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A Study on the Potential of the Periostracum of *Perna viridis* as a Biomonitoring Material for Pb in Tropical Coastal Waters Based on Correlation Analysis

(Kajian Potensi Periostracum *Perna viridis* Sebagai Bahan Biopenunjuk Pb di Perairan Pantai Tropika Berasaskan Analisis Korelasi)

C.K. YAP* & S.G. TAN

ABSTRACT

The periostracum is the outermost layer overlying the inner prismatic and nacreous layers of the shells of bivalves. In the present study, the distributions of Cd and Pb in the soft tissues (ST) and periostracum of the green-lipped mussel Perna viridis *sampled from 15 sampling sites in the coastal waters of Peninsular Malaysia were determined. The concentrations of Cd (0.21-10.87 mg/g dry weight) and Pb (1.16-40.20 mg/g dry weight) in the periostracum were generally higher than those in the ST (Cd: 0.10-5.55 mg/g dry weight; Pb: 2.53-18.62 mg/g dry weight). Based on correlation analysis from nine geographical populations, the higher correlation coefficients (R values) between the periostracum-geochemical fractions of the sediments than between the ST-geochemical fractions of the sediments indicated that the periostracum could be a potential biomonitoring material for Pb. Hence, the present results supported the use of the periostracum of* P. viridis *as a potential biomonitoring material for Pb but not for Cd. However, more studies are warranted to verify its usefulness for the biomonitoring of heavy metal pollution in tropical coastal waters.*

Keywords: Biomonitoring material; Cd; Pb; periostracum; Perna viridis

ABSTRAK

Periostrakum adalah lapisan luar yang meliputi perismatik dalaman dan lapisan nakreus bagi cangkerang bivavia. Di dalam kajian ini, taburan Cd dan Pb di dalam tisu lembut dan periostrakum telah ditentukan pada kupang Perna viridis *yang disampel dari 15 kawasan di pantai Semenanjung Malaysia. Kepekatan bagi Cd (0.21-10.87 mg/g berat kering) dan Pb (1.16-40.20 mg/g berat kering) di dalam periostrakum secara amnya adalah lebih tinggi daripada tisu lembut (Cd: 0.10-5.55 mg/g berat kering; Pb: 2.53-18.62 mg/g berat kering). Berdasarkan analisis korelasi daripada 9 populasi geografi, korelasi koefisien (nilai R) yang lebih tinggi di antara periostrakum- fraksi geokimia bagi sedimen daripada tisu lembut-fraksi geokimia bagi sedimen, menunjukkan potensi periostrakum sebagai bahan biopenunjuk bagi Pb. Oleh itu, keputusan ini menyokong penggunaan periostrakum* P. viridis *sebagai bahan biopenunjuk bagi Pb tetapi bukan bagi Cd. Walau bagaimanapun, kajian lanjutan perlu dilakukan untuk mengesahkan penggunaannya dalam kajian pemantauan logam berat di kawasan persisiran pantai tropika.*

Kata kunci: Bahan biopenunjuk; Cd; Pb; periostrakum; Perna viridis

INTRODUCTION

Coastal wetlands which include marine and estuarine wetlands are unique because of their hydrologic properties and their roles as ecotones between terrestrial and aquatic systems (Donald 2001). They potentially receive impacts from land-based activities through riverine inputs, thus, their use of a suitable biomonitoring material for pollutants is important for this intertidal resourceful ecosystem.

In Malaysia, the green-lipped mussel *Perna viridis* is chosen and had proven to be a good biomonitoring organism of heavy metals especially for Cd, Cu and Pb (Yap et al. 2003a) in the west coast waters of Peninsular Malaysia. However, the accumulation of metals in the total soft tissues (ST) of marine mussels is affected by both biotic and abiotic factors (Phillips 1980; Phillips & Rainbow 1993). The fact that metals are accumulated differentially in the tissues (soft and hard) of *P. viridis* indicated that certain organs or materials are better at accumulating metals than others (Yap et al. 2003b). Recently, the byssus (Yap et al. 2003c) and total shell (Yap et al. 2003d) were studied for their potential as biomonitoring organs/materials for Zn. In the literature, there is no report on heavy metal concentrations in the periostracum and its potential as a biomonitor of metals has also not yet been determined.

The use of the shell of mussel for the biomonitoring of metal contamination in coastal waters is not popular among researchers. Most researchers used total ST since there is a lot of such information available in the literature. However, recently some researchers reported the use of mussel shells for biomonitoring studies although their use is still open to argument (Yap et al. 2003d). Several authors had investigated heavy metal concentrations in the shells of mussels (Al-Dabbas et al. 1984; Carell et al. 1987; Galloway et al. 1983; Goldberg 1980; Koide et al. 1982; Phillips 1980; Puente et al. 1996; Richardson et al. 2001; Sturesson 1976, 1978; Szefer & Szefer 1985; Wilbur & Saleuddin 1983) and other molluscs (Carvo et al. 2002; Dermott & Lum 1986; Foster & Chacko 1995; Szefer & Szefer 1985; Walsh et al. 1995). Most of the studies concluded that shells could be potential biomonitoring indicators due to their ability to accumulate a wide range of metals (Dodd 1963; Foster & Chacko 1995) and some trace metals are incorporated into the shells through the substitution of calcium ions in the crystalline phase of the shell or are associated with the organic matrix during shell growth and must have been assimilated by the mussel (Wilbur & Saleuddin 1983; Watson et al. 1995). According to Simkiss (1983), the suitability of *Perna viridis* shell is enhanced by its complex polylayer shell which increases the potential for mineral and chemical variations.

The periostracum of bivalves consists of proteins that have been sclerotized by quinone tanning to give the characteristic horny texture. Furthermore, the quinone tanning of the periostracum has been postulated to be an essential prerequisite for an orderly deposition of calcium carbonate crystals (Taylor & Kennedy 1969). However, the periostracum layer covers only a small portion (about 5%) of the total shell weight and the mussel shell consists mainly of two calcareous inorganic layers (prismatic and nacreous). The periostracum is the first newly formed layer and is an exposed layer that protects against abrasive conditions such as physical forces (Akberali & Trueman 1985; Imlay 1982).

Most researchers used the shells for biomonitoring studies but did not mention which layers of the shells were analysed. It is assumed that total shells were homogenised and analysed. Since the metal levels in the periostracum of *P. viridis* have not yet been reported, the objectives of this study were to report the concentrations of Cd and Pb in the periostracum of *P. viridis* and to determine the potential use of the periostracum as a biomonitoring material for Cd and Pb, based on correlation analysis between shell-geochemical fractions of metals in the sediments.

MATERIALS AND METHODS

The mussel *Perna viridis* samples were collected from 15 geographical sampling sites along the west coast of Peninsular Malaysia (Figure 1). Sediments were only collected from 9 of the 15 geographical sampling sites due to unavoidable problems such as whether conditions. The description of each site and the number of mussels analysed and their shell lengths are presented in Table 1. The sampling sites in this study included different backgrounds such as aquacultural area, agricultural area, recreational site, port and urban areas, mooring and pristine sites. Samplings for all the sites were conducted between 1998-2004. The mussels were transported to the laboratory and stored at -10°C prior to analysis.

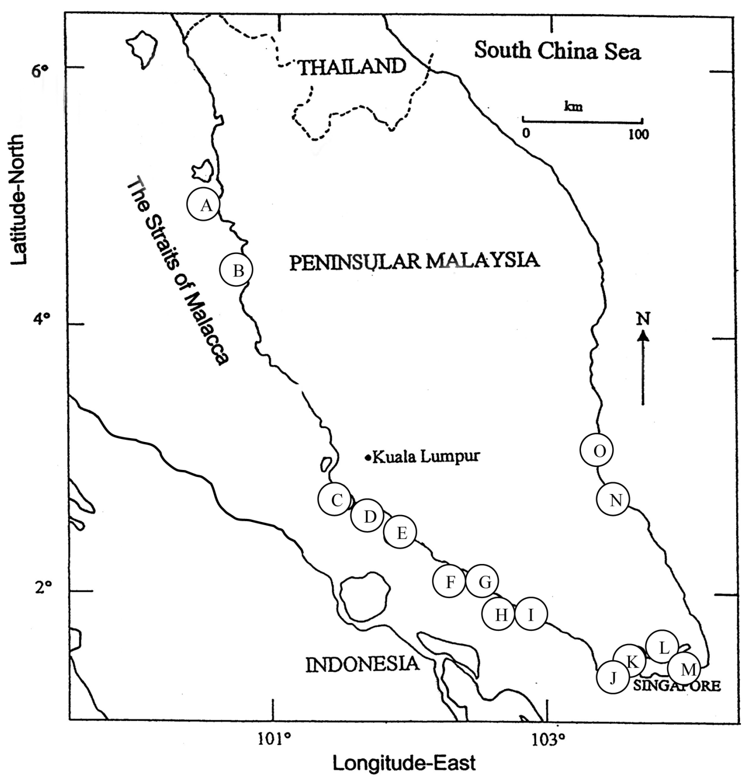

FIGURE 1. Sampling sites of green-lipped mussel Perna viridis from the west coast of Peninsular Malaysia. Names of sampling sites represented by alphabets follow those in Table 1

TABLE 1. Sampling dates and shell lengths of Perna viridis analyzed and descriptions of sampling sites in the coastal waters of Peninsular Malaysia. Shell lengths are in mm. About 15-20 individuals were analysed from each sampling site

	Location	Sampling date	Shell length (mean: mm)	Description of sampling site
A	*Pulau Aman	11 Sep1999	91.50	A fish aquacultural area
B	Bagan Tiang	01 April 2002	135	Aquacultural area
C	*Bagan Lalang	08 Jun 1998	91.2	Recreational and agricultural areas
D	*Lukut	08 Aug 1998	93.9	An aquacultural area
E	*Pasir Panjang	22 Sep 1998	88.6	A mussel aquacultural area
F	*Kuala Linggi	21 Nov 2000	80.0	Fish and mussel aquacultural area
G	Merlimau-1	19 April 2002	68.55	Aquaculture
	Merlimau-2	09 April 2004	82.30	Aquaculture
H	Telok Emas	09 April 2004	84.61	Aquaculture
I	*Sebatu-1	12 Aug 2000	85.4	Agricultural and mussel aquacultural areas
	Sebatu-2	19 February 2002	63.04	Agricultural and mussel aquacultural areas
J	*Tanjung Kupang	19 Jan 2000	83.6	Port and aquacultural areas
K	*Pantai Lido-1	23 Sep 1998	59.4	Urban and agricultural areas
	Pantai Lido-2	17 April 2002	89.08	Urban and agricultural areas
L	*Kg. Pasir Puteh-1	19 Jan 2000	61.10	Industrial and mooring activities and urban areas
	Kg. Pasir Puteh-2	17 April 2002	92.84	Industrial and mooring activities and urban areas
M	Kuala Belungkor	18 April 2002	64.94	Pristine area
N	Kuala Pontian	08 April 2004	70.11	Mussel aquacultural site
O	Nenasi	08 April 2004	76.11	A light house near shore; pristine water.

Note: *indicated where sediments were also collected in the mussel habitats. The numbers(1 and 2) after the names of sampling sites indicated different sampling periods.

SAMPLE PREPARATION

In the laboratory, the STs of mussels were thawed and then carefully separated from the shell and the byssus was pulled out. The shells were cleaned under a jet of tap water to remove the encrusting algae, barnacles, mud or bryozoa. After rinsing with double distilled water (DDW) and 0.5% of concentrated HNO_3 (AnalaR grade, BDH 69%), the mussels were dried for 72 h at 105°C to a constant dry weight (Mo & Neilson 1994). The periostracum layer on the edge of the mantle was taken for analysis since it was easily isolated from the other shell components after drying. From each site, the periostracum from 20 mussels were pooled while the STs were analysed individually.

Duplicates from each sampling site were analysed. For the sequential extraction technique (SET) of Pb from the sediments, the modified SET described by Badri & Aston (1983) was followed. The four fractions extracted were as follows:

1. EFLE: Around 10 g of powdered sample was agitated for 3 h in 50 mL of 1 *M* ammonium acetate (NH_4CH_3COO), maintained at pH 7 under room temperature.
2. Acid-reducible: The residue from (1) was agitated for 3 h in 50 mL of 0.25 *M* hydroxylammonium hydrochloride ($NH_2OH.HCL$), maintained at pH 2 under room temperature.
3. Oxidisable-organic: The residue from (2) was first oxidized with H_2O_2 (30% v/v) in a water bath at 90-95°C. After cooling, the reaction mixture was agitated for 3 h after adding 50 mL of 1 *M* ammonium acetate (NH_4CH_3COO) acidified to pH 2.
4. Resistant: The residue from (3) was digested in a mixture of concentrated nitric acid (69%) and perchloric acid (60%).

The residue used in each leaching step was weighed before the next fractionation was carried out. The residue was washed with 20 mL double distilled water, then filtered through Whatman No 1 filter-paper, and the filtrate was stored until metal determination. At each leaching step, a reagent blank was also subjected to the extraction and finally used as a calibration blank to account for any external contamination. The metal concentrations are presented in mg/g on dry weight basis.

SAMPLE DIGESTION AND DETERMINATION OF CD AND PB LEVELS

Concentrated HNO_3 (AnalaR grade, BDH 69%) was added to the samples in long test tubes. They were placed in a hot-block digester first at a low temperature (40°C) for one hour and then were fully digested at high temperature (140°C) for at least 3 h. The samples were completely digested after the acidic solutions were clear (Yap et al. 2003b). The digested samples were then diluted to a certain volume (40 mL) with DDW. After filtration, the prepared samples were determined for Cd and Pb by using the flame-acetylene technique of an atomic absorption spectrophotometer (AAS) Perkin-Elmer Model 4100. The data are presented in mg/g dry weight basis. To avoid possible contamination, all glassware and equipment were acid-washed. Procedural blanks were analysed once for every five samples. Quality control samples made from standard solutions of Cd and Pb were analysed once for every five samples to check for the metal recoveries. The percentages of recoveries were 110% for Cd and 115% for Pb.

The relationships of metal concentrations between the geochemical fractions of the sediment and the total soft tissue/periostracum of *Perna viridis* were based on double-log scale.

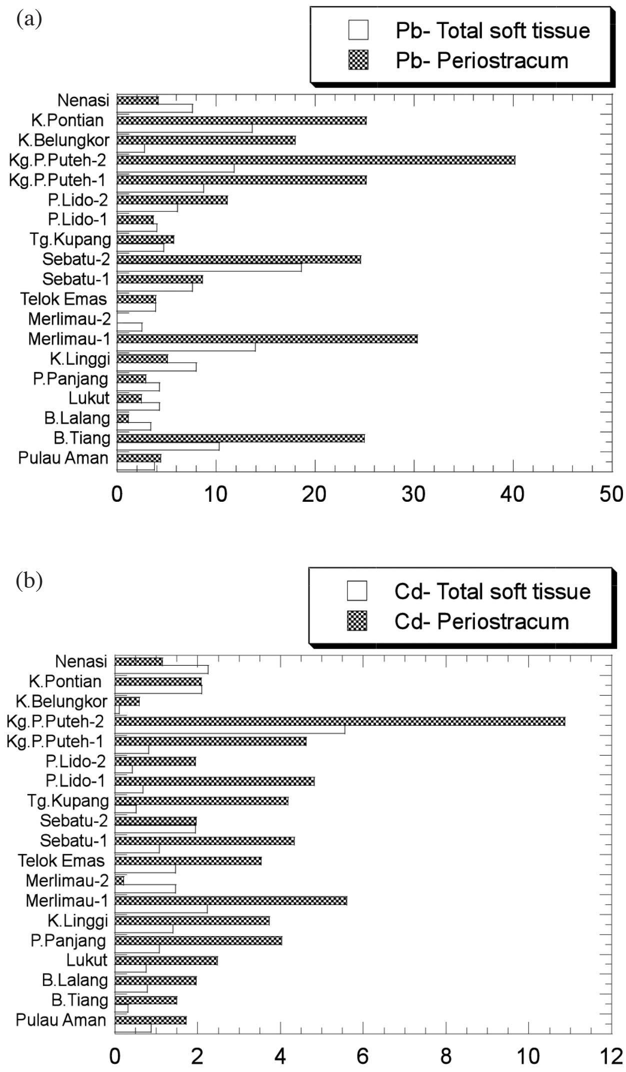

FIGURE 2. Concentrations of (a) Pb and (b) Cd and Pb in the total soft tissues and periostracum of the green-lipped mussel *Perna viridis* collected from the coastal waters of Peninsular Malaysia

RESULTS

The concentrations of Cd and Pb in the total STs and periostracum of *P. viridis* are presented in Figure 2 and Tables 2 and 3. Mussels from all the sampling sites showed higher concentrations of Cd in the periostracum (0.21-10.87 mg/g dry weight) than in the ST (0.10-5.55 mg/g dry weight) while the concentrations of Pb in mussels from the sampling sites also showed higher levels of Pb in the periostracum (1.16-40.20 mg/g dry weight) than in the ST (2.53-18.62 mg/g dry weight). The maximum to minimum ratios of metals in the periostracum and total ST were 0.14-8.19 and 0.34-6.48 for Cd and Pb, respectively.

The relationships between the different geochemical fractions in the sediment with the periostracum and with the total ST of *P. viridis* are shown in Figures 3 and 4, for Cd and Pb, respectively. The relationships were best fitted to exponential equations since they gave higher *R* values when compared to linear and logarithmic equations. For Cd (Figure 3), the *R* values for periostracum-EFLE, periostracum-'acid-reducible', periostracum-'oxidisable-organic', periostracum-nonresistant, periostracum-resistant and periostracum-total Cd were 0.321, 0.172, 0.234, 0.216, 0.305 and 0.369, respectively. Most of these *R* values were lower than those found for the relationships between ST- EFLE (*R*= 0.702), ST-nonresistant (R= 0.626),

TABLE 2. Cd concentrations (mean µg/g dry weight) in total soft tissue (ST) and periostracum (perios) of *Perna viridis*

	Location	Total ST	Perios	$\frac{Cd_{perios}}{Cd_{ST}}$
A	Pulau Aman	0.87	1.73	1.98
B	Bagan Tiang	0.32	1.49	4.71
C	Bagan Lalang	0.78	1.96	2.51
D	Lukut	0.76	2.48	3.26
E	Pasir Panjang	1.08	4.03	3.73
F	Kuala Linggi	1.41	3.73	2.64
G	Merlimau-1	2.24	5.60	2.50
	Merlimau-2	1.46	0.21	0.14
H	Telok Emas	1.47	3.54	2.41
I	Sebatu-1	1.08	4.34	4.02
	Sebatu-2	1.95	1.96	1.01
J	Tanjung Kupang	0.51	4.18	8.19
K	Pantai Lido-1	0.68	4.82	7.09
	Pantai Lido-2	0.43	1.94	4.52
L	Kg.Pasir Puteh-1	0.82	4.62	5.63
	Kg.Pasir Puteh-2	5.55	10.87	1.96
M	Kuala Belungkor	0.11	0.59	5.51
N	Kuala Pontian	2.10	2.09	1.00
O	Nenasi	2.25	1.15	0.51

Note: NA-not available

TABLE 3. Pb concentrations (mean µg/g dry weight) in total soft tissue (ST) and periostracum (perios) of *Perna viridis*

	Location	Total ST	Perios	$\frac{Pb_{perios}}{Pb_{ST}}$
A	Pulau Aman	3.76	4.40	1.17
B	Bagan Tiang	10.32	24.99	2.42
C	Bagan Lalang	3.41	1.16	0.34
D	Lukut	4.31	2.47	0.57
E	Pasir Panjang	4.28	2.87	0.67
F	Kuala Linggi	7.98	5.12	0.64
G	Merlimau-1	13.99	30.30	2.17
	Merlimau-2	2.53	NA	NA
H	Telok Emas	3.92	3.93	1.00
I	Sebatu-1	7.59	8.63	1.14
	Sebatu-2	18.62	24.60	1.32
J	Tanjung Kupang	4.70	5.71	1.21
K	Pantai Lido-1	4.03	3.68	0.91
	Pantai Lido-2	6.09	11.13	1.83
L	Kg.Pasir Puteh-1	8.76	25.15	2.87
	Kg.Pasir Puteh-2	11.85	40.20	3.39
M	Kuala Belungkor	2.78	18.02	6.48
N	Kuala Pontian	13.65	25.17	1.84
O	Nenasi	7.64	4.15	0.54

Note: NA-not available

ST-resistant (*R*= 0.620) and ST-total Cd (*R*= 0.719). For Pb (Figure 4), the *R* values for periostracum-EFLE, periostracum-'acid-reducible', periostracum-'oxidisable-organic', periostracum-nonresistant, periostracum-resistant and periostracum-total Pb were 0.136, 0.012, 0.912, 0.943, 0.637 and 0.794, respectively. These *R* values were higher than those found for the relationships between ST-EFLE (*R*= 0.059), ST-'oxidisable-organic' (*R*= 0.643) and ST-nonresistant (*R*= 0.567) while the *R*values for periostracum-resistant (*R*= 0.637) and periostracum-total ST (*R*= 0.794) were close to those between ST-resistant (*R*= 0.752) and ST-total Pb (*R*= 0.834) although they were lower.

Discussion

The Cd and Pb concentrations found in the periostracum of *P. viridis* indicated that the material could accumulate both metals. The wider range, ratios of maximum to minimum, for Cd and Pb found in the periostracum than those in the ST indicated that it could accumulate higher levels of Cd and Pb than the ST. Yap et al. (2003a) reported that Kg. Pasir Puteh was highly contaminated by Pb and this phenomenon was supported since the highest levels of Pb were found in the total ST and most obviously in the periostracum of *P. viridis* collected from this site. The significantly (P< 0.001) higher levels of Cd and Pb found in the periostracum than in the total ST at Kg. Pasir Puteh indicated that the periostracum is a better accumulator for Cd and Pb than the ST.

The higher accumulation of Cd and Pb indicated that the biodeposition of these metals was more concentrated in the periostracum than in the ST. This also supports the hypothesis that the metals were first assimilated in the ST before biomineralization in the newly secreted periostracum that was being biodeposited in the crystalline lattices of the inner prismatic and nacreous shell matrice (Yap et al. 2003d). Such being the case, the periostracum accumulates higher levels of metals and is a more useful monitor of recent metal exposures compared to the inner shell layers.

The higher correlation coefficients found between the periostracum and some of the different Pb geochemical fractions in the sediment indicated that the periostracum of *P. viridis* was more closely related to Pb EFLE, Pb 'oxidisable-organic' and Pb 'non-resistant' fractions in the sediment, when compared to those in the ST of the mussel. The higher *R* values of the relationships indicated that the periostracum of *P. viridis* could be a better biomonitoring material for Pb than the ST of the mussel. Based on this reasoning, the periostracum seems to be a poor biomonitoring material for Cd since the correlation coefficients that were found between the periostracum and all the different Cd geochemical fractions of the sediment were poor and lower than those between the ST and the geochemical fractions of the sediment.

One of the criteria when an organism is proposed as a biomonitoring organism is a simple correlation between the pollutant levels accumulated in the body of organism and those in its environment (Rainbow 1997; Yap et al. 2002). Reports on the use of correlation analysis between pollutant levels in an organism and its environment in order to determine the potential of the organism as a potential biomonitor have been reported in the literature. For example, Yap et al. (2002) found significant (p< 0.05) correlations between Cd in *P. viridis* and Cd in the sediment (EFLE fraction and total Cd), Cu in *P. viridis* and Cu in the sediment (EFLE and 'acid-reducible' fractions and total Cu) and Pb in *P. viridis* and Pb in the sediment ('oxidisable-organic' fraction and total Pb). This indicated that the ST of *P. viridis* is a suitable biomonitoring agent for Cd, Cu and Pb. Yap et al. (2004) used correlation analysis to determine that the shell of *P. viridis* as a good biomonitoring material for Zn since the shell correlated better and significantly with some of the geochemical fractions of Zn in the sediments. By using similar correlation analysis, Yap et al. (2005) reconfirmed the use of the byssus as an alternative organ to monitor Zn contamination in coastal waters. Based on the above literature, the periostracum can be considered to be good biomonitoring material for Pb.

In comparison to the metal levels in the total shell of *P. viridis* (Table 4), wider ranges of Cd and Pb were found in the periostracum than those in the total shell of the mussels.

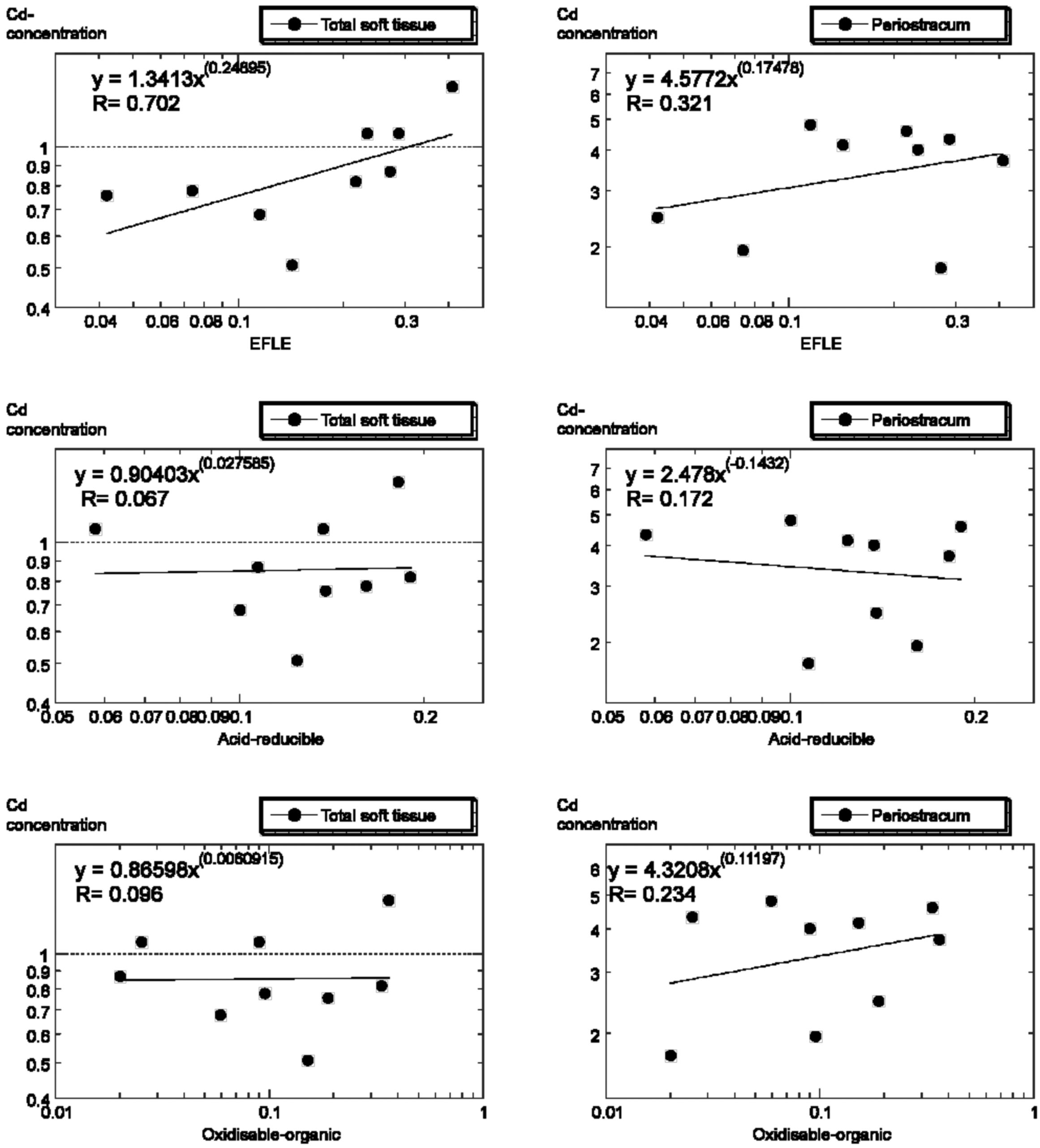

FIGURE 3. Relationships of Cd concentration (µg/g) between the geochemical fractions of the sediment and the total soft tissue/periostracum of *Perna viridis* (N= 9) based on double-log scale

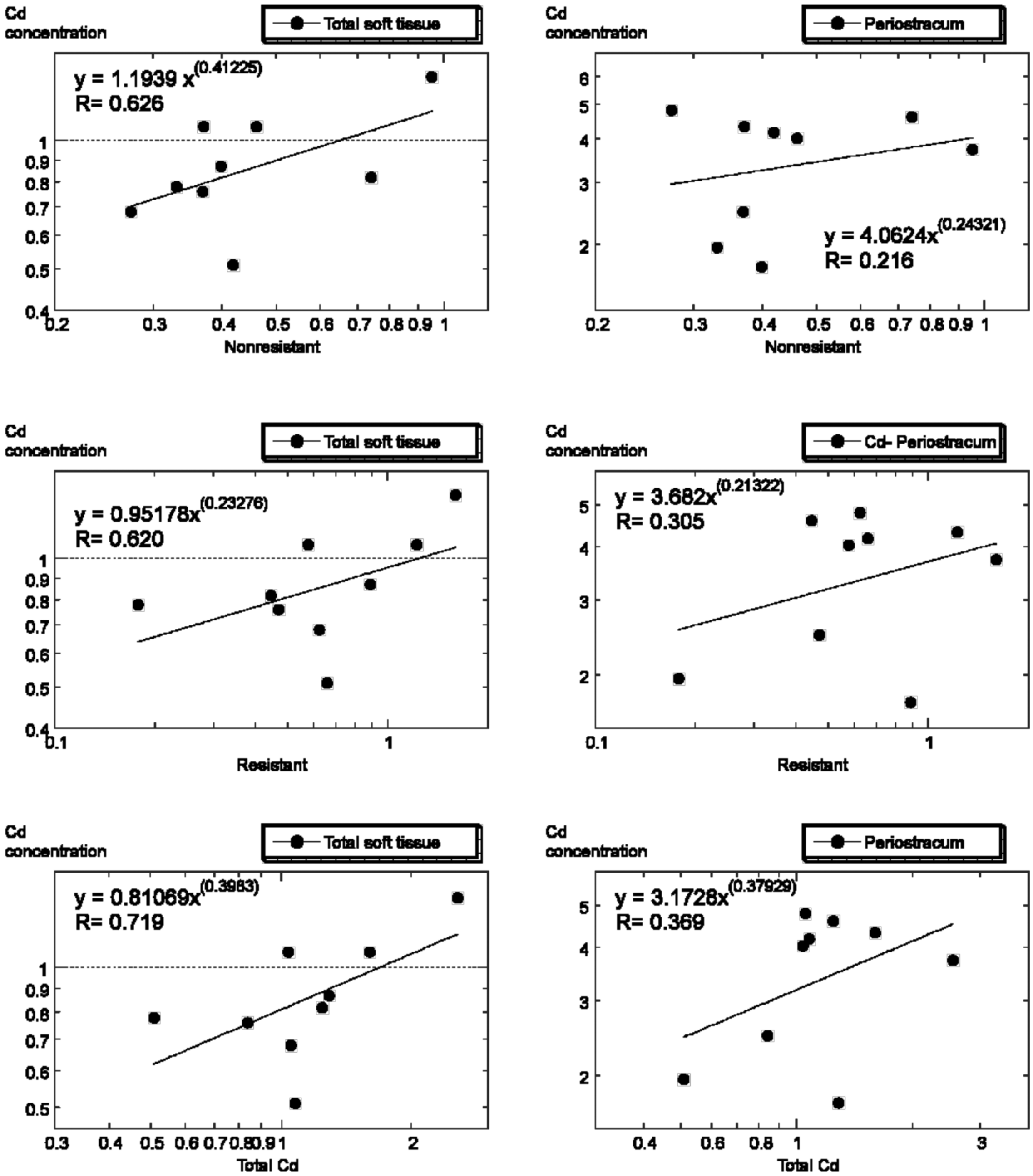

FIGURE 3. Continued

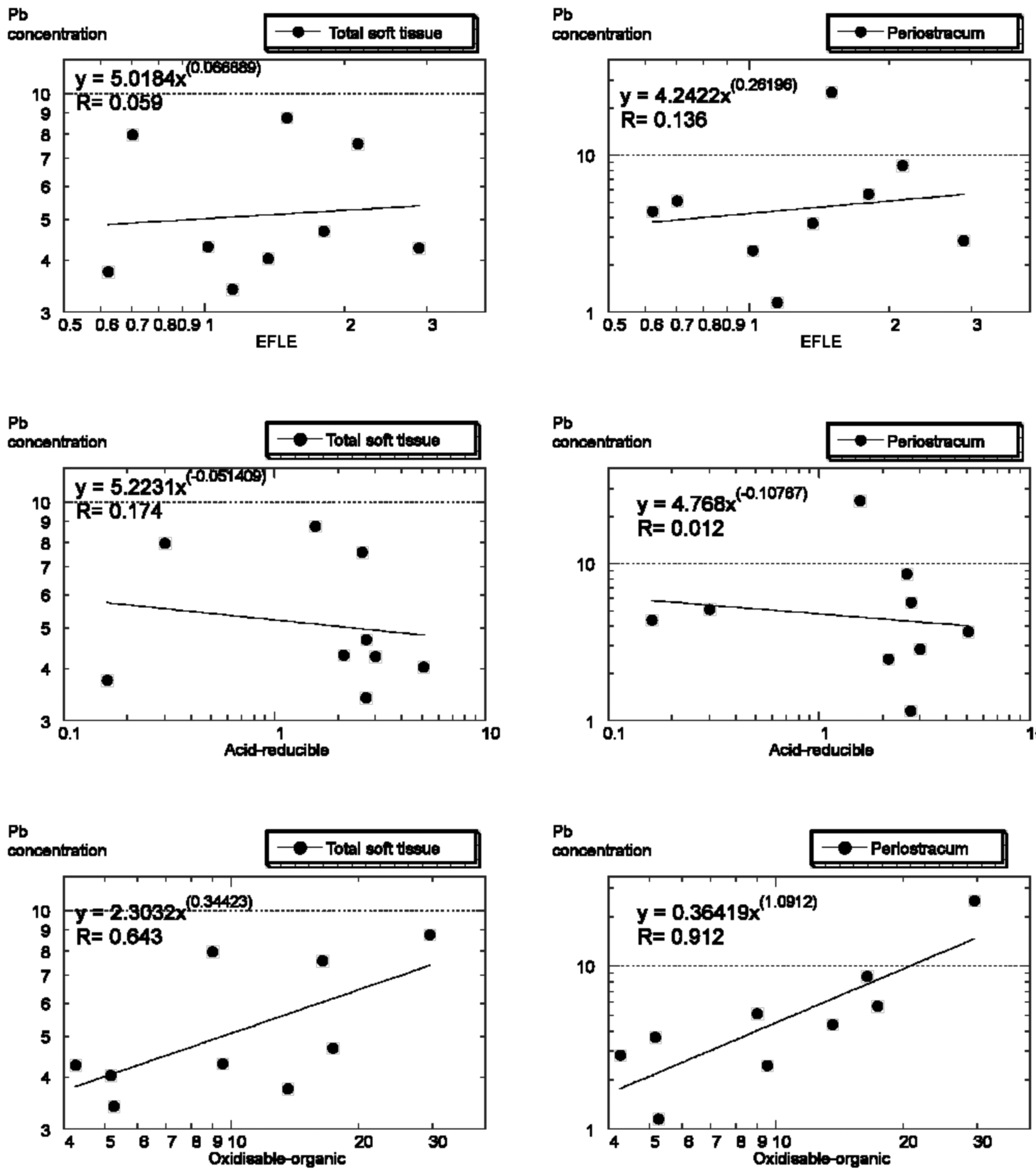

FIGURE 4. Relationships of Pb concentration (μg/g) between the geochemical fractions of the sediment and the total soft tissue/periostracum of *Perna viridis* (N= 9) based on double-log scale

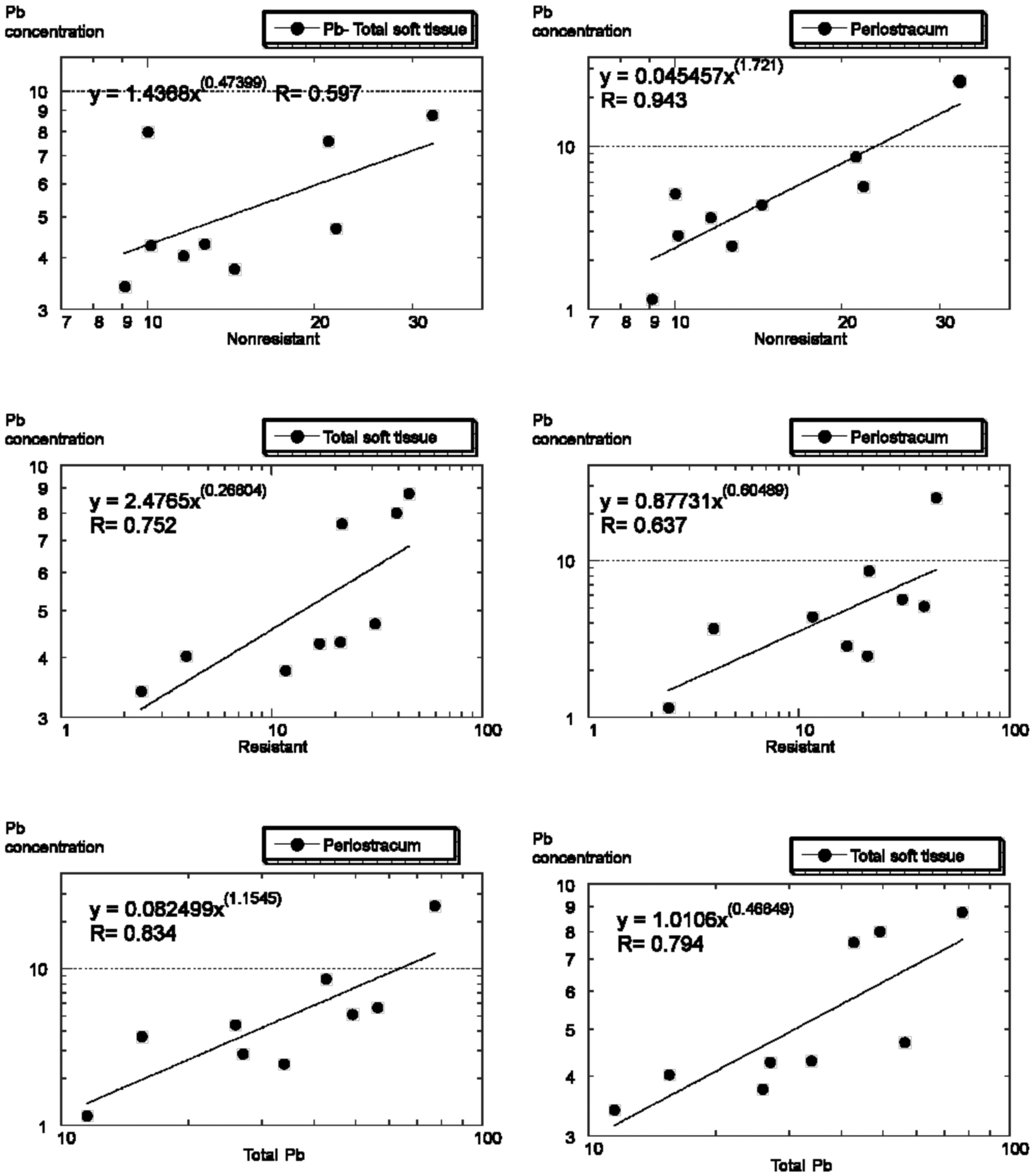

FIGURE 4. Continued

TABLE 4. Comparisons of shell-metal to soft tissue-metal ratios for Cd and Pb concentrations (µg/g) of other molluscs species

	Species	Mshell	M tissue	$\frac{M_{shell}}{M_{tissue}}$	Reference
Pb	Total shells of *P. viridis* of Peninsular Malaysia	17.67-32.56	2.51-8.76	2.65-8.53	Yap et al. (2003)
	P. viridis periostracum of Peninsular Malaysia (15 sites)	1.16-40.2 (13.43 ± 2.83)	2.78-18.62 (7.65 ± 1.05)	0.34-6.48 (1.69 ± 0.35)	This study
Cd	*P. viridis* (Hong Kong)	11.98-13.94	1.58-2.81	4.96-7.58	Cheung & Wong (1995)
	Total shells of *P. viridis* of Peninsular Malaysia	9.34-10.88	0.51-1.41	7.07-20.14	Yap et al. (2003)
	P. viridis periostracum of Peninsular Malaysia (15 sites)	0.21-10.87 (3.23 ± 0.55)	0.11-5.55 (1.36 ± 0.28)	0.14-8.19 (3.33 ± 0.50)	This study

Note: Values are expressed on a dry weight basis.

This phenomenon indicated that the metals found in the periostracum were more dependent on the bioavailable metal variation in the intertidal waters. However, it should be noted that the metals accumulated in the periostracum were all assimilated from the soft tissues of the mussels and therefore unlikely to be affected by physiological conditions such as spawning.

Since the periostracum of *P. viridis* taken for analysis were those that were easily isolated from the edge of the mantle or the periphery of the shell, it is believed that the metals accumulated in the periostracum had been previously assimilated in the soft tissues and the periostracum were formed from the mantle. Therefore, it is the newly formed layer of the shell from the mantle. The periostracum could therefore be used as a biomonitoring material for the current levels of Pb contamination in coastal waters. Differing from the inner layers of the shells, such as the prismatic and the nacreous layers, the periostracum is unlikely to record the environmental history of Pb levels that the *P. viridis* had been exposed to during its lifetime. The present results supported the use of the periostracum of *P. viridis* as a potential biomonitoring material for Pb but not for Cd. However, more studies are warranted to verify usefulness for biomonitoring studies.

CONCLUSION

The present study found that Cd and Pb were accumulated in the periostracum of *P. viridis*. The spatial variation of the Cd and Pb found in the mussel shells indicated the variations of the bioavailabilities of Cd and Pb in coastal waters. Based on correlation analysis, the significant correlation coefficients found between some Pb geochemical fractions and Pb-periostracum indicated the potential of the periostracum to be used as a biomonitoring material Pb. However, since the correlation coefficients found in this study were based on a small sample size (N= 9, as sampling for geographical populations is not an easy task), cautions should be exercised and further studies should be done to compare with the results of the present work.

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