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Research Article

Shells of Intertidal Mudflat Snails: A Promising Biomonitoring Materials of Nickel Pollution

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Abstract: Monitoring the level of metal pollution in a water body, especially in polluted areas, is crucial. Gastropod shells have been used as a biomonitor for metal pollution. The goal of this study was to evaluate the utilisation of the mudflat snails, *Telescopium telescopium* shells, as biomonitoring materials for nickel (Ni) pollution in the intertidal area. The snails and their habitat surface sediments were sampled from 17 sites in Peninsular Malaysia. Up to 21 individuals from each site were sampled and dissected. In addition to the shells, six parts of the soft tissues (cephalic tentacle, foot, gill, muscle, mantle, and remaining soft tissues) were analysed for Ni. The snail shell was found to be a potential biomonitoring material for Ni pollution based on four positive points: (i) higher value of shell/soft tissue ratios (> 1.00); (ii) categorisation as a 'microconcentrator' based on bioaccumulation factor; (iii) significant correlation coefficients (at least $P < 0.05$) and significant influential total Ni levels in the sediments to the shell Ni; and (iv) higher precision of Ni in the shells based on the lowest value of the coefficient of variation of Ni. The described results indicated that the shell of *T. telescopium* would be suitable for assessing Ni pollution in the intertidal areas.

Keywords: *Telescopium telescopium*, shells, Ni, biomonitoring material

1. Introduction

The impact of metals on the environment is an increasing problem worldwide. Malaysia itself is facing metal pollution, particularly caused by anthropogenic activities [1].

Nickel (Ni) was thought to have no biological functions before 1975, but it was later revealed to be important in

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a variety of living organisms, despite the lack of a well-defined biochemical mechanism for Ni's participation [2]. Ni usually occurs in water bodies in trace amounts. The release of human-induced effluents significantly contributes to the amount of Ni in the soil and water, but its relative concentration depends on the source of the effluent [3]. Recently, Yap et al. [4] proposed the use of specific parts or organs of mollusks to be more reflective of potential toxic metal pollution in the intertidal area. The focus of this paper was on Ni because it is currently regarded as an emerging potentially toxic metal and thus of high ecotoxicological concerns. Meanwhile, depending on the organism, Ni absorption and metabolism are crucial for certain enzymatic activities [3]. Many harmful impacts of Ni in mollusks encompass Fe metabolism disturbances, and Ni is attached to protein and nucleic acid [5]. Ni has been reported in mussels [6,7], sediments [8,9], plants [10,11], and snails [10].

Mollusk shells have been proposed as biomonitoring materials for metal pollution in the mangrove snail *Nerita lineata* [12], green-lipped mussel *Perna viridis* [13-15], rocksnail *Reishia clavigera* and chameleon nerite snail *Nerita chamaeleon* [16], and spiny frog shell *Bufonaria echinata* [17]. Specifically, Ni has been the primary focus of biomonitoring studies in *P. viridis* [6], *Telescopium telescopium* [10,18], and *N. lineata* [12]. However, the use of *T. telescopium* shells as potential biomonitoring materials of Ni has not been well investigated.

Telescopium telescopium, commonly known as a mudflat snail, is from the class Gastropoda, which is a common species that lives on muddy shores up to high tide level [18]. This species is abundant and relatively easy to sample.

Yap et al. [18] proposed using the digestive cecum of *T. telescopium*, but not the shells, as a biomonitoring organ for Ni pollution in the tropical intertidal zone. Positive correlations with the ambient sediment of the gastropod habitats backed up the conclusion. In the literature, there has been a lot of evidence that the potential of mollusks as biomonitoring materials for heavy metal pollution has been documented [19-21]. However, monitoring studies using *T. telescopium* shells have remained limited and scarce. Shell analyses may be beneficial to include in routine monitoring techniques since the shells of these species can be maintained for a long time after harvesting. The shells can be well preserved to record the geological variability of elements [22].

Owing to a variety of minerals and chemistries in the shell, gastropod shells can acquire a wide spectrum of metals to varying degrees [23]. The metals accumulated in the shells are indicative of the overall digested metals by the mollusks since the metals must have been absorbed into the shells by calcium ion substitution in the crystalline phase [19]. Furthermore, the shell chemical composition has been reported to be highly reflective of its exposure to the habitat environments [24].

Studies on metal pollution using local organisms in Malaysia are gaining extensive attention. However, limited information exists in the literature concerning the effects of Ni on this mudflat snail. Since the snail is accessible along the intertidal areas of Peninsular Malaysia and can easily be sampled, it can be suggested as a biomonitor for metal pollution. Thus, the goal of this study is to evaluate the utilisation of *T. telescopium* shells as biomonitoring materials for Ni pollution in the tropical intertidal area.

2. Materials and methods

The sampling map for the snails and surface sediments is presented in Figure 1. The habitat surface sediments (0 to 10 cm) were also sampled at the same time. Upon collection, all samples were transported to the laboratory for further analysis.

Between 6 to 21 of the mudflat snails from each site were sampled and dissected. The soft tissues of snails were separated into gill, cephalic tentacle (CT), foot, mantle, muscle, and remaining soft tissues (REST). For the shells, they were pounded by using a pestle and mortar.

Later, the samples were dried at 80 °C for 72 hours to constant dry weights [13]. The snail tissues were digested in concentrated nitric acid (BDH: 69%) while the sediments were digested in a combination of concentrated HNO_3 (AnalaR grade; BDH 69%) and $HClO_4$ (AnalaR grade; BDH 60%) (4:1). After digestion, they were determined for Ni by using an air-acetylene flame Atomic Absorption Spectrophotometer (AAS) Perkin Elmer Model AAnalyst 800.

The sequential extraction technique used in the current study followed the one detailed by Badri and Aston [25]. Four geochemical fractions involved include: easily, freely, leachable or exchangeable (EFLE); acid-reducible (AR); oxidisable-organic (OO); and resistant (RES). The summation of four geochemical fractions of Ni was also known as total Ni concentrations (SUM).

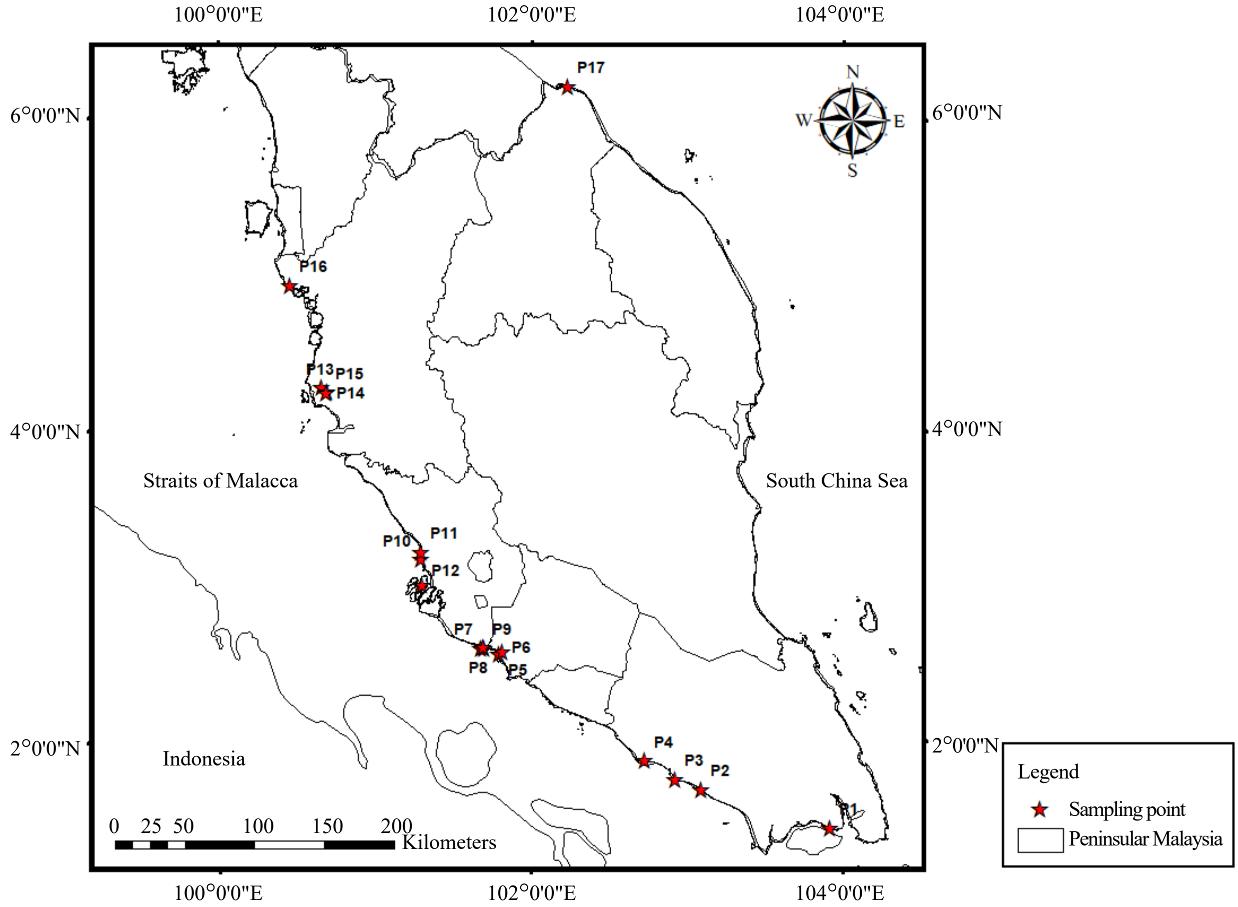

Figure 1. Sampling map of *Telescopium telescopium* and their habitat surface sediments in Peninsular Malaysia. Sampline sites: P1 = Kampung Pasir Puteh (KPP); P2 = Pantai Punggur (PP); P3 = Kuala Sungai Ayam (KSA); P4 = Sungai Balang Laut (SBL); P5 = Kuala Lukut Kecil (KLK); P6 = Kuala Lukut Besar (KLB); P7 = Sungai Sepang Kecil (SK); P8 = Bagan Lalang (BL); P9 = Sungai Sepang Besar (SB); P10 = Sungai Janggut (SJ); P11 = Kampung Pantai Jeram (KPJ); P12= Pulau Indah (PI); P13 = Jambatan Permaisuri Bainun (JPB); P14 = Kampung Deralik (KD); P15 = Kampung Setiawan (KS); P16 = Kuala Gula (KG); and P17 = Tumpat (T)

Quality assurance and quality control were conducted throughout the analytical procedures. The quality of the methods used was checked with the Certified Reference Materials for Dogfish Liver (DOLT-3, National Research Council Canada), and Soil (NCS DC73319-Soil, China National Analysis Center for Iron and Steel 2004). Their recoveries were satisfactory (95.6 to 125%).

By using the Statistical Program for Social Science (SPSS) for Windows (Version 15), correlation coefficients (CA) and multiple linear stepwise regression analysis (MLSRA) were used to assess the relationships of Ni levels between the sediment geochemical fractions and different parts of the snails. Prior to the statistical analysis, all data for the CA and MLSRA analyses were $\log_{10}$(mean + 1) transformed in order to reduce the variance [26].

The coefficient of variation (CV) value is an indicator of variability of parameters [27] with their respective standard deviation. An increase in standard deviation would result in a higher variability of the parameter investigated [28]. The CV value was calculated based on untransformed data as shown in equation (1):

$$CV(\%) = \frac{standard\ deviation}{mean} \times 100 \quad (1)$$

The bioaccumulation factors (BCF) value was calculated in the different tissues of the snails, according to equation (2):

$$BCF = \frac{mean\ Ni\ concentration\ in\ the\ tissue}{mean\ Ni\ concentration\ in\ the\ associated\ sediment} \quad (2)$$

According to Dallinger [29], the BCF values can be categorised as 'deconcentrators (< 1)', 'microconcentrator (1 < BCF < 2)', and 'macroconcentrators (BCF > 2)'.

3. Results and discussion

The sizes of snails (shell heights and widths) analysed in the current study ranged from 4.96 ± 0.06 to 9.20 ± 0.08 cm and 2.83 ± 0.04 to 4.82 ± 0.04 cm respectively. The ratios of Ni levels between shell/different snail soft tissues from this study are shown in Figure 2. It was obvious and significant that the shell/soft tissues ratios indicated that the accumulation of Ni in the different tissues and the shells of the snails were different [30]. The ratios of tissues to shells are > 1 in all soft tissue parts of the snails. This evidently indicated that the shells accumulated more Ni than the different soft tissue parts of the snails. The higher Ni concentration in the shell compared to the six soft tissue parts could be attributed to the Ni affinity to compete or replace calcium for binding sites in the shell structures [19]. The current finding was supported by previous work on *Angulyagra oxytropis* by Gupta [31]. Since the shell layer is secreted by the shell glands and epithelial lining of the mantle, Ni accumulated in the mantle may eventually be mobilised and sequestered or stored almost permanently in the shell structures [31]. Mollusk shells have been used or proposed as potential biomonitoring materials for metals in numerous studies [21,22,32,33]. However, discussion on Ni has been limited in the literature.

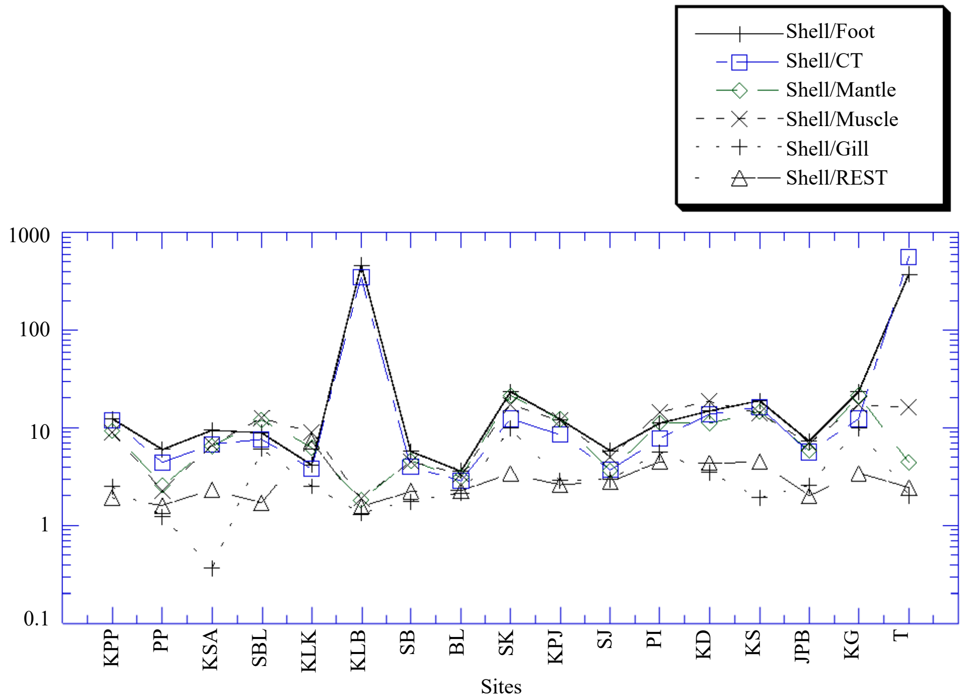

Figure 2. Mean ratios (shell/different soft tissues) of Ni concentrations in *Telescopium telescopium* from current study (N = 3). Y-axis refers to ratio values in log scale. Note: KPP = Kampung Pasir Puteh; PP = Pantai Punggur; KSA = Kuala Sungai Ayam; SBL = Sungai Balang Laut; KLK Kuala Lukut Kecil; KLB = Kuala Lukut Besar; SB = Sungai Sepang Besar; BL = Bagan Lalang; SK = Sungai Sepang Kecil; KPJ = Kampung Pantai Jeram; SJ = Sungai Janggut; PI = Pulau Indah; KD = Kampung Deralik; KS = Kampung Setiawan; JPB = Jambatan Permaisuri Bainun; KG = Kuala Gula; and T = Tumpat

The BCF values in the shells and all the tissues were established to determine the accumulation capacity of Ni uptake from the environmental sedimentary matrices (Figure 3). It was found that different soft tissue parts of the snails were classified as 'deconcentrator' of Ni [29]. In contrast, the shells were classified as 'microconcentrators' of Ni. This could be attributable to differences in the internal regulations of Ni between the soft tissues and shells [34]. These BCF results indicated that the shells of *T. telescopium* accumulated more Ni from environmental sediments when compared to other soft tissues.

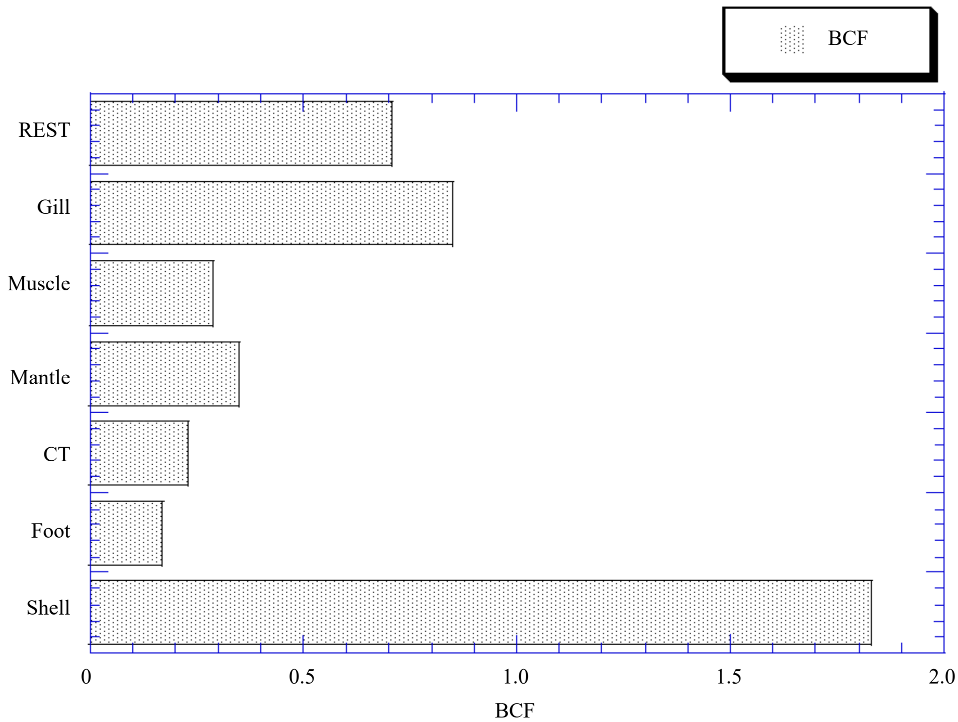

Figure 3. Bioaccumulation factor (BCF) values of Ni levels in different tissues and shells of *Telescopium telescopium* from current study. Y-axis represents different tissues of snails, while X-axis represents BCF values (unitless) of Ni. Note: REST = remaining soft tissues; and CT = cephalic tentacle

The comparison of CA of Ni levels between the different sedimentary geochemical fractions and the shells are given in Figure 4. The CA of Ni of shell-AR, shell-NonRES, shell-resistant, and shell-SUM pairs were found to be significant ($P < 0.05$). These significant relationships indicated that the snail shells reflected the level of Ni pollution in the intertidal area. The significant CA was also well supported by the statistical outcome of the MLSRA. It shows that the shell Ni levels are significantly ($P < 0.05$) influenced by total Ni in the surface sediments (Table 1). Earlier, Yap et al. [14] reported higher CA of Zn between the shell of *P. viridis* and surface sediments (EFLE, AR, OO, NonRES, and SUM of Zn). They concluded that the mussel shells were good biomonitoring materials of Zn pollution.

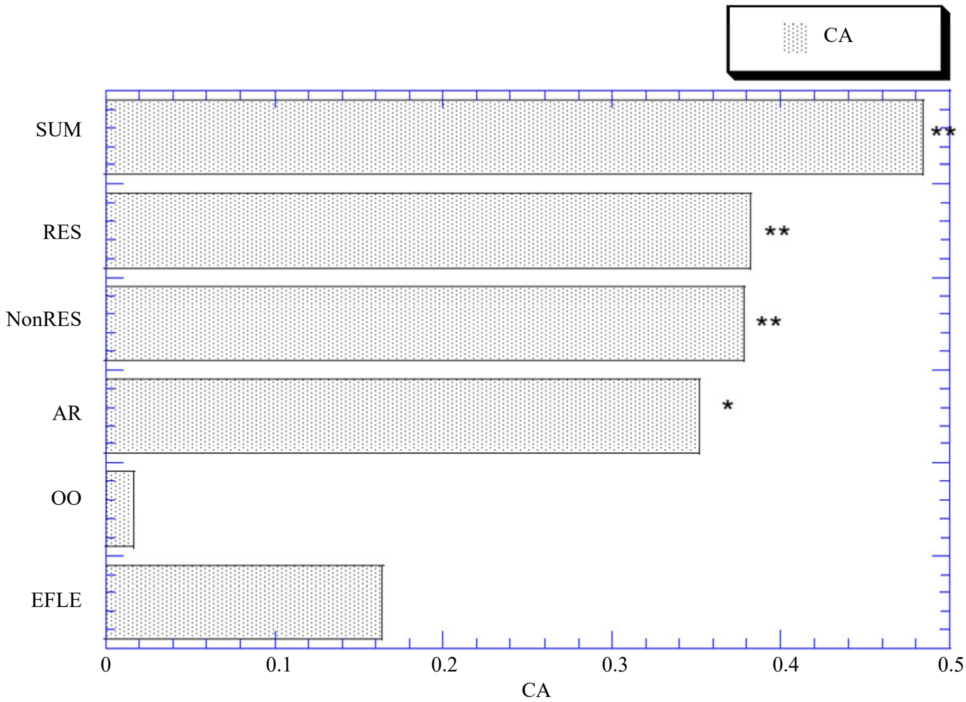

Figure 4. Correlation coefficients (CA) of Ni between surface sedimentary geochemical fractions and shells of *Telescopium telescopium* (N = 17). Y-axis represents sedimentary geochemical fractions, while X-axis represents correlation coefficient values of Ni. Note: * = $P < 0.05$; ** = $P < 0.01$; EFLE = easily, freely, leachable or exchangeable; OO = oxidisable-organic; AR = acid-reducible; NonRES = non-resistant; RES = resistant; and SUM = total summation of all fractions

Table 1. Output of multiple linear stepwise regression analysis of Ni levels ($\log_{10}$[mean + 1]) between different tissues of *Telescopium telescopium* and geochemical fractions of surface sediment (N = 17)

	Statistical values			
Correlation equations	R	R^2	F	p
$\log_{10}$ shell = 0.377 $\log_{10}$ Total Ni + 1.046	0.537	0.288	4.373	0.042

Figure 5 shows the CV values (%) in different soft tissue parts and shells to illustrate the precision in the Ni data. It appeared that the snail shell had the lowest CV value (2.24%) when compared to those of other different soft tissues (7.59 to 16.5%). Hence, the current results indicated lower variability degrees and higher precision of Ni levels in the shells than in the different soft tissue parts of the snails. Lower degrees of variability (lower CV values) for Pb concentrations have been reported in the shells of *Mytilus edulis* [35], *M. galloprovincialis* [36], and *P. viridis* [13]. The lower degrees of shell metal variability could be attributed to the dissimilarities in the biochemical behaviour and biological half-lives of Ni between the mollusk shells and their soft tissue parts [13,37].

In biomonitoring studies of metal pollution, shells have more advantages over soft tissues of mollusks. The shells can integrate metal concentrations over the lifespan of mollusks [17]. As a result, they can preserve metals over a geological period, allowing them to provide more accurate profiles of metal pollution history [38]. This is due to shell metal levels being less subjective to physiological changes such as spawning [39]. The shells are also acting as a potential sink for heavy metals with their capacity to reduce metal bioavailability from the intertidal area [40]. Hence, the use of shells as biomonitoring materials to record environmental levels of metal pollution in the intertidal area holds a promising future [16,24].

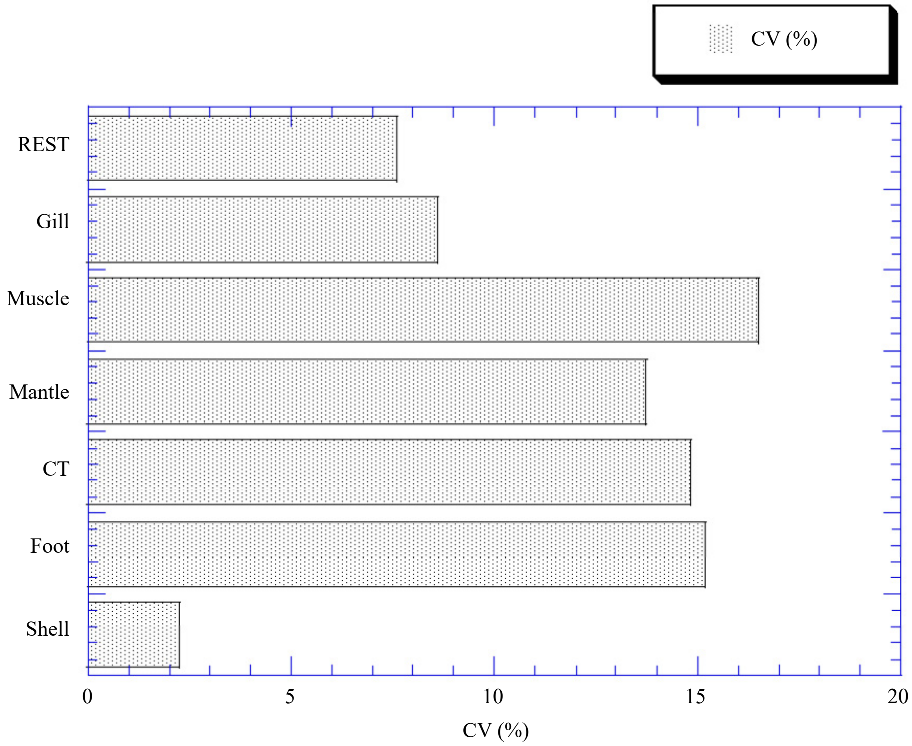

Figure 5. Coefficient of variation (CV) of Ni levels in different tissues and shells of the mudflat snails (*Telescopium telescopium*) collected from Peninsular Malaysia. Y-axis represents different tissues of snails, while X-axis represents CV values (%). Note: REST = remaining soft tissues; and CT = cephalic tentacle

4. Conclusions

The mudflat snail is widely distributed in intertidal areas and is relatively easy to identify and collect, making it suitable for ecotoxicology studies. These findings indicated that the *T. telescopium* shells could be a potential biomonitoring material for Ni pollution in the intertidal areas. Nevertheless, this proposal needs to be validated through experimental studies in the field and laboratory conditions.

Conflict of interest

The authors declare no conflict of interest.

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