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ASSESSMENT OF BIOAVAILABILITY AND CONTAMINATION BY Cd IN THE TROPICAL INTERTIDAL AREA, USING DIFFERENT SOFT TISSUES OF *Telescopium telescopium*: STATISTICAL MULTIVARIATE ANALYSES

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Abstract: The mudflat snails *Telescopium telescopium* were collected from 18 geographical sampling sites in the intertidal area of Peninsular Malaysia. In this paper, the concentrations of Cd were determined in seven different soft tissues of the snails, namely foot, cephalic tentacles, mantle, muscle, gill, digestive caecum and remaining soft tissues. From this study, it was found that different concentrations of Cd were found in different soft tissues, indicating different mechanisms of sequestration and regulations of Cd in these different tissues. By comparing the Cd concentrations in the similar tissues, spatial variation of Cd was found in the different sampling sites although Kuala Juru always showed higher Cd levels based on the data of both snails and surface sediments. This is an indication of the polluted condition by Cd at Kuala Juru and localisation of Cd contamination in the intertidal area of Peninsular Malaysia. Digestive caecum was the main target organ for Cd storage and accumulation based on accumulation pattern of Cd in the seven soft tissues. The present study had revealed the importance of employing multivariate analyses in finding reliable relationships of Cd concentrations between the different soft tissues of *T. telescopium* and sediments. Cephalic tentacle and mantle were identified as good biomonitoring organs for Cd contamination in snails based on correlation analysis and multiple linear stepwise regression analysis, in which significant ($P< 0.05$) relationships were found between both tissues and sediments. Different bioaccumulation patterns of Cd were found in the different soft tissues of snails where tissue redistributions of Cd in cephalic tentacle and foot were good indicators of high Cd bioavailability of the sampling sites. This study may be the most comprehensive study on Cd tissue distribution in the different soft tissues of *T. telescopium* in relation to sediment in the tropical intertidal areas.

KEYWORDS: *Telescopium telescopium*, Cd distribution, different soft tissues

Introduction

From the literature, a lot of reports can be found employing intertidal gastropods (eg. Gundacker, 2000; Liang *et al*., 2004; Daka, 2005; Chandran *et al*., 2005; Storelli and Marcotrigiano, 2005; Hamed and Emara, 2006; Daka and Hawkins, 2006, Berto *et al*., 2006) and particularly in *Telescopium telescopium* snails (Peerzada *et al*., 1990; Jones *et al*., 2000; Ismail and Safahieh, 2004; Dang *et al*., 2005; Yap and Noorhaidah, 2008; Yap *et al.,* 2008, 2009), being used as biomonitors of heavy-metal pollution. This is because they can be employed as biomonitors of the bioavailability and contamination of heavy metals in the intertidal area (Rainbow, 1995, 1997; Yap *et al*., 2006a, 2006b). Furthermore, the telescope mudflat snail is large, relatively immobile and serves as a good model for examining the effects of pollution on populations because this snail is in direct contact with polluted bottom sediments (Lefcort *et al.,* 2004). However, most of these reports were based on total soft tissues of the snails. This could be due to difficulties in separating the different soft tissues. The relatively large size of the *T. telescopium* facilitates the dissection of different soft tissues.

To date, knowledge on the distribution of metals in isolated organs/tissues of marine organisms is useful in the identification of specific

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organs that may tend to accumulate higher levels of heavy metals (Szefer *et al*., 2002). Therefore, the metal distribution in the different parts of gastropods is more informative than the analysis of total soft tissues. The use of molluscs has been widely accepted nowadays as biomonitors of trace-metal bioavailabilities in the coastal waters (Rainbow and Blackmore, 2001; Yap *et al.,* 2009). The measurements of pollutants in the biomonitors are said to be integrated measures of the ecologically-significant fraction of ambient metals in the habitat (Phillips and Rainbow, 1993; Rainbow and Phillips, 1993). Once there is an egg spawning in the molluscs, the metal contents in the individual would be drastically changed (Taylor and Maher, 2006.).

The metal data based on the total soft tissues is therefore always subject to much argument and the question on whether the metal data could truly reflect the metal bioavailabilities could be raised. One of the methods to reduce the effects of spawning condition and problem of defaecation, is the use of different soft tissues in the biomonitors including separation/dissection into different soft tissues such as muscle (since all molluscs contain muscles) (Yap *et al.,* 2006a, 2006b). Previously, Yap *et al.* (2006a) used the different soft tissues of *Perna viridis* to indicate the bioavailabilities and contamination of heavy metals in the semi-enclosed Straits of Johore which is a potentially-receiving anthropogenic source.

Recently, Yap *et al.* (2009) proposed *T. telescopium* as potential biomonitors of bioavailabilities and contamination of Cu, Pb and Zn in the Malaysian intertidal areas. This study was a comprehensive work on Cd covering 18 geographical populations from intertidal areas of Peninsular Malaysia. Thus, the objectives of this paper were to determine Cd-accumulation patterns, the relationships of Cd between different soft tissues of *T. telescopium* and the surface sediment based on multivariate analysis, besides provision of an extensive data set for Cd accumulation in the different soft tissues of the snails in relation to sediment data.

Materials and Methods

The site descriptions are given in Table 1. Snails were collected by hand picking from 18 geographical sampling sites along the intertidal areas of Peninsular Malaysia (Figure 1). In addition to snails, sediment samples were also collected using a clean plastic spatula from every sampling site of the snails. These samples were brought back to the laboratory for Cd analysis.

About 6-21 individuals of *T. telescopium* from every site were dissected and pooled into seven different soft tissues, namely foot, cephalic tentacle (CT), mantle, muscle, gill, digestive caecum (DC) and remaining soft tissues (REST). The shell lengths (from anterior to posterior) and shell widths (from ventral to dorsal) 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 72h until constant dry weights. Three replicates of each different part of soft tissues and shells 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 shaken vigorously to produce homogeneity. For the analyses of total Cd concentrations in the sediment samples, three replicates were analysed by using the direct aqua-regia method. About 1g 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 1hr and then were fully digested at 140°C for at least 3hrs. The prepared samples were then analysed for Cd 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

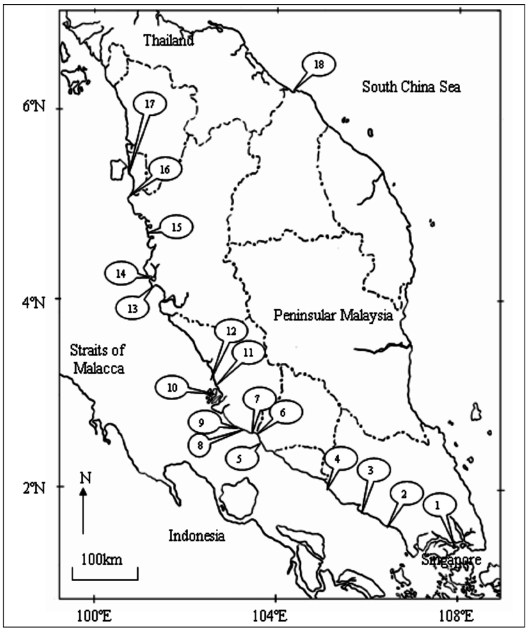

Figure 1: Map showing of *Telecopium telescopium* from 18 sampling sites of the intertidal area of Peninsular Malaysia.

fractions, extraction solutions and conditions employed by each fraction were:

1. Easily, freely, leacheable or exchangeable (EFLE): About 10g of the sample was shaken continuously with 50ml of 1.0M ammonium acetate (NH_4CH_3COO), at pH 7.0 and at room temperature pH 7.0 for 3 hours.
2. Acid-reducible (AR): The residue from the first step was shaken continuously with 50ml of 0.25M hydroxylammonium chloride ($NH_2OH{\cdot}HCl$) that had been acidified with HCl to pH 2, at room temperature for 3 hours.
3. Oxidisable-organic (OO): The residue from the second step was first oxidised with 30% H_2O_2 in a water bath at 90-95°C. After being oxidised, the residue was shaken continuously with 50ml of 1.0M ammonium acetate (NH_4CH_3COO) that had been acidified with HCl to pH 2, at room temperature for 3 hours.
4. Resistant (RES): The residue from the third step was digested with 10ml of an aqua-regia solution that comprised a combination of nitric acid (AnalaR grade, BDH 69%) and perchloric acid (AnalaR grade, BDH 60%) in the ratio of 4:1 for 1 hour at a temperature of 40°C. The temperature was then increased to 140°C for an additional 3 hours.

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 20ml 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 prepared using an identical procedure to ensure that the samples and chemicals used were not contaminated (Yap *et al*., 2002). The nonresistant (NR) fraction was 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 1000ppm stock solution for Cd were analysed once every five samples in order to check for sample accuracy. The quality of the methods used were checked with Certified Reference Materials for Soil (NCS DC73319-Soil, China National Analysis Centre for Iron and Steel 2004) [certified Cd value: 4.30 µg/g dw, measured Cd value: 4.84 µg/g dw; recovery= 112.6%], while tissues of snails were verified using Dogfish Liver (DOLT-3, National Research Council Canada) [certified Cd value: 19.4 µg/g dw, measured Cd value: 19.7 ± 0.13 µg/g dw; recovery= 101.7%].

For the statistical analysis, the relationships of Cd 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) and

Table 1: Sampling dates and sampling sites (Global Positioning System), for the intertidal sediments and *Telescopium telescopium* along intertidal areas of Peninsular Malaysia.

No.	Sampling Sites	Sampling dates	Longitude	Latitude	Sites description
1.	Kg Pasir Puteh (KPPuteh), Johore	30 Apr 2006	N 01° 26’ 05.8”	E 101° 56’ 02.4”	Fishing area, mangrove and industrial area at Pasir Gudang
2.	Pantai Punggur (PPunggur), Johore	29 Apr 2006	N 01° 41’ 07.2	E 103° 05’ 54.6”	A recreational area
3.	Kuala Sg Ayam (KSAyam), Johore	29 Apr 2006	N 01° 45’ 12.5”	E 102° 55’ 45.4	A recreational beach and a muddy area
4.	Sg. Balang Laut (SBLaut), Johore	29 Apr 2006	N 01° 52’ 21.0	E 102° 44’ 16.5	A busy jetty, housing area, fishing village, mangrove swamp and an estuary
5.	Kuala Lukut Kecil (KLukutK), Negeri Sembilan	28 Apr 2006	N 02° 33’ 42.2”	E 101° 48’ 00.2”	A prawn aquaculture, mangrove swamp, water irrigation
6.	Kuala Lukut Besar (KLukutB), Negeri Sembilan	28 Apr 2006	N 02° 34’ 49.2”	E 101° 49’ 34.4”	Under construction of jetty, mangrove swamp, prawn farm
7.	Sg Sepang Kecil (SepangK), Selangor	18 Aug 2006	N 02º 36’ 4.11”	E 101º 41’ 7.79”	An aquaculture of prawn and muddy area,
8.	Bagan Lalang (BLalang), Selangor	15 Sep 2006	N 02º 35’ 57.52”	E 101º 42’ 31.41”	An aquaculture of prawn, water gate and near Dragon fruit farm, muddy area
9.	Sg Sepang Besar (SepangB), Selangor	7 Jan 2006	N 02º 36’ 19.41”	E 101º 42’ 11.51”	A restaurant, jetty, water irrigation and thousands of *T. Telescopium* are found
10.	Sg Janggut (SJanggut), Selangor	20 Mar 2006	N 03° 10’ 20.0”	E 101° 18’ 1.4”	A housing area, muddy, chicken farm, palm oil plantation and prawn culture activities
11.	Kg Pantai Jeram (KPJeram), Selangor	24 Feb 2006	N 03° 13’ 14.6”	E 101° 18’ 19.5”	A jetty and sea-food restaurant
12.	Pulau Indah (PIndah), Selangor	16 Aug 2006	N 03º 0’ 22.94”	E 101° 18’ 22.5”	An irrigation water, a small jetty and muddy area
13.	Jambatan Permaisuri Bainun (JPBainum), Perak	27 Feb 2006	N 04° 16’ 46.0”	E 100° 39’ 50.2”	A residential area (kampong), recreational area (kayak) and an estuary.
14.	Kg Deralik (KDeralik), Perak	25 Feb 2006	N 04° 14’ 53.8”	E 100° 42’ 09.1”	A busy traffic and road to west port of Klang
15.	Kg Setiawan (KSetiawan), Perak	25 Feb 2006	N 04° 14’ 44.3”	E 100° 41’ 35.6”	A residential area, mangrove (very muddy) with no direct polluton observed
16.	Kuala Gula (KGula), Perak	12 Jan 2007	N 04º 55’ 89.6”	E 100º 26’ 79.1”	Under the bridge and near the Port of Lumut.
17.	Jetty Kuala Juru (KJuru), Penang	20 Apr 2005	N 05º20.410'	E 100º24.518'	An industrial area in Penang
18.	Tumpat, Kelantan	15 Dec 2006	N 06º 12’ 55.21”	E 102º 14’ 14.21”	A pristine area

Note: Number of sites followed those in Figure 1.

cluster analysis (Yap *et al*., 2010). 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 Cd concentrations in the four geochemical fractions and NR fractions for the surface sediments collected from 18 sampling sites, was done by using STATISTICA 99” 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 and Discussion

The mean concentrations of Cd in the different soft tissues of *T. telescopium*, collected from 18 sampling sites on the intertidal area of Peninsular Malaysia, are presented in Figure 2 and the overall Cd concentrations are given in Table 3. Most obviously, the highest Cd concentrations (µg/g dry weight) are consistently found in DC (6.36), gill (2.48), mantle (2.68) and muscle (1.81) from KJuru population. Populations of KPunggur, KDeralik and KPPuteh had the highest Cd levels in CT (1.81), foot (1.96) and

Table 2: Mean values of shell heights (cm) and widths (cm) (± standard error) of *Telescopium telescopium* analysed and descriptions of sampling sites in the intertidal area of Peninsular Malaysia.

No.	Sites	N	Height			Width		
1	KPPuteh	11	8.60	±	0.11	4.28	±	0.07
2	PPunggur	8	7.30	±	0.18	3.08	±	0.04
3	KSAyam	11	6.58	±	0.14	3.18	±	0.04
4	SBLaut	15	5.65	±	0.15	3.24	±	0.07
5	KLukutK	17	5.35	±	0.07	2.83	±	0.04
6	KLukutB	6	8.98	±	0.13	4.52	±	0.05
7	SepangK	9	4.96	±	0.06	2.89	±	0.06
8	BLalang	10	8.35	±	0.08	4.56	±	0.12
9	SepangB	12	7.81	±	0.12	3.71	±	0.04
10	SJanggut	10	8.41	±	0.17	3.89	±	0.07
11	KPJeram	10	7.64	±	0.06	3.72	±	0.05
12	PIndah	21	8.74	±	0.16	4.73	±	0.09
13	JPBainum	13	7.15	±	0.13	3.51	±	0.07
14	KDeralik	8	9.20	±	0.08	4.68	±	0.21
15	KSetiawan	6	7.83	±	0.07	3.90	±	0.08
16	KGula	8	8.47	±	0.14	4.16	±	0.04
17	KJuru	20	8.96	±	0.99	4.37	±	0.44
18	Tumpat	9	9.03	±	0.13	4.82	±	0.04

REST (2.28), respectively. By comparing the Cd concentrations in the similar tissues, spatial variation of Cd was found in the different sampling sites although there is no consistent pattern of Cd in these sampling sites (Figure 2). The well-reported metal-polluted sites from KPPuteh and KJuru are manifest in the high Cd bioavailability to the remaining soft tissues of *T. telescopium* while most soft tissues indicated that *T. telescopium* in Kuala Juru has high Cd bioavailability to *T. telescopium*.

Based on a dendrogram using cluster analysis on the geochemical fractions of the surface sediments (Figure 3), it was found that KJuru was ranked on the top in which it was subclustered together with KSAyam. This may indicate Cd contamination at KJuru and KSAyam. It should be noted that Kuala Juru has been reported as a metal-polluted site according to some previous studies (Lim and Kiu, 1995; Yap *et al.,* 2002, 2003) and the Juru River is one of the polluted rivers in Malaysia according to a recent report by the Malaysian Department of Environment (Malaysian DOE 2008). On the other hand, Tumpat ranked the lowest with low concentrations (Figure 2), indicating less Cd contamination.

When the dendrogram is based on the cluster analysis on the Cd concentrations in all seven tissues of *T. telescopium* collected from 18 sampling sites (Figure 4), it is found that higher Cd bioavailability is evidenced to KJuru population. This is well clustered as one single entity. It is almost expected for KJuru to be highly contaminated by Cd as KJuru had been reported as a metal-polluted site in Peninsular Malaysia (Lim and Kiu, 1995; Yap *et al.,* 2003) and the highest Cd concentrations found in DC, gill, mantle and muscle at KJuru population (Figure 2). From the two dendrograms in Figures 3 and 4, the higher Cd contamination in the sampling site as represented by the sediments, the higher Cd bioavailability to the biomonitor *T. telescopium* may be expected.

It is found that the overall accumulation patterns for Cd based on 18 populations are as follows: DC> REST> gill> mantle> CT> muscle> foot (Table 3). From Table 3, ratio of maximum to the minimum values is useful to understand which tissue has the highest Cd variation. It is found that mantle has the highest ratio (134), followed by foot, muscle, REST, CT, DC and gill. This might point to the fact that mantle has the high Cd variation in response to environmental Cd bioavailability.

The Cd concentration (mean µg/g dry weight ± SE) of four geochemical fractions of surface sediments in the snail habitats from 18 sampling sites are given in Table 4. It is found that KPPuteh recorded the highest EFLE Cd while the highest AR and OO are found in JPBainun and KSetiawan, respectively. KSAyam recorded the highest RES and summation of Cd. The only site recording dominance (> 50%) of NR fraction of Cd is SepangB, based on NR/R ratio.

In order to investigate the phenomenon of Cd redistribution due to high Cd bioavailability to the biomonitor *T. telescopium*, increasing orders of Cd concentrations in the different soft tissues of *T. telescopium* from 18 sampling sites are presented in Table 5. Overall, DC showed the highest Cd levels among all the seven soft tissues studied. Cd redistribution in DC at KJuru, which was found to have the highest Cd level in DC, was not evidenced. This was because DC from 14 out of 18 populations also recorded the highest Cd concentrations, ranging from different sampling-site backgrounds. Similarly, KJuru population that recorded the highest Cd concentrations in gill, mantle and muscles also showed no evidence of Cd redistribution, indicating the consistency of higher Cd level found in one soft tissue, the higher Cd levels accumulated in other soft tissues would follow. One interesting characteristic found is the significant highest Cd level in CT from KPunggur population which had exceeded the DC level, thus, indicating Cd redistribution phenomenon due to high accumulation of Cd in the CT. The highest Cd level found in REST from KPPuteh population is not likely to be redistributed due to the fact that REST is also similarly ranked second to DC in other lower Cd REST populations when compared to other populations. On the other hand, muscle and foot always show the lowest Cd levels among all the seven soft tissues investigated. Either muscle or foot from 7 out of 18 populations recorded the lowest Cd concentrations. Thus, KDeralik population that recorded the highest Cd concentrations in foot is indicative of Cd redistribution since foot is ranked second to DC due to high Cd accumulation in foot.

Correlation coefficients of Cd concentrations between the different soft tissues of the snails and geochemical fractions of the surface sediment are presented in Table 6. It is clearly seen that the only significant ($P< 0.05$) pairwises are found for CT-AR ($R= -0.53$) and mantle-EFLE ($R= 0.57$). This indicated that only CT and mantle could significantly ($P< 0.05$) reflect one of the Cd NR fractions of the surface sediment while other soft tissues are found insignificant ($P> 0.05$). The negative correlation coefficients found, although not significant, $P> 0.05$) between the different soft tissues and the geochemical fraction of Cd in sediments should not be regarded as a point to conclude that the different soft tissues are not good biomonitoring tissues for Cd bioavailabilities and contamination. This could be due to low Cd bioavailability and contamination or rapid detoxification of Cd (Negri *et al.,* 2006) by the selected soft tissues of *T. telescopium*. Also, this can be due to Cd levels being low in sediments, probably lower than the threshold below which *T. telescopium* is able to regulate the Cd accumulation in its different soft tissues (Usero *et al.,* 2005).

The high variations of Cd concentrations in mantle (Table 3) could be the reason why only mantle is significantly ($P< 0.05$) influenced by EFLE, AR and OO fractions of the surface sediments as shown in MLSRA (Table 7). However, based on MLSRA (Table 7), although CT has a lower ratio when compared to foot, muscle and REST (Table 3), it is also significantly ($P< 0.05$) influenced by EFLE, AR and OO fractions of the surface sediments. Therefore, high Cd variation in the soft tissues might not necessarily be influenced by the Cd levels in the sediment geochemical fractions.

Different bioaccumulation of Cd in the different soft tissues in the different populations

Different Cd concentrations in the different soft tissues are indicative of different mechanisms of sequestration and regulations of Cd in these different tissues. The different ratios of maximum to minimum Cd concentrations in the different soft tissues are also evidence to prove that the accumulative capacity for Cd is different in every tissue. All the ratios ranged from 9.9-134 with gill having the narrowest range while mantle having the widest range. Thus, this implies that certain tissue is better in the biomonitoring for Cd. The different degrees of Cd accumulation may also indicate that the mechanism of detoxification and metallothionein synthesis for this non-essential Cd (Viarengo *et al.,* 1985) is different in each soft tissue of *T. telescopium*.

Accumulation of Cd in the soft tissues of molluscs is always related to the metal binding by metallothionein (Talbot and Magee, 1978)

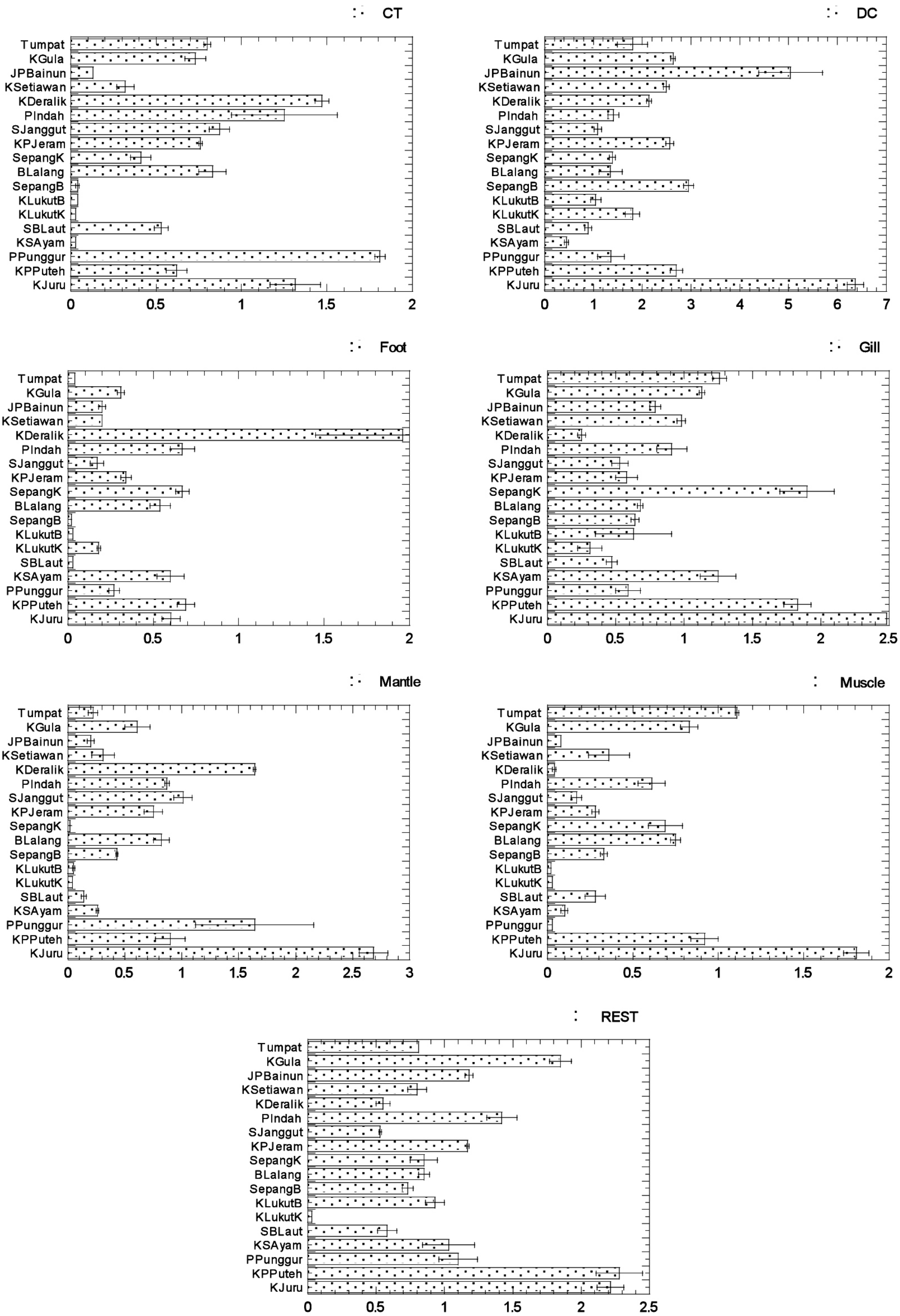

Figure 2: Cadmium concentrations (mean ± standard error, µg/g dry weight) in the different soft tissues of *Telescopium telescopium* collected from 18 sampling sites in the intertidal area of Peninsular Malaysia. Note: CT= cephalic tentacle; DC= digestive caecum; REST= remaining soft tissues. All values in X-axis are concentrations, µg/g dry weight.

Table 3: Overall concentrations (µg/g dry weight) of Cd in the different soft tissues of *Telescopium telescopium* collected from 18 sampling sites in Peninsular Malaysia.

	Minimum	Maximum	Max/Min	Mean	Std Error
CT	0.03	1.81	60.33	0.67	0.13
DC	0.45	6.36	14.13	2.19	0.35
Foot	0.02	1.96	98.00	0.42	0.11
Gill	0.25	2.48	9.92	0.96	0.14
Mantle	0.02	2.68	134.00	0.70	0.17
Muscle	0.02	1.81	90.50	0.47	0.11
REST	0.03	2.28	76.00	1.05	0.14

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

Table 4: The Cd concentration (mean µg/g dry weight ± SE) of four geochemical fractions of surface sediments in the snail habitats from 18 sampling sites of Peninsular Malaysia (N= 3).

No.	Sites	EFLE	AR	OO	RES	SUM	NR	NR/R
1	KPPuteh	**0.30**	0.28	0.64	1.38	2.60	1.22	0.88
2	PPunggur	0.22	0.02	0.21	2.65	3.10	0.45	0.17
3	KSAyam	0.17	0.21	0.58	**3.53**	**4.49**	0.96	0.27
4	SBLaut	0.11	0.70	0.33	2.98	4.12	1.14	0.38
5	KLukutK	0.11	0.08	0.54	1.38	2.11	0.73	0.53
6	KLukutB	0.16	0.44	0.54	0.92	2.06	1.14	1.24
7	SepangK	0.05	0.04	0.09	0.86	1.04	0.18	0.21
8	BLalang	0.13	0.09	0.24	1.12	1.58	0.46	0.41
9	SepangB	0.11	0.22	0.32	0.56	1.21	0.65	**1.16**
10	SJanggut	0.22	0.02	0.21	1.79	2.24	0.45	0.25
11	KPJeram	0.23	0.08	0.35	2.05	2.71	0.66	0.32
12	PIndah	0.11	0.01	0.27	0.79	1.18	0.39	0.49
13	JPBainum	0.16	**0.92**	0.55	2.25	3.88	1.63	0.72
14	KDeralik	0.19	0.03	0.42	2.01	2.65	0.64	0.32
15	KSetiawan	0.23	0.70	**0.85**	2.01	3.79	**1.78**	0.89
16	KGula	0.14	0.03	0.07	1.05	1.29	0.24	0.23
17	KJuru	0.23	0.11	0.50	2.83	3.67	0.84	0.30
18	Tumpat	0.02	0.01	0.02	0.12	0.17	0.05	0.42

Note: EFLE– easily, freely, leacheable or exchangeable; AR– acid-reducible; OO- oxidisable-organic; RES– resistant; SUM- summations of four geochemical fractions; NR= summation of EFLE, AR and OO. Values in bold are the maximum levels.

or by other molecules (Dallinger and Wieser, 1984) or compartmentalisation of metals into specific cellular structures like organelles or vesicles (Simkiss and Mason, 1984). According to Sri Lakshmi and Prabhakara Rao (2002), the intertidal gastropod *Turbo intercostalis* has a good capacity of accumulation and depuration of Cd and is thus considered as a suitable species for biomonitoring of the coastal environment. On the subject of the toxicity of Cd to aquatic organisms, deleterious effects have also been reported in limpets, where correlations between increased levels of Cd and reduced ability to utilise glucose were found and the organism may excrete all the metal that enters in excess of metabolic requirements, thereby maintaining a relatively constant body content corresponding to the physiological need of the organism (Rainbow, 1997). Although Cd is not considered to be internally regulated in marine invertebrates, some biological processes might appear to control Cd concentration in the different soft tissues (Sokolowski *et al.,* 2005).

Digestive caecum is the main target organ for Cd storage and accumulation

Out of 18 sampling sites, 14 sites showed digestive caecum accumulated the highest Cd concentration, followed by the remaining soft tissues, gill and other soft tissues. The higher Cd concentrations in digestive caecum, remaining soft tissues and gills had indicated that they were the main target organs for Cd accumulation.

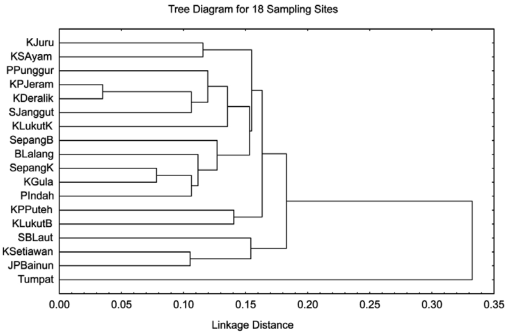

Figure 3: Cluster analysis based on Single Linkage Euclidean distances, on the geochemical fractions of Cd (EFLE, acid-reducible, oxidisable-organic, resistant and nonresistant) and its summation in the surface sediments from 18 sampling sites, based on $\log_{10}$(mean + 1) transformed data.

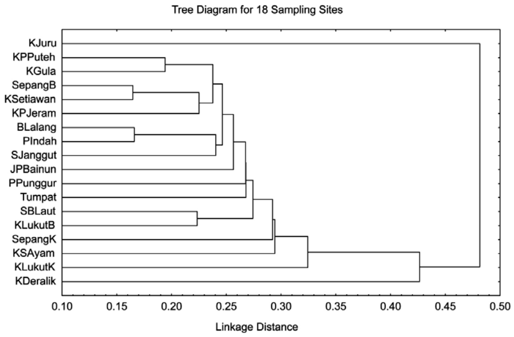

Figure 4: Cluster analysis based on Single Linkage Euclidean distances, on the Cd concentrations in 7 tissues of *Telescopium telescopium* collected from 18 sampling sites, based on $\log_{10}$(mean + 1) transformed data.

The higher concentration of Cd in the digestive caecum of *T. telescopium* was supported by reported studies. For example, some researchers reported high concentration of metals in the digestive gland of marine gastropods (Nott and Nicolaidou, 1989) and *Patella caerulea* in particular (Yüzereroğlu *et al.,* 2010). The metals are accumulated within intracellular mineralised granules as phosphates and within lysosomal residual bodies in association with sulphur (Nott and Nicolaidou 1989). These results could explain the high Cd levels found in the DC of *T. telescopium*, which formed part of the digestive glands. The elevated Cd concentration found in this DC indicated that DC is a storage site for this nonessential Cd, possibly in the form of metallothionein or granules as a Cd detoxification mechanism (Lobel *et al.,* 1982). However, the highest Cd concentrations found in DC was not supported by the correlation analysis and MLSRA, which showed insignificant correlations between DC with any of the geochemical fractions in the sediments and there were not any geochemical fraction selected as influential parameters (Table 7). This indicated that a high Cd contamination in the environmental habitat may not be necessarily have high Cd bioavailability to the biomonitor *T. telescopium,* especially in the DC which is very accumulative of Cd. This suggests that the Cd bioaccumulation and bioavailability to the biomonitors are very much dependent on other factors, such as metal speciation, multiple routes of exposure (diet and solution) and geochemical effects (Luoma and Rainbow, 2005).

One of the major sources of Cd in the marine environment is sewage. The *T. telescopium* collected from most sampling sites were potentially receiving large amounts of it, and therefore, had the highest Cd concentrations in the DC (due to increased bioavailabilities), if anthropogenic inputs were significant.

Cephalic tentacle and mantle are biomonitoring organs for Cd contamination

Based on correlation analysis and MLSRA, CT and mantle significantly correlated with one of the NR fractions while all the three NR fractions (EFLE, AR and OO) were selected as significant influential factors in the accumulation of Cd in both tissues. Thus, besides reflecting the Cd bioavailability, CT and mantle of *T. telescopium* are also biomonitoring organs for Cd contamination in the tropical intertidal area. The availability of Cd to living organisms from their immediate physical chemical environment depends on numerous factors, including adsorption and desorption rates of Cd from terrigenous materials, pH, chemical speciation

Table 5: Increasing order of Cd concentrations (mean µg/g dry weight) in the different soft tissues of *Telescopium telescopium* from 18 sampling sites.

No.	Sampling sites	Orders of Cd accumulation patterns						
1.	KJuru	Foot	CT	Muscle	REST	Gill	Mantle	DC
		0.60	1.31	1.81	2.21	2.48	2.68	6.36
2.	KPPuteh	CT	Foot	Mantle	Muscle	Gill	REST	DC
		0.62	0.69	0.9	0.92	1.83	2.28	2.7
3.	PPunggur	Muscle	Foot	Gill	REST	DC	Mantle	CT
		0.03	0.27	0.59	1.1	1.36	1.64	1.81
4.	KSAyam	CT	Muscle	Mantle	DC	Foot	REST	Gill
		0.03	0.1	0.26	0.45	0.6	1.03	1.25
5.	SBLaut	Foot	Mantle	Muscle	Gill	CT	REST	DC
		0.03	0.14	0.28	0.47	0.53	0.58	0.89
6.	KLukutK	CT	Muscle	REST	Mantle	Foot	Gill	DC
		0.03	0.03	0.03	0.04	0.18	0.31	1.8
7.	KLukutB	Muscle	Foot	CT	Mantle	Gill	REST	DC
		0.02	0.03	0.04	0.05	0.63	0.93	1.05
8.	SepangB	Foot	CT	Muscle	Mantle	Gill	REST	DC
		0.02	0.04	0.33	0.43	0.64	0.73	2.95
9.	BLalang	Foot	Gill	Muscle	Mantle	CT	REST	DC
		0.54	0.68	0.75	0.82	0.83	0.85	1.35
10.	SepangK	Mantle	CT	Foot	Muscle	REST	DC	Gill
		0.02	0.41	0.67	0.69	0.85	1.39	1.9
11.	KPJeram	Muscle	Foot	Gill	Mantle	CT	REST	DC
		0.28	0.34	0.58	0.75	0.76	1.17	2.56
12.	SJanggut	Muscle	Foot	REST	Gill	CT	Mantle	DC
		0.17	0.17	0.53	0.53	0.87	1.01	1.09
13.	PIndah	Muscle	Foot	Mantle	Gill	CT	DC	REST
		0.61	0.67	0.87	0.91	1.25	1.41	1.42
14.	KDeralik	Muscle	Gill	REST	CT	Mantle	Foot	DC
		0.04	0.25	0.55	1.47	1.64	1.96	2.14
15.	KSitiawan	Foot	Mantle	CT	Muscle	REST	Gill	DC
		0.2	0.31	0.32	0.36	0.8	0.98	2.49
16.	JPBainun	Muscle	CT	Foot	Mantle	Gill	REST	DC
		0.08	0.13	0.2	0.2	0.79	1.18	5.04
17.	KGula	Foot	Mantle	CT	Muscle	Gill	REST	DC
		0.31	0.61	0.73	0.83	1.13	1.85	2.63
18.	Tumpat	Foot	Mantle	CT	REST	Muscle	Gill	DC
		0.04	0.22	0.8	0.81	1.11	1.26	1.80

Note: CT – cephalic tentacle, REST – remaining soft tissues, DC – digestive caecum

Table 6: Pearson's correlation coefficients of Cd concentrations (based on log10[mean+1]) between the different soft tissues of *Telescopium telescopium* and geochemical fraction of the surface sediment. N= 18.

	EFLE	AR	OO	RES	SUM	NR
CT	0.23	**-0.53**	-0.40	0.10	-0.08	-0.44
DC	0.27	0.18	0.21	0.01	0.09	0.23
Foot	0.25	-0.37	0.09	0.22	0.13	-0.11
Gill	0.05	-0.07	-0.03	-0.08	-0.10	-0.08
Mantle	**0.57**	-0.43	-0.03	0.32	0.20	-0.13
Muscle	-0.11	-0.27	-0.29	-0.32	-0.38	-0.33
REST	0.41	-0.01	0.02	0.08	0.08	0.05

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; 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 Cd concentrations (based on log10[mean+1]) between the different soft tissues of *Telescopium telescopium* and the geochemical fractions of sediment. N= 18.

Soft tissue	Multiple linear stepwise regression equation	R	R^2
CT	CT = 0.180 + 3.463 (EFLE) – 0.370 (AR) – 1.294 (OO)	0.737	0.543
DC	No significant variable was selected.		
Foot	No significant variable was selected.		
Gill	No significant variable was selected.		
Mantle	0.034 + 4.658 (EFLE) – 0.0709 (AR) – 0.567 (OO)	0.791	0.625
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; SUM- summations of four geochemical fractions; NR– nonresistant; CT– Cephalic tentacle; REST– remaining soft tissues; DC– digestive caecum. Those dependent variables were significantly ($P < 0.05$) influenced by the selected independent variables.

and other modifiers (Eisler, 2000). Changes in physico-chemical conditions, especially pH and redox potential, that occur during dredging and disposal of Cd-polluted sediments may increase chemical mobility and hence bioavailability of sediment-bound Cd (Khalid *et al.,* 1978).

Tissue redistributions of Cd in cephalic tentacle and foot can be used as indicators of high Cd bioavailability

CT and foot can be redistributed due to high Cd bioavailability while Cd redistribution is not clear for other soft tissues. However, it should be noted that PPunggur and KDeralik are two sampling sites which are not highly contaminated by Cd, based on sediment data. Therefore, we can conclude tissue redistributions of Cd in CT and foot can be indicators of high Cd bioavailability, but contamination by Cd is not quite evidenced from the present study.

Conclusions

Present study revealed the importance of employing multivariate analyses in finding reliable relationships of Cd concentrations between the different soft tissues of *T. telescopium* and sediments. Based on multivariate analyses, CT and mantle were identified as biomonitoring organs for Cd contamination. From the different bioaccumulation of Cd found in the different soft tissues of snails, DC was found as the main target organ for Cd storage and accumulation. Tissue redistributions of Cd in CT and foot are proposed as indicators of high Cd bioavailability of the sampling sites.

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