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Gill and Digestive Caecum of *Telescopium telescopium* as Biomonitors of Pb Bioavailability and Contamination by Pb in the Tropical Intertidal Area

(Insang dan Secum Pencernaan *Telescopium telescopium* sebagai Penunjuk Biologi bagi Ketersediaan dan Pencemaran Pb di Kawasan Pasang Surut Tropika)

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ABSTRACT

In this paper we investigated the concentrations of Pb in seven different soft tissues (foot, cephalic tentacles, mantle, muscle, gill, digestive caecum and remaining soft tissues) of 17 geographical populations of Telescopium telescopium *collected from the intertidal area of Peninsular Malaysia. Two points can be presented based on the present study. First, as expected, different concentrations of Pb were found in the different soft tissues, indicating different mechanisms of bioaccumulation and regulations of Pb in these different tissues. By comparing the Pb concentrations in the similar tissues, spatial variation of Pb was found in the different sampling sites although there is no consistent pattern of Pb contamination in these sampling sites. Second, based on the correlation coefficients and multiple linear stepwise regression analysis between Pb concentrations in the different soft tissues and Pb concentrations in geochemical factions in the surface sediments, it is found that gill and digestive caecum can truly reflect Pb contamination and Pb bioavailabilities in the tropical intertidal mudflats. To our knowledge, this is the most comprehensive study on Pb in the different soft tissues of* T. telescopium*, in relation to the habitat sediments of the snails.*

Keywords: Different soft tissues; Pb distribution; Telescopium telescopium

ABSTRAK

Kertas ini melaporkan kajian mengenai kepekatan Pb di dalam tujuh bahagian tisu lembut (kaki, tentakel sefalon, mantel, otot, insang, secum pencernaan dan tinggalan tisu lembut) bagi 17 populasi geografi Telescopium telescopium *yang disampel dari kawasan pasang surut di Semenanjung Malaysia. Terdapat dua perkara boleh dikemukakan berdasarkan kajian ini. Pertama, seperti yang dijangkakan, kepekatan Pb yang berlainan diperoleh dalam pelbagai tisu lembut, menunjukkan mekanisme biopenimbunan dan regulasi bagi Pb yang berlainan dalam pelbagai tisu ini. Dengan membandingkan kepekatan Pb pada tisu yang sama, variasi secara tempat bagi Pb diperoleh di kawasan pensampelan yang berlainan walaupun tiada pola yang tekal bagi pencemaran Pb di kawasan pensampelan tersebut. Kedua, berdasarkan korelasi koefisen dan pelbagai analisis regresi linear peringkat demi peringkat di antara kepekatan Pb di dalam tisu lembut yang berlainan dan kepekatan Pb di fraksi geokimia di permukaan sedimen, didapati insang dan secum pencernaan boleh menunjukkan pencemaran Pb dan Pb ketersediaan di tempat pensampelan pasang surut berlumpur. Pada pengetahuan kami, ini adalah kajian yang paling lengkap bagi Pb di tisu lembut berlainan di* T. telescopium *yang berkaitan dengan sedimen habitat bagi siput ini.*

Kata kunci: Taburan Pb; tisu lembut berlainan; Telescopium telescopium

Introduction

Compared with Cd, Zn and Cu, Pb is typically shown to have the least impact on ecological processes and few toxic effects on the invertebrates or microorganisms critical to decomposition and nutrient transfer (Knigge & Kohler 2000). Pb had not caused significant decrease in survival of aquatic snails, *Physa integra* after 28 days of exposure and it is possible that medium lethal concentrations for this species would decrease if they were exposed for longer period (Spehar et al. 1978). Indirectly, a significant toxic effect of Pb might indicate if organisms showed some form of adaptive tolerance to the metal (Posthuma & van Straalen 1993). Based on a toxicity study by Yap et al. (2004) using mortality as endpoint in *Perna viridis*, the LC50 values for Pb is higher compared to Cu, Zn and Cd. This indicated that Pb toxicity was the lowest among these four metals. Therefore, acute Pb exposure is lessening (Eisler 2000).

In this study, we focused on Pb because this element is one of the most ubiquitous toxic metals that has been known for centuries to be a cumulative metabolic poison (Sadiq 1992). Molluscs can accumulate Pb from water, soil and sediment, with organic forms being more easily absorbed than inorganic. In general, the highest Pb concentrations are found in aquatic molluscs that live near to Pb mining, smelting, and refining facilities, storage battery recycling plants, areas affected by high automobile and truck traffic, sewage sludge and spoil disposal areas, sites where dredging has occurred and in urban and industrialized areas (USPHS 1997). Generally, Pb has no known nutrition, biochemical or physiological function, and demonstrated no biological need and it is toxic to most living things (Eisler 2000). Furthermore, Pb is one of the popular anthropogenic metals in Malaysia (Malaysian DOE 2008).

In many reported literature, it is well known that molluscs are used as biomonitors of trace metal bioavailabilities and contamination in the coastal waters (Hamed & Emara 2006; Liang et al. 2004; Rainbow 1995, 1997; Taylor & Maher 2006; Yap et al. 2009). The measurements of pollutants in the biomonitors can be considered as integrated measures of the ecologically significant fraction of ambient metals in the habitat (Phillips & Rainbow 1993). The gastropods generally can be employed a biomonitor because they fulfill most of the characteristics for a good biomonitor including almost sedentary lifestyle, abundance, of relative longevity, large, easy sampling (Rainbow 1997; Rainbow & Phillips 1993), in contact with polluted bottom sediments.

Nonetheless, most of the biomonitoring studies are based on total soft tissues of gastropods, this could be due to difficulties in separating the different soft tissues. Therefore, the issue of which tissue or organ is most vulnerable to contamination remains poorly known. One of the methods to increase the validity of metal data in order to truly reflect bioavailabilities and contamination by metals is the use of different soft tissues in the biomonitors including separation/dissection into different soft tissues (Yap et al. 2006a, 2006b). Furthermore, knowledge on the distribution of metals in isolated organs/tissues of marine organisms is useful in the identification of specific organs that may tend to accumulate higher levels of heavy metals (Szefer et al. 2002).

Some studies on metals in *Telescopium telescopium* (Family: Potamididae) had been reported (Dang et al. 2005; Ismail & Safahieh 2004; Jones et al. 2000; Lefcort et al. 2004; Peerzada et al. 1990; Yap & Noorhaidah 2008; Yap et al. 2008, 2009). However, the relationships of metals between the snails and sediments were not conclusive since they were based on limited geographical populations.

In fact, this study follows up our earlier biomonitoring work using *Perna viridis* in Malaysia, aiming to study the spatial variation of Pb bioavailability to intertidal mudflat snail *T. telescopium* which were dissected into seven different soft tissues, collected from 17 geographical populations at the intertidal waters of Peninsular Malaysia, relation to Pb levels in the geochemical fractions in the surface sediments.

MATERIALS AND METHODS

The site descriptions are given in Table 1. Snails were collected from 17 geographical sampling sites along the intertidal area of Peninsular Malaysia (Figure 1). In addition to snails, sediment samples were also collected from every sampling site of the snails. The samples were brought back to the laboratory for Pb analysis.

About 15-20 individuals of *T. telescopium* from every site were dissected and pooled into seven different soft tissues, namely foot, cephalic tentacle CT), mantle, muscle,

TABLE 1. Sampling dates, positions (Global positioning system) for the surface sediments and *T. telescopium* collected along intertidal area of Peninsular Malaysia

No.	Sampling sites	Sampling dates	Longitude	Latitude
1	Kg Pasir Puteh (KPPuteh), Johore	30 Apr 2006	N 01° 26’ 05.8”	E 101° 56’ 02.4”
2	Pantai Punggur (PPunggur), Johore	29 Apr 2006	N 01° 41’ 07.2	E 103° 05’ 54.6”
3	Kuala Sg Ayam (KSAyam), Johore	29 Apr 2006	N 01° 45’ 12.5”	E 102° 55’ 45.4
4	Sg. Balang Laut (SBLaut), Johore	29 Apr 2006	N 01° 52’ 21.0	E 102° 44’ 16.5
5	Kuala Lukut Kecil (KLukutK), Negeri Sembilan	28 Apr 2006	N 02° 33’ 42.2”	E 101° 48’ 00.2”
6	Kuala Lukut Besar (KLukutB), Negeri Sembilan	28 Apr 2006	N 02° 34’ 49.2”	E 101° 49’ 34.4”
7	Sg Sepang Kecil (SepangK), Selangor	18 Aug 2006	N 02º 36’ 4.11”	E 101° 41’ 7.79”
8	Bagan Lalang (BLalang), Selangor	15 Sep 2006	N 02º 35’ 57.52”	E 101° 42’ 31.41”
9	Sg Sepang Besar (SepangB), Selangor	7 Jan 2006	N 02º 36’ 19.41”	E 101° 42’ 11.51”
10	Sg Janggut (SJanggut), Selangor	20 Mar 2006	N 03° 10’ 20.0”	E 101° 18’ 1.4”
11	Kg Pantai Jeram (KPJeram), Selangor	24 Feb 2006	N 03° 13’ 14.6”	E 101° 18’ 19.5”
12	Pulau Indah (PIndah), Selangor	16 Aug 2006	N 03º 0’ 22.94”	E 101° 18’ 22.5”
13	Jambatan Permaisuri Bainun (JPBainum), Perak	27 Feb 2006	N 04° 16’ 46.0”	E 100° 39’ 50.2”
14	Kg Deralik (KDeralik), Perak	25 Feb 2006	N 04° 14’ 53.8”	E 100° 42’ 09.1”
15	Kg Setiawan (KSetiawan), Perak	25 Feb 2006	N 04° 14’ 44.3”	E 100° 41’ 35.6”
16	Kuala Gula (KGula), Perak	12 Jan 2007	N 04º 55’ 89.6”	E 100º 26’ 79.1”
17	Tumpat, Kelantan	15 Dec 2006	N 06º 12’ 55.21”	E 102º 14’ 14.21”

Note: Number of sites followed those in Figure 1

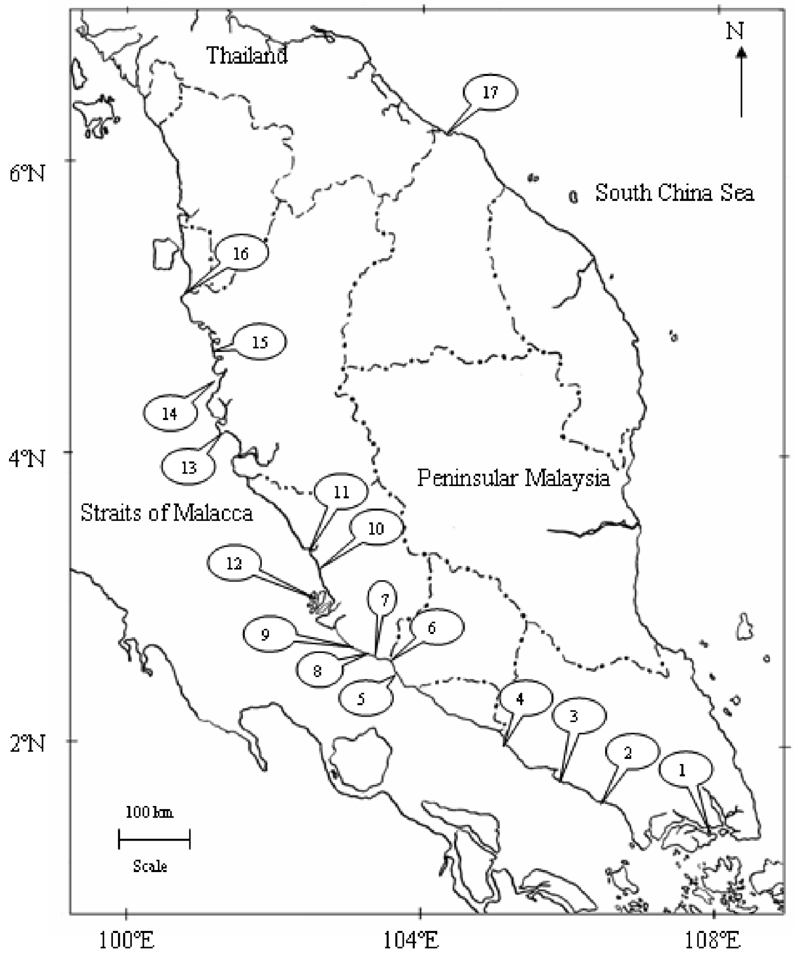

FIGURE 1. Map showing 17 sampling sites (in numbers) of the intertidal area of Peninsular Malaysia

gill, digestive caecum (DC) and remaining soft tissues (REST). The shell heights and shell widths for the snails measured for the metal analysis are given in Table 2.

All of the snail and sediment samples were dried at 80°C for 72 h until constant dry weights. Three replicates of each different part of soft tissues of snails were then digested in concentrated nitric acid (BDH: 69%). The dried sediment samples were crushed by using a mortar and pestle and sieved through a 63 µm aperture stainless steel sieve and were shaken vigorously to produce homogeneity. For the analyses of total Pb concentrations in the sediment samples, three replicates were analyzed by using the direct aqua-regia method. About 1 g of each dried sample was digested in a combination of concentrated HNO_3 (AnalaR grade; BDH 69%) and $HClO_4$ (AnalaR grade; BDH 60%) in the ratio of 4:1. The snail and sediment samples were put into a hot-block digester first at low temperature (40°C) for 1 h and then were fully digested at 140°C for at least 3 h. The prepared samples were then analyzed for Pb by an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Model AAnalyst 800. The sample concentrations are presented as µg/g dry weight.

For the analytical procedures for four geochemical fractions of the surface sediments, sequential extraction technique (SET) described by Badri and Aston (1983) and slightly modified by Yap et al. (2002) was used. These four fractions, extraction solutions and conditions employed by each fraction were:

1. Easily, freely, leacheable or exchangeable (EFLE): About 10 g of the sample was shaken continuously with 50 mL of 1.0 M ammonium acetate (NH_4CH_3COO), at pH 7.0 and at room temperature pH 7.0 for 3 h.
2. Acid-reducible (AR): The residue from the first step was shaken continuously with 50 mL of 0.25 M hydroxylammonium chloride ($NH_2OH{\cdot}HCl$) that had been acidified with HCl to pH 2, at room temperature for 3 h.
3. Oxidisable-organic (OO): The residue from the second step was first oxidized with 30% H_2O_2 in a water bath at 90-95°C. After being oxidized, the residue was shaken continuously with 50 mL of 1.0 M ammonium acetate (NH_4CH_3COO) that had been acidified with HCl to pH 2, at room temperature for 3 h.
4. Resistant (RES): The residue from the third step was digested with 10 mL of an aqua-regia solution that

comprised of a combination of nitric acid (AnalaR grade, BDH 69%) and perchloric acid (AnalaR grade, BDH 60%) in the ratio of 4:1 for 1 h at the temperature of 40°C. The temperature was then increased to 140°C for an additional 3 h.

For each fraction, the resulting solution obtained at the end of each step was filtered through a Whatman No. 1 filter paper into a clean, acid-washed polyethylene bottle. The residue was then washed with 20 mL of double distilled water and filtered through a Whatman No. 1 filter paper into the same polyethylene bottle. The filtrate was stored until metal determination. The residue used for each step was first dried and weighed before the next step was carried out. At each step of the SET, a blank was done using an identical procedure to ensure that the samples and chemicals used were not contaminated (Yap et al. 2002). The nonresistant (NR) fraction is calculated based on the summation of EFLE, AR and OO while total summation (SUM) is the summation of EFLE, AR, OO and RES.

To avoid possible contamination, all the glassware and equipment used were acid-washed and the accuracy of the analysis was checked using the standard addition testing procedure. Procedural blanks and quality control samples made from standard solutions with each 1000 ppm stock solution for Pb were analyzed once every five samples in order to check for sample accuracy (Yap et al. 2006a). The quality of the methods used were checked with Certified Reference Materials for Soil (NCS DC73319-Soil, China National Analysis Center for Iron and steer 2004) (certified Pb value: 98 μg/g dry weight, measured Pb value: 96.1 μg/g dry weight; recovery= 98.1%).

For the statistical analysis, the relationships of Pb concentrations between the different parts in the snails and geochemical factions in the sediments were elucidated by using correlation coefficients and multiple linear stepwise regression analysis (MLSRA), cluster analysis. All the multivariate analyses were performed by using Statistical Program for Social Science (SPSS) for Windows, version 15.0 software, except for cluster analysis. The cluster analysis based on Single Linkage Euclidean distances, on the Pb concentrations in the four geochemical fractions and NR fractions for the surface sediments collected from 17 sampling site, was done by using STATISTICA 99"

TABLE 2. Mean values (± standard error) of shell heights (Hei, cm) and shell widths (Wid, cm) of *T. telescopium* and descriptions of 17 sampling sites in the intertidal area of Peninsular Malaysia

No.	Sites	N	Hei		SE	Wid		SE	Sites description
1	KPPuteh	11	8.6	±	0.11	4.28	±	0.07	Fishing area, mangrove and industrial area at Pasir Gudang
2	PPunggur	8	7.3	±	0.18	3.08	±	0.04	A recreational area
3	KSAyam	11	6.58	±	0.14	3.18	±	0.04	A recreational beach and a muddy area
4	SBLaut	15	5.65	±	0.15	3.24	±	0.07	A busy jetty, housing area, fishing village, mangrove swamp and an estuary
5	KLukutK	17	5.35	±	0.07	2.83	±	0.04	A prawn aquaculture, mangrove swamp, water irrigation
6	KLukutB	6	8.98	±	0.13	4.52	±	0.05	Under construction of jetty, mangrove swamp, prawn farm
7	SepangK	9	4.96	±	0.06	2.89	±	0.06	An aquaculture of prawn and muddy area,
8	BLalang	10	8.35	±	0.08	4.56	±	0.12	An aquaculture of prawn, water gate and near Dragon fruit farm, muddy area
9	SepangB	12	7.81	±	0.12	3.71	±	0.04	A restaurant, jetty, water irrigation and thousands of *T. Telescopium* are found
10	SJanggut	10	8.41	±	0.17	3.89	±	0.07	A housing area, muddy, chicken farm, palm oil plantation and prawn culture activities
11	KPJeram	10	7.64	±	0.06	3.72	±	0.05	A jetty and sea-food restaurant
12	PIndah	21	8.74	±	0.16	4.73	±	0.09	An irrigation water, a small jetty and muddy area
13	JPBainum	13	7.15	±	0.13	3.51	±	0.07	A residential area (kampong), recreational area (kayak) and an estuary.
14	KDeralik	8	9.2	±	0.08	4.68	±	0.21	A busy traffic and road to west port of Klang
15	KSetiawan	6	7.83	±	0.07	3.9	±	0.08	A residential area, mangrove (very muddy) with no direct polluton observed
16	KGula	8	8.47	±	0.14	4.16	±	0.04	Under the bridge and near the Port of Lumut.
17	Tumpat	9	9.03	±	0.13	4.82	±	0.04	A pristine area

Note: N= number of individuals analyzed

Edition (Version 5.5). All the data for the three multivariate analyses were $\log_{10}$(mean + 1) transformed in order to reduce the variance (Zar 1996).

RESULTS

The Pb concentrations in the different soft tissues of *T. telescopium* collected from 17 sampling sites are presented in Figure 2 and the overall Pb concentrations is given in Table 4. Most obviously, the highest Pb concentrations (µg/g dry weight) are consistently found in DC (69.6), gill (95.8) and REST (27.1) from KSAyam population. PIndah population had the highest Pb levels in CT (16.8) and mantle (17.2). The highest Pb levels in foot (17.3) and muscle (13.9) are found in SepangB and KSetiawan, respectively. By comparing the Pb concentrations in the

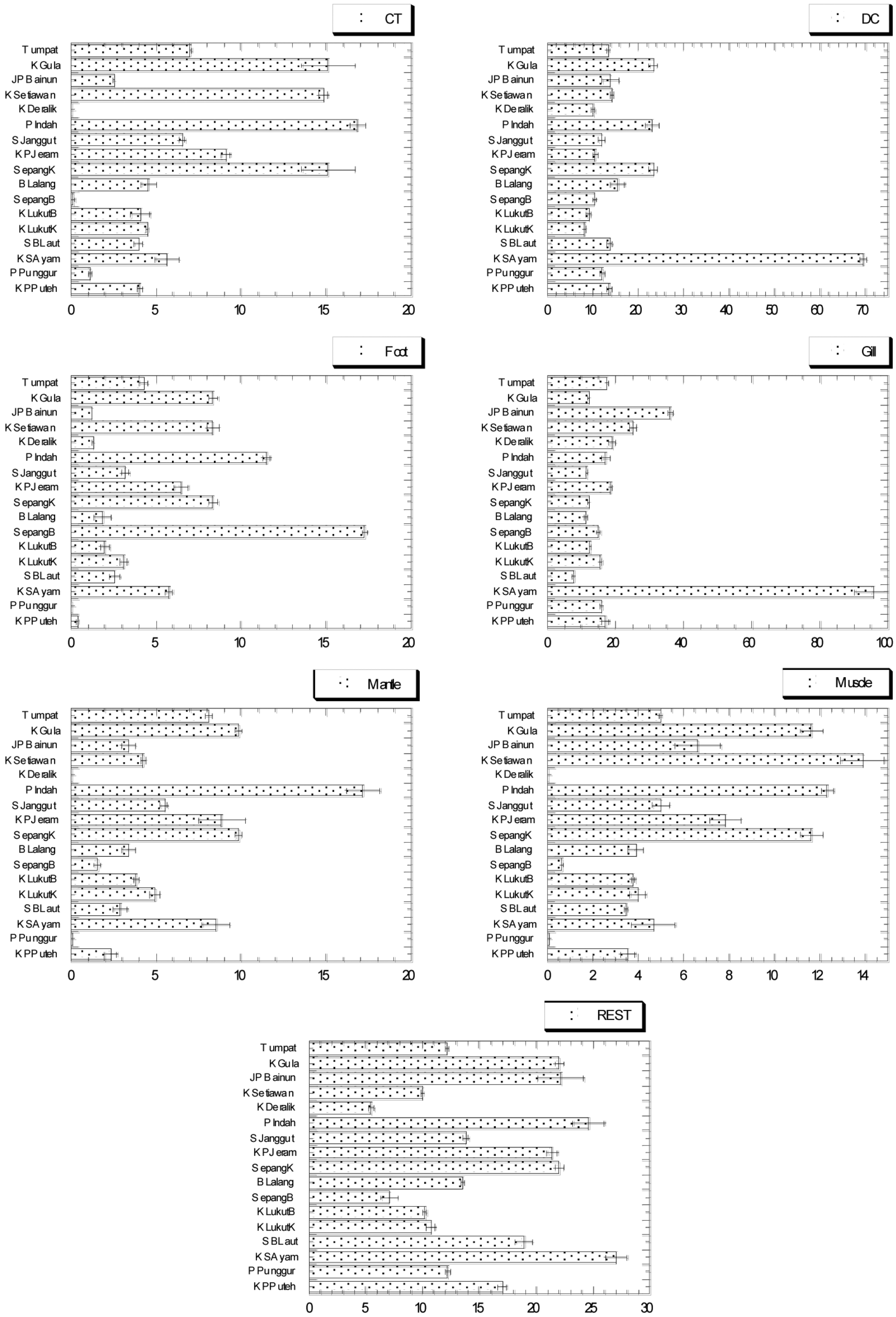

FIGURE 2. Lead concentrations (µg/g dry weight) in different soft tissues of *T. telescopium* collected from 17 sampling sites in Peninsular Malaysia

similar soft tissues (Figure 2), spatial variation of Pb was found in the different geographical populations although there is no consistent pattern of Pb accumulation in these sampling sites. However, the well reported metal-polluted site from KPPuteh did not manifest to have distinctly high Pb bioavailability to *T. telescopium* if it is based on individual soft tissues.

Based on a dendrogram using cluster analysis on the geochemical fractions of the surface sediments (Figure 3), it is found that KSAyam is ranked on the top in which it is subclustered as a single entity. This may indicate the Pb contamination at KSAyam. On the other hand, KPPuteh ranked the lowest with the low concentrations, indicating less Pb contamination.

When the dendrogram is based on the cluster analysis on the Pb concentrations in all seven tissues of *T. telescopium* collected from 17 sampling sites (Figure 4), it is found that higher bioavailability of Pb was found in the KSAyam population. This is well clustered as one single entity. This is almost unexpected for KPPuteh to be uncontaminated by Pb as indicated by a number of previously reported. The high Pb bioavailability to *T. telescopium* is well indicated by the highest Pb levels found DC< gill and REST at KSAyam population (Figure 2). In contrast, KPPuteh population is ranked the lowest, indicating lowest Pb bioavailability to this population although previous studies (Yap et al. 2002, 2003) found that KKPuteh had high bioavailability of Pb to the biomonitor green-lipped mussel *Perna viridis* (Yap et al. 2003).

From Table 3, the ratio of maximum to the minimum values is useful to understand the Pb variations in the different soft tissues of snails. It was found that the foot have the highest ratio (863), followed by muscle (463), mantle (429), CT (420), gill (12.3), DC (8.39) and REST

TABLE 3. Overall concentrations (μg/g dry weight) of Pb in the different soft tissues of *T. telescopium* collected from 17 sampling sites in Peninsular Malaysia

	Minimum	Maximum	Max/min	Mean	Std Error
CT	0.04	16.82	420	6.78	1.33
DC	8.29	69.58	8.39	17.44	3.46
Foot	0.02	17.26	863	5.07	1.11
Gill	7.77	95.76	12.3	21.24	4.91
Mantle	0.04	17.16	429	5.57	1.06
Muscle	0.03	13.89	463	5.77	1.05
REST	5.49	27.05	4.93	15.91	1.57

Note: CT– Cephalic tentacle; REST– remaining soft tissues; DC– digestive caecum.

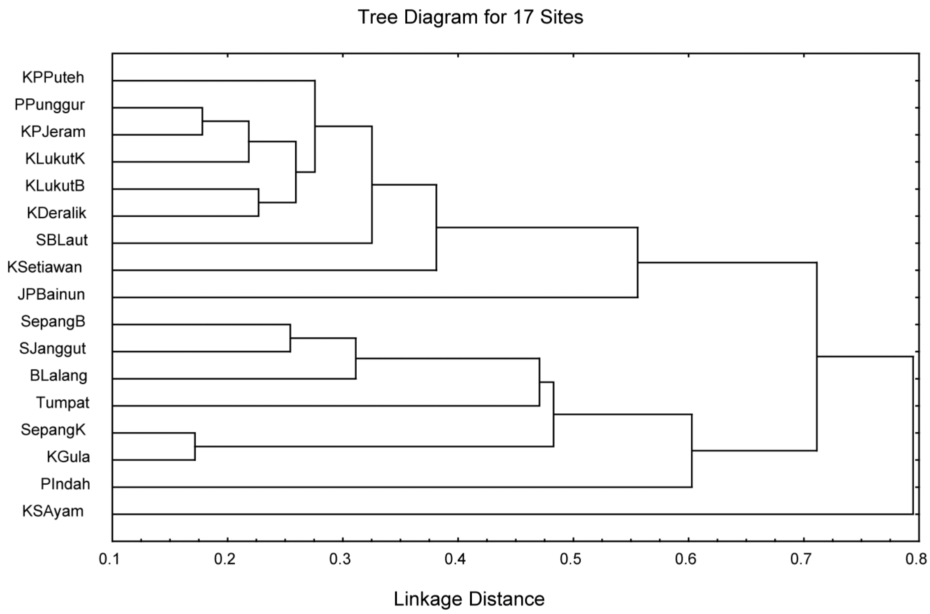

FIGURE 3. Cluster analysis based on Single Linkage Euclidean distances, on the geochemical fractions of Pb (EFLE, acid-reducible, oxidiable-organic, resistant and nonresistant) and its summation in the surface sediments, based on $\log_{10}$(mean + 1) transformed data

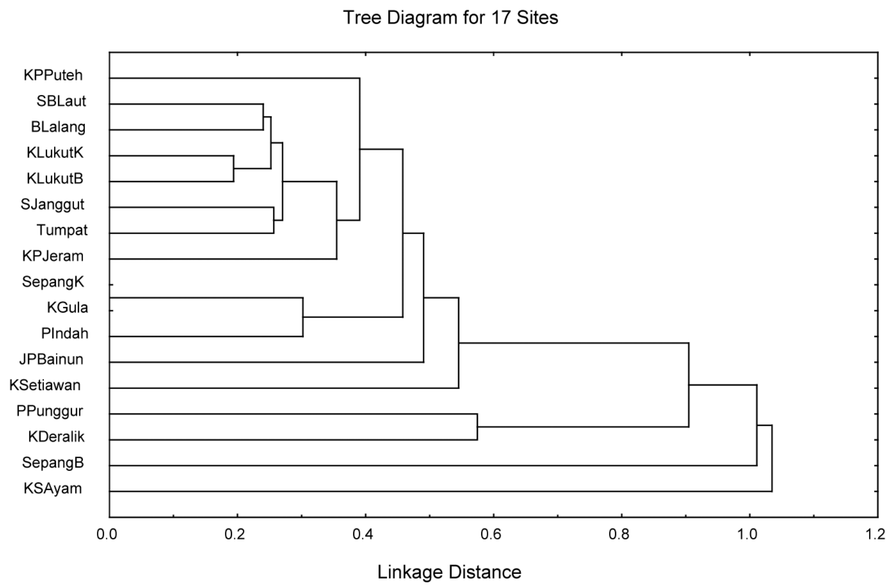

FIGURE 4. Cluster analysis based on Single Linkage Euclidean distances, on the Pb concentrations in seven tissues of *T. telescopium* collected from 17 sampling sites, based on $\log_{10}$(mean + 1) transformed data

(4.93). This might point to the fact that the foot has the highest Pb variation in response to environmental Pb bioavailability. Generally, it is shown that the Pb accumulation pattern in the different soft tissues is as follows: gill> DC> REST> C> muscle> mantle> foot.

The Pb concentrations of four geochemical fractions of surface sediments in the snail habitats from 17 sampling sites are given in Table 4. It was found that KGula recorded the highest EFLE. The highest levels of AR, RES, SUM and NR fraction were found in KSAyam. The highest OO was found in KDeralik. The only sampling site at KPPuteh recorded the dominance (> 50%) of NR fraction of Pb, based on NR/R ratio. This indicated that the total Pb was dominated (> 50%) by NR fractions which were mostly related to anthropogenic inputs (Badri & Aston 1983; Yap et al. 2002).

To understand the relationships of Pb levels between the different soft tissues, correlation coefficients between the soft tissues are performed in (Table 5). It is found that significant (P< 0.05) positive correlations are found between pairwises for CT-mantle, CT-muscle, CT-REST, DC-gill, DC-REST, foot-mantle, foot-muscle, mantle-muscle, mantle-REST and muscle-REST. This indicated that these soft tissues had strong relationships in their Pb accumulations.

TABLE 4. Mean Pb concentrations (μg/g dry weight) of four geochemical fractions of surface sediments in the snail habitats from 17 sampling sites of Peninsular Malaysia (N=3)

No	Sites	EFLE	AR	OO	Res	SUM	NR	NR/Res
1	KPPuteh	1.69	0.64	22.61	19.26	44.2	24.94	1.29
2	PPunggur	1.20	0.38	14.78	36.07	52.43	16.36	0.45
3	KSAyam	1.93	3.97	30.09	132.68	168.67	35.99	0.27
4	SBLaut	2.23	1.77	18.44	29.87	52.31	22.44	0.75
5	KLukutK	1.21	0.32	12.97	53.92	68.42	14.5	0.27
6	KLukutB	1.25	1.21	11.51	23.78	37.75	13.97	0.59
7	SepangK	0.44	0.25	1.04	22.26	23.99	1.73	0.08
8	BLalang	0.58	1.19	0.67	27.86	30.3	2.44	0.09
9	SepangB	2.53	0.76	0.58	15.61	19.48	3.87	0.25
10	SJanggut	0.95	0.29	11.51	31.77	44.52	12.75	0.40
11	KPJeram	0.58	0.22	2.11	19.15	22.06	2.91	0.15
12	PIndah	2.73	2.35	3.28	11.32	19.68	8.36	0.74
13	JPBainum	1.53	0.44	14.35	21.16	37.48	16.32	0.77
14	KDeralik	1.30	1.72	34.18	30.46	67.66	37.2	1.22
15	KSetiawan	0.42	1.57	5.34	53.39	60.72	7.33	0.14
16	KGula	3.03	0.83	0.50	20.60	24.96	4.36	0.21
17	Tumpat	0.29	3.68	0.24	17.57	21.78	4.21	0.24

Note: EFLE– easily, freely, leacheable or exchangeable; AR– acid-reducible; OO- oxidisable-organic; RES– resistant; SUM- summations of EFLE, AR, OO and RES; NR= nonresistant fraction or summation of EFLE, AR and OO

In order to see the relationships of Pb concentrations between the seven different soft tissues and the different geochemical fractions in the surface sediment, correlation coefficients and MLSRA are established and the results are presented in Tables 6 and 7. From Table 6, it is found that the significant (P< 0.05) pairwises are DC-AR, gill-RES and gill-SUM. The correlation coefficients close to zero or negative indicating that the concentrations of any geochemical fractions of studied metals were not directly reflected (Usero et al. 2005) in the selected soft tissues of *T. telescopium*. This can be explained that concentrations of metals were low in sediments, probably lower than the threshold below which *T. telescopium* was able to regulate the Pb accumulation in its different soft tissues (Usero et al. 2005). The strong correlation could be due to their physiological roles in snail metabolism.

Based on MLSRA (Table 7), the geochemical fractions in the surface sediments that could significantly (P< 0.05) influence the accumulation in the different soft tissues of *T. telescopium* are given in Table 7. It was also found that gill and DC are the only two soft tissues which are significantly influenced by EFLE, AR, OO and RES fractions. However, the low variations of Pb in gill and DC (Table 3) might be the reason why these two soft tissues are significantly (P< 0.05) influenced by EFLE, AR, OO and RES fractions of the surface sediments as shown in MLSRA (Table 7).

TABLE 5. Pearson's correlations of Pb concentrations (based on log10[mean +1]) among the different parts of *T. telescopium*. N=17

	CT	DC	Foot	Gill	Mantle	Muscle	REST
CT	1.00	0.41	0.44	-0.03	0.87	0.94	0.65
DC		1.00	0.31	0.59	0.48	0.37	0.66
Foot			1.00	0.06	0.63	0.50	0.15
Gill				1.00	0.11	0.07	0.25
Mantle					1.00	0.89	0.69
Muscle						1.00	0.65
REST							1.00

Note: CT– Cephalic tentacle; REST– remaining soft tissues; DC– digestive caecum. Values in bold are significant at P< 0.05

TABLE 6. Pearson's correlations of Pb concentrations (based on log10[mean +1]) in the different soft tissues of *T. telescopium* and geochemical fractions in the sediment. N=17

	EFLE	AR	OO	RES	SUM	NR
CT	0.41	0.36	-0.18	-0.19	-0.15	0.01
DC	0.48	0.61	0.00	0.35	0.30	0.16
Foot	0.17	0.17	-0.38	-0.19	-0.28	-0.32
Gill	-0.05	0.48	0.41	0.71	0.70	0.44
Mantle	0.27	0.44	-0.34	-0.10	-0.18	-0.18
Muscle	0.29	0.38	-0.19	-0.11	-0.10	-0.04
REST	0.42	0.40	-0.06	0.18	0.13	0.07

Note: EFLE– easily, freely, leachable or exchangeable; AR– acid-reducible; OO- oxidisable-organic; RES– resistant; SUM- summations of four geochemical fractions; NR– nonresistant fraction; CT– Cephalic tentacle; REST– remaining soft tissues; DC– digestive caecum. Values in bold are significant at P< 0.05

TABLE 7. Multiple linear stepwise regression analysis of Pb concentrations (based on log10[mean+1]) between the different soft tissues of *T. telescopium* and the geochemical fractions of sediment. N= 17

Soft tissues	Multiple linear stepwise regression equation	R	R^2
CT	No significant variable was selected		
DC	0.234 + 0.804 (EFLE) + 0.473 (AR) – 0.183 (OO) + 0.474 (RES)	0.866	0.750
Foot	No significant variable was selected		
Gill	0.325 – 0.076 (EFLE) + 0.419 (AR) + 0.049 (OO) + 0.546 (RES)	0.783	0.614
Mantle	No significant variable was selected		
Muscle	No significant variable was selected		
REST	No significant variable was selected		

Note: EFLE– easily, freely, leacheable or exchangeable; AR– acid-reducible; OO- oxidisable-organic; RES– resistant; CT– Cephalic tentacle; REST– remaining soft tissues; DC– digestive caecum. Those dependent variables were significantly (P< 0.05) influenced by the selected independent variables

Discussion

The different concentrations of Pb were found in the different soft tissues in the snail populations indicating different mechanisms of sequestration and regulations of Pb in these different tissues (Phillips & Rainbow 1993). The different Pb accumulation patterns and the different ratios of maximum to minimum Pb concentrations in the different soft tissues are also evidence to prove that the accumulative capacity for Pb is different in every tissue. From the general Pb accumulation patterns: gill> DC> REST> CT> muscle> mantle> foot, this may indicate that the gills, DC and REST were the main target organs for Pb accumulation, ranging at 15.9-21.4 µg/g dry weight while other soft tissues seem to be less accumulative of Pb, only ranging at 5.57-6.78 µg/g dry weight.

The above accumulation patterns and ratios also implied that certain tissue is better in the biomonitoring for Pb. The different degrees of Pb accumulation may also indicate that the mechanism of detoxification and metallothionein synthesis for this nonessential Pb is different from one tissue to another (Viarengo et al. 1985).

Even though Pb is not considered as one of the most environmentally mobile or toxic of metals, there is still appreciable evidence showing the bioavailability of sediment-bound Pb to deposit feeding species (Bryan & Langston 1992). In addition, Pb can be accumulated directly from sea, especially in organisms that utilize gill tissue as the major nutrient uptake route (Sadiq 1992). Hence, the present study did show an evidence that *T. telescopium* used gill as major Pb uptake direct from environmental media.

It is suggested that the gill and DC of *T. telescopium* can truly reflect Pb contamination of the sampling site. This point is particularly based on the correlation analysis and MLSRA of Pb concentrations between the different soft tissues and geochemical fractions of the surface sediments. The positive significant correlation coefficients and four selected geochemical fractions as influential variables in the Pb accumulations suggest that gill and DC are good biomonitor of contamination by Pb.

The high concentration of Pb in the gills of *T. telescopium* was in accordance with the previous study on *Anodonta* sp. and *Unio pictorum* (Gundacker 2000), *Anodonta anatina* (Manly & George 1977) and *Lymnaea stagnalis* (Balogh et al. 1988). It may be concluded that the continual exposure to water and suspended particles may facilitate accumulation of some metals in the gill (Gundacker 2000). Furthermore, the gills of molluscs are generally considered to be the main site for the exchange of bivalent cations (Pynnonen 1991). As the gills serve also as breeding chambers, its involvement in metal storage may have harmful effects on the reproductive success of bivalve species (Gundacker 1999). In addition to that, the important accumulation of metals in gills could be due to its contact with the ambient external medium (seawater and sediment) (Yap et al. 2006c) and considered responsible of the metal transfer to organism (Saha et al. 2006).

Ii is noteworthy that elevated Pb concentration was found in the DC, which formed part of the digestive glands, and this agrees well with some reported studies in the digestive glands of marine gastropods (Nott & Nicolaidou 1989) and *Patella caerulea* in particular (Yüzereroğlu et al. 2010). The high Pb concentration found in this DC indicated that DC is a Pb storage site, possibly in the form of metallothionein or granules as detoxification mechanism for Pb (Lobel et al. 1982).

Nonetheless, the high metal accumulation in gill and DC should not be considered as good biomonitor of metal contamination. Hence, the relationship of the metal between the snails and sediment is investigated. In this study, the high Pb concentrations found in gill and DC are also supported by the correlation coefficients and MLSRA which showed significant ($P< 0.05$) relationships of Pb between the two soft tissues and geochemical fractions of the sediments. This indicated that high Pb contamination in the environmental habitat may have high Pb bioavailability to the biomonitor *T. telescopium* especially in the gill and DC which are very accumulative of Pb. Thus, we suggest, gill and DC are good biomonitoring tissues for Pb contamination. However, cautions should be exercised since Pb bioaccumulation and bioavailability to the biomonitor are also dependent to the metal speciation, multiple routes of exposure (diet and solution) and geochemical effects (Luoma & Rainbow 2005).

Conclusion

The present study revealed the importance of employing multivariate analyses in finding reliable relationships of Pb concentrations between the different soft tissues of *T. telescopium* and sediments. Based on multivariate analyses, gills dan DC were good biomonitoring organs for bioavailability and contamination by Pb.

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