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Shells of *Telescopium Telescopium* as Biomonitoring Materials of Ni Pollution in the Tropical Intertidal Area

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ABSTRACT

The objective of this paper is to propose the use of the shells of *Telescopium telescopium* as good biomonitoring materials of Ni pollution. The snail and surface sediment samples were collected from 17 geographical sites in Peninsular Malaysia and they were dissected into six different soft tissues, namely foot, cephalic tentacle (CT), mantle, muscle, gill and remaining soft tissues (REST). The total shells, pooled and dissected soft tissues and sediments were determined for Ni using an air-acetylene flame Atomic Absorption Spectrophotometer. The present findings based on 1) ratios of shell/soft tissues (ratios> 1.0), 2) bioaccumulation factor values (shell as microconcentrator), 3) statistical outcomes of correlation analysis [significant correlations between shells and geochemical fractions (acid-reducible, non-resistant, resistant and total Ni concentrations) in the environmental sediments] and multiple linear stepwise regression analysis [significantly influenced by total Ni concentrations in the sediment], and 4) lowest value of coefficient of variation [thus higher precision], do indicate the very potential of shells of *T. telescopium* as good biomonitoring material of Ni pollution in the tropical intertidal area.

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1. INTRODUCTION

Recently, Yap et al. (2012) proposed the use of digestive cecum of *T. telescopium* as a biomonitoring organ of Ni contamination in the tropical intertidal area [1]. The finding was fundamentally supported by positive correction with the environmental sediment of the gastropod habitats. However, the paper by Yap et al. (2012) did not report the gastropod shells due to some technical problems during preparation of the manuscript [1]. In fact, the potentials of mollusks as biomonitoring materials of heavy metal pollution have been widely reported in the literature (Walsh et al. 1995; Yap et al. 2003) but that in the shells of *T. telescopium* awaits further studies [2],[3]. Inclusion of shell analyses in routine monitoring procedures may be advantageous because the shell of these organisms can be stored after harvest for long period of time and can be potentially compared with shells preserved in the geological record [4],[5]. In addition, molluscan shell is a convenient tool for assessing the condition of the coastal environment [6],[7].

Moreover, gastropod shells can accumulate a wide range of metals to varying extents because of different minerals and chemistries in the shell [8]. Some trace metals are incorporated into the shells through the substitution of the calcium ion in the crystalline phase of the shell or associated with the organic matrix during shell growth must have been assimilated by the animal [9]. The concentration of an element found in the shell at a particular stage of growth critically depends upon the extent to which the element is accumulated in the tissue during the formative growth process and its concentration in the weight of shell tissue added during subsequent growth [6]. Moreover, which elements are preferentially incorporated into the shell during the early stages of growth and the magnitudes of such incorporation are sensitive to the chemical composition to which they are exposed [7].

Since the idea of using shells of *T. telescopium* has not been reported in the literature, the objective of this study was to propose the use of *T. telescopium* as biomonitoring materials of Ni pollution in the tropical intertidal area based on four important points discussed in this paper.

2. MATERIALS AND METHODS

Snails were randomly hand-picked from 17 geographical sampling sites from the intertidal areas of Peninsular Malaysia. The site descriptions are given in Table 1In addition to snails, surface sediment samples (0-10 cm) were also collected by using a clean stainless spatula from every sampling site of the snails. All the snails and sediment samples were put into clean plastic bags and stored in an ice compartment (< 10ºC) until transportation to the laboratory for analysis.

Table 1. Positions (Global positioning system), sampling dates, for the intertidal sediments and *T. telescopium* collected along the west coast of Peninsular Malaysia

No.	Sampling Sites	Date	GPS	GPS
1.	Kg Pasir Puteh (KPP)	30 Apr 2006	N 01° 26’ 05.8”	E 101° 56’ 02.4”
2.	Pantai Punggur (PP)	29 Apr 2006	N 01° 41’ 07.2	E 103° 05’ 54.6”
3.	Kuala Sg Ayam (KS)	29 Apr 2006	N 01° 45’ 12.5”	E 102° 55’ 45.4
4.	Sg Balang Laut (SBL)	29 Apr 2006	N 01° 52’ 21.0	E 102° 44’ 16.5
5.	Kuala Lukut Kecil (KLK)	28 Apr 2006	N 02° 33’ 42.2”	E 101° 48’ 00.2”
6.	Kuala Lukut Besar (KLB)	28 Apr 2006	N 02° 34’ 49.2”	E 101° 49’ 34.4”
7.	Sg Sepang Kecil (SK)	18 Aug 2006	N 02º 36’ 4.11”	E 101º 41’ 7.79”
8.	Bagan Lalang (BL)	15 Sep 2006	N 02º 35’ 57.52”	E 101º 42’ 31.41”
9.	Sg Sepang Besar (SB)	7 Jan 2006	N 02º 36’ 19.41”	E 101º 42’ 11.51”
10.	Sg Janggut (SJ)	20 Mar 2006	N 03° 10’ 20.0”	E 101° 18’ 1.4”
11.	Kg Pantai Jeram (KPJ)	24 Feb 2006	N 03° 13’ 14.6”	E 101° 18’ 19.5”
12.	Pulau Indah (PI)	16 Aug 2006	N 03º 0’ 22.94”	E 101º 18’ 22.5”
13.	Jambatan Permaisuri Bainun (JPB)	27 Feb 2006	N 04° 16’ 46.0”	E 100° 39’ 50.2”
14.	Kg Deralik (KD)	25 Feb 2006	N 04° 14’ 53.8”	E 100° 42’ 09.1”
15.	Kg Setiawan (KS)	25 Feb 2006	N 04° 14’ 44.3”	E 100° 41’ 35.6”
16.	Kuala Gula (KG)	12 Jan 2007	N 04º 55’ 89.6”	E 100º 26’ 79.1”
17.	Tumpat (T)	15 Dec 2006	N 06º 12’ 55.21”	E 102º 14’ 14.21”

Note: Number of sites followed those in Figure 1.

The shell heights and shell widths for the snails used for metal analysis are given in Table 2. About 6-21 individuals of *T. telescopium* from every site were dissected and pooled into six different soft tissues, namely foot, cephalic tentacle (CT), mantle, muscle, gill and remaining soft tissues (REST). The total shells were crushed into pieces by using a pestle and mortal.

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

No.	Sites	N	Height			Width			Sites description
1	KPP	11	8.6	±	0.11	4.28	±	0.07	Fishing area, mangrove and industrial area at Pasir Gudang
2	PP	8	7.3	±	0.18	3.08	±	0.04	A recreational area
3	KSA	11	6.58	±	0.14	3.18	±	0.04	A recreational beach and a muddy area
4	SBL	15	5.65	±	0.15	3.24	±	0.07	A busy jetty, housing area, fishing village, mangrove swamp and an estuary
5	KLK	17	5.35	±	0.07	2.83	±	0.04	A prawn aquaculture, mangrove swamp, water irrigation
6	KLB	6	8.98	±	0.13	4.52	±	0.05	Under construction of jetty, mangrove swamp, prawn farm
7	SB	9	4.96	±	0.06	2.89	±	0.06	An aquaculture of prawn and muddy area,
8	BL	10	8.35	±	0.08	4.56	±	0.12	An aquaculture of prawn, water gate and near Dragon fruit farm, muddy area
9	SK	12	7.81	±	0.12	3.71	±	0.04	A restaurant, jetty, water irrigation and thousands of *T. Telescopium* are found
10	KPJ	10	8.41	±	0.17	3.89	±	0.07	A housing area, muddy, chicken farm, palm oil plantation and prawn culture activities
11	SJ	10	7.64	±	0.06	3.72	±	0.05	A jetty and sea-food restaurant
12	PI	21	8.74	±	0.16	4.73	±	0.09	An irrigation water, a small jetty and muddy area
13	KD	13	7.15	±	0.13	3.51	±	0.07	A residential area (kampong), recreational area (kayak) and an estuary.
14	KS	8	9.2	±	0.08	4.68	±	0.21	A busy traffic and road to west port of Klang
15	JPB	6	7.83	±	0.07	3.9	±	0.08	A residential area, mangrove (very muddy) with no direct polluton observed
16	KG	8	8.47	±	0.14	4.16	±	0.04	Under the bridge and near the Port of Lumut.
17	T	9	9.03	±	0.13	4.82	±	0.04	A pristine area

All of the snail and sediment samples were dried at 80°C for 72hrs until constant dry weights. Three replicates of different 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, sieved through a 63 µm aperture stainless steel sieve and shaken vigorously to produce homogeneity. For the analyses of total Ni concentrations in the sediment samples, three replicates were analyzed 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 analyzed for Ni 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 the four geochemical fractions of the surface sediments, the sequential extraction technique (SET) described by Badri and Aston (1983) and slightly modified by Yap et al. (2002) was used [10],[11]. The four fractions, extraction solutions and the conditions employed for each fraction were 1) Easily, freely, leacheable or exchangeable (EFLE), 2) Acid-reducible (AR), 3) Oxidisable-organic (OO) and 4) Resistant (RES).

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 done using an identical procedure to ensure that the samples and chemicals used were not contaminated [11]. The nonresistant (NR) fraction was calculated based on the summation of EFLE, AR and OO while the total summation (SUM) was 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 Ni were analyzed once every five samples in order to check for sample accuracy. The quality of the methods used were checked with the Certified Reference Materials for Soil (NCS DC73319-Soil,China National Analysis Center for Iron and Steel 2004) [certified Ni value: 20.4 µg/g dw, measured Ni value: 19.5 µg/g dw; recovery= 95.6%] while for the tissues of snails, they were verified using Dogfish Liver (DOLT-3, National

Research Council Canada) [certified Ni value: 2.72 µg/g dry weight, measured Ni value: 3.41 µg/g dry weight; recovery= 125.3%].

For the statistical analysis, the relationships between the Ni concentrations of the different parts of the snails and the geochemical factions of the sediments were elucidated by using correlation coefficients (CA) and multiple linear stepwise regression analysis (MLSRA). All the above analyses above were performed by using the Statistical Program for Social Science (SPSS) for Windows, version 15.0 software. All the data for the CA and MLSRA analyses were $\log_{10}$(mean + 1) transformed in order to reduce the variance [12].

Coefficient of variation

The coefficient of variation (CV) value is a well-known unitless index of 'relative' variation [13]. It is useful to relate the arithmetic means and the standard deviation together. The CV value is most useful in comparing the variability of several different samples, each with different arithmetic means. This is because a higher variability is usually expected when the standard deviation increases, and the CV value is a measure that accounts for the variability [14],[15]. In each individual, the CV value was calculated from the untransformed data as :-

$$\text{CV (\%)} = \frac{\text{standard deviation}}{\text{mean}} \times 100$$

To estimate the proportion in which metal occurs in mussel and in the associated sediment, bioaccumulation factors (BCFs) were calculated for the Ni in the different tissues of the snails, according to a formula as below:-

$$\text{BCF} = \frac{\text{mean Ni concentration in the tissue}}{\text{mean Ni concentration in the associated sediment}}$$

The value of BCF was defined as macroconcentrators (BCF > 2), microconcentrator (1 < BCF < 2) and deconcentrators (less than 1), according to Dallinger (1993) [16].

3. RESULTS AND DISCUSSION

Comparison of Ni concentrations between the different soft tissues and in the total shells of *T. telescopium* collected from 17 sampling locations are shown in Table 3. The shell/soft tissues ratios indicate that the accumulation of Ni in the different soft tissues and the shells of *T. telescopium* are different. The ratios of tissues to shells are > 1 in all the different soft tissues, indicating that the shells are more accumulative of Ni than the different soft tissues. The higher concentration of Ni in shell, when compared to those in foot, CT, mantle, muscle, gill and remaining soft tissues, could be due to the tendency of Ni to compete with Ca for binding sites [9]. The higher ratios of shell/soft tissues exhibit that the shells are more accumulative of Ni than the soft tissues. This finding is similar to the result obtained for *Angulyagra oxytropis* [17]. As the shell is secreted by the shell glands and the general epithelial lining of mantle, metals accumulated in the mantle may eventually be mobilized and sequestered in the shell [17]. In fact, the use of mollusks shells as potential biomonitoring materials for heavy metals have been long discussed in the literature [4]-[7],[18],[19].

Table 3. Mean concentrations (µg/g dry weight) of Ni, in the different soft tissues, total shell and ratio (shell/different soft issues) of *T. telescopium* collected from 17 sampling locations. N= 3

No	Sites	Shell	Foot	Shell/Foot	CT	Shell/CT	Mantle	Shell/ Mantle	Muscle	Shell/ Muscle	Gill	Shell/ Gill	REST	Shell/ REST
1	KPP	20.41	1.64	12.47	1.70	12.03	2.21	9.25	2.29	8.92	8.23	2.48	10.57	1.93
2	PP	16.52	2.78	5.95	3.75	4.40	6.49	2.55	7.45	2.22	13.36	1.24	10.33	1.60
3	KSA	27.24	2.87	9.48	4.03	6.75	4.21	6.48	4.06	6.70	76.23	0.36	11.78	2.31
4	SBL	16.68	1.87	8.91	2.20	7.59	1.39	12.03	1.33	12.54	2.75	6.07	9.83	1.70
5	KLK	15.64	3.75	4.17	4.13	3.79	2.51	6.24	1.73	9.02	6.23	2.51	2.15	7.26
6	KLB	13.82	0.03	460.56	0.04	345.42	7.59	1.82	7.35	1.88	10.29	1.34	8.87	1.56
7	SB	22.12	3.86	5.73	5.60	3.95	4.84	4.57	4.96	4.46	12.75	1.74	9.90	2.23
8	BL	15.69	4.37	3.59	5.52	2.84	5.03	3.12	4.72	3.33	7.37	2.13	6.95	2.26
9	SK	20.99	0.90	23.41	1.68	12.47	0.99	21.28	1.22	17.21	2.11	9.95	6.17	3.40
10	KPJ	25.14	2.07	12.12	2.96	8.49	2.07	12.14	2.13	11.78	8.72	2.88	9.61	2.62
11	SJ	15.51	2.68	5.79	4.18	3.71	4.29	3.61	3.11	4.98	5.25	2.95	5.50	2.82
12	PI	24.87	2.23	11.17	3.22	7.73	2.20	11.32	1.73	14.40	4.39	5.66	5.52	4.50
13	KD	16.01	1.08	14.87	1.16	13.84	1.43	11.22	0.86	18.55	4.63	3.46	3.67	4.37
14	KS	15.77	0.84	18.85	0.97	16.26	1.09	14.52	1.12	14.04	8.20	1.92	3.51	4.49
15	JPB	27.42	3.75	7.32	4.84	5.67	4.68	5.86	3.98	6.88	10.76	2.55	13.66	2.01
16	KG	20.99	0.90	23.41	1.68	12.47	0.99	21.28	1.22	17.21	2.11	9.95	6.17	3.40
17	T	14.95	0.04	373.83	0.03	560.75	3.41	4.39	0.92	16.31	7.47	2.00	6.06	2.47

Note : CT – cephalic tentacle; REST remaining soft tissues

In order to see the potential accumulation capacity of Ni uptake from the environment, the BCF values in the shells and all the tissues are established in Table 4. It appears that different soft tissues of *T. telescopium* are deconcentrator of Ni. The shells are microconcentrators since they fall within 1-2 BCF values. The differences could be due to differences in the internal concentrations regulations between the soft and hard tissues of the snails [20]. These BCF results show that the shells of the snails have a better accumulation potential from the environmental sediments when compared to other different soft tissues.

Table 4. Macroconcentrators, microconcentrators and deconcentrators and bioaccumulation factor (BCF) values of the shell and different soft tissues of *T. telescopium* for Ni

Tissues		BCF Value
Shell	MiC	1.83
Foot	DeC	0.17
CT	DeC	0.23
Mantle	DeC	0.35
Muscle	DeC	0.29
Gill	DeC	0.85
REST	DeC	0.71

Note: MaC – macroconcentrator, MiC – microconcentrator, DeC - deconcentrator

In order to see the relationships between metals concentration in the shell of *T. telescopium* and the different geochemical fraction in the surface sediment, Pearson's correlation coefficient analysis was established and the results were presented in Table 5. The significant ($p < 0.05$) correlation coefficients are found for shell – acid reducible, shell – non-resistant, shell – resistant and shell – total Ni concentration. These significant relationships between shell and the geochemical fractions in the environmental sediments indicated that the shell of *T. telescopium* can reflect the Ni levels in the study area. In addition, the significant correlation results are also supported by the statistical outcome of multiple linear stepwise regression analysis which shows that the Ni levels in the shells are significantly ($P< 0.05$) influenced by total Ni in the surface sediments (Table 6). Earlier, Yap et al. (2004) found higher correlation coefficients between the shell of *Perna viridis* with EFLE, acid reducible, oxidisable organic, non-resistant and total concentration of Zn in the sediment and concluded that the mussel shells are good biomonitoring materials of Zn pollution [21].

Table 5. Pearson's Correlation Coefficients Analysis between Ni (log_{10} transformed) in the shell and geochemical fraction (EFLE, oxidisable organic, acid reducible, non-resistant, resistant and total concentrations) of the surface sediment

	Ni in shell
EFLE	0.164
Oxidisable organic	0.017
Acid reducible	0.352*
Non-resistant	0.378**
Resistant	0.382**
Total Ni concentrations	0.484**

***=** Correlation is significant at the 0.05 level (2-tailed).
****=** Correlation is significant at the 0.01 level (2-tailed).

Table 6. Multiple linear stepwise regression analysis of Ni concentrations (based on log10[mean + 1]) between the different tissues of *T. telescopium* and the geochemical fractions of sediment (N= 17)

	Statistical values				
Correlation equations	R	R^2	Adjusted R^2	F	P
log_{10} shell = 0.377 log10 Total Ni + 1.046	0.537	0.288	0.191	4.373	0.042

Note : Independent variables included are EFLE – easily, freely and leachable, AR – acid reducible, OO, oxidisable organic, R – resistant, NR – non resistant, CT – cephalic tentacle, REST – remaining soft tissues. Dependent variable is shell.

Table 7. Coefficient of variation (CV%) of Ni concentrations in the different soft tissues and total shell of *Telescopium telescopium* collected from 17 sampling sites from Peninsular Malaysia

Tissues	CV(%)
Shell	2.24
Foot	15.21
CT	14.83
Mantle	13.73
Muscle	16.50
Gill	8.59
REST	7.59

Note: CT – cephalic tentacle; REST – remaining soft tissues

Lastly, the CV values (%) in the shells and different soft tissues of the snails are established in Table 7, in order to see the precision in the Ni data. It appears that the snail shell has the lowest CV value (2.24%) when compared to those (7.59-16.5%) of other different soft tissues. Hence, the results indicated lower degrees of variability (thus, higher precision) of Ni data in the shells of *T. telescopium* than in the different soft tissues of *T. telescopium*. Lower variability (based on CV values) for Pb concentrations was found in *Mytlus edulis* (Bourgoin, 1990), *Mytilus galloprovincialis* (Puente et al. 1996) and in *Perna viridis* (Yap et al. 2003), and all the above findings concluded that a greater precision when the shell was used as a biomonitoring material for heavy metals [23],[3]. The lower CV values in the shell could be due to the differences in the biological half-lives and biochemical behaviours of Ni found in the different soft tissues and the shell of molluscs [3],[5],[24]. According to Carriker et al. (1980), the different distribution of chemical elements within the structure of the shell could be understood by looking at the chemical properties of trace elements in the shells [25]. Moreover, shells have important practical advantages over the use of the soft tissues to monitoring metal contamination of the aquatic environment since they less variability (Bourgoin 1990; Lingard et al. 1992) and shells are a potential sink for metals due to their capacity to remove bioavailable metals from the environment (Langston et al. 1998) [22],[26],[27]. It is therefore, the accumulation of heavy metals in shells has prompted some researchers to promote the concept that the metal composition of shells may be promising as a record of environmental levels of heavy metals [7],[21]. In addition to that, shells integrate elemental concentrations over the life of the animal, preserve the metals after the death of the organisms giving information about the concentrations that they were exposed in the pas [25].

Table 8. Established Sediment Quality Guidelines for Ni concentrations (µg/g dry weight) in marine sediments

1.	SQV-Low	40.0	Chapman et al. (1999)
2.	Effect Range Low (ERL)	20.9	Long et al. (1995)
3.	Effect Range Median (ERM)	51.6	Long et al. (1995)

Since significant and positive correlations of Ni between shells and surface sediments are found, the levels of Ni in the shells (Table 3) are compared to two well established sediment quality values (SQVs) and Effect Ranges (Low and Median), as shown in Table 8. It is found that all the Ni levels in the shells are lower than SQV-low (Chapman et al. 1999), indicating low polluted status while the Ni levels are between Effect Range Low and Effect Range Median (Long et al. 1995), indicating 'non-low polluted' status [28],[29]. These comparisons show that the Ni levels in the intertidal areas of Peninsular Malaysia should not pose a toxicological risk to living organisms.

4. CONCLUSION

In conclusion, the present findings based on ratios of shell/soft tissues, BCF values, statistical outcomes of correlation analysis and multiple linear stepwise regression analysis, and low CV values, do indicate the very potential of shells of *T. telescopium* as good biomonitoring material of Ni pollution in the tropical intertidal area. Therefore, the use of total shells of *T. telescopium* as good biomonitoring materials of Ni pollution in the tropical intertidal area, is suggested.

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