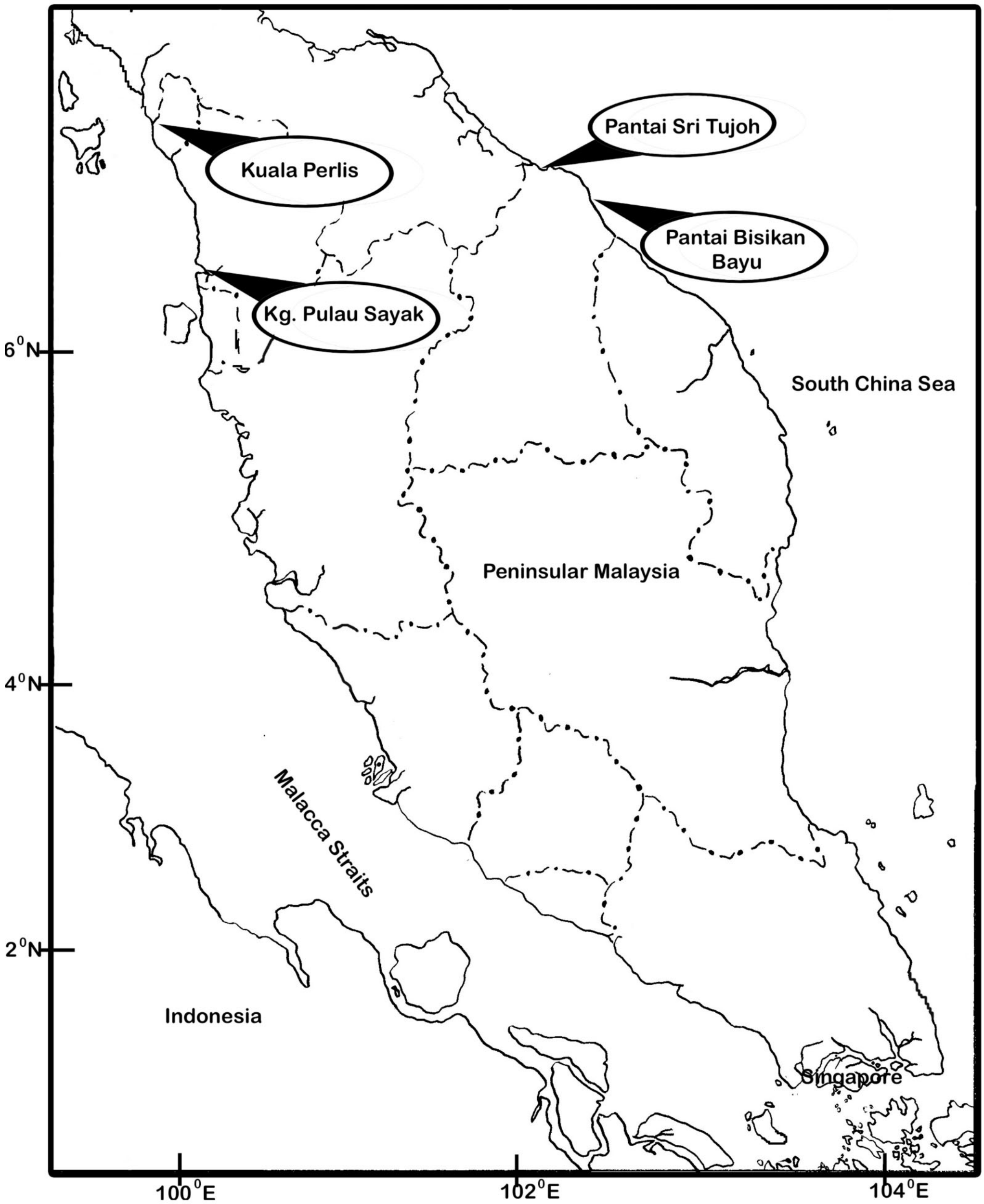

Figure 1: Map showing the sampling sites of *Perna viridis* in the northern parts of Peninsular Malaysia.

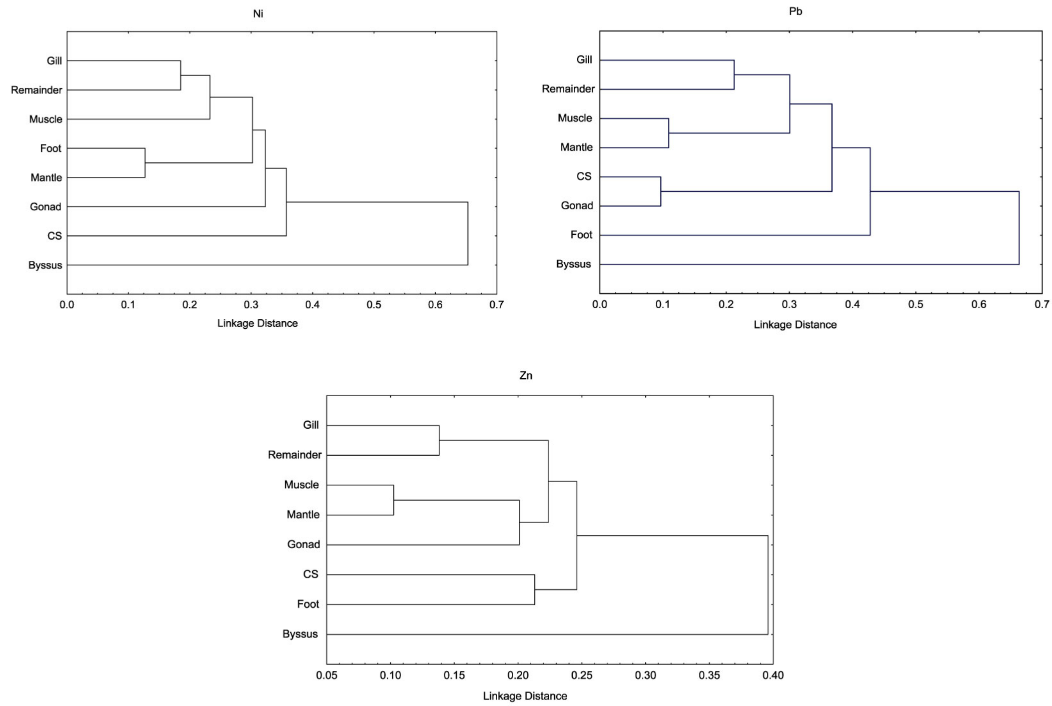

Figure 2: Euclidean distance cluster analysis of different parts of *Perna viridis*, based on samples collected from Kg. Pulau Sayak, Kuala Perlis, Pantai Sri Tujoh and Pantai Bisikan Bayu. Cluster made based on log_{10} (X+1) transformed data.

Note: CS= Crystalline style