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Spatial distribution of zinc, lead and cadmium in snails, leaves and soils from diverse sites

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ABSTRACT: The potentials of snails, leaves and soils to act as indicators for mapping environmental concentrations of toxic heavy metals was assessed in diverse sites in Benin metropolis, Nigeria. Steady state metal concentrations (Zn, Pb and Cd) were measured in the snail (*Limicolaria aurora*), plant leaf (*Zanchezia nobilis*) and soil samples from rural and urban sites. Each replicate snail was partitioned into two soft tissues (kidney and entire rest) and their Zn, Pb and Cd levels compared with those of soil and leaves of a food plant *Zanchezia nobilis*.

In all sites, the concentrations of all metals were highest in the pooled snail fractions in an increasing order of snail > soil > leaf. Metal concentrations also showed rural-urban differential, being higher in the urban sites in all matrices. Zinc, which is an essential metal, had the highest concentration in all the matrices. Monthly variations were species and metal specific. Mean metals concentrations in snail tissue fractions correlated with soil levels. Thus, the use of snail (*L. aurora*) as a sentinel for the quantification of metal contamination is further recommended in mapping environmental metal pollution in Nigeria.

Key Words: Heavy metals; Environmental pollution; Bioconcentration; Bioaccumulation; Snail tissue fractions.

Introduction

Possible infiltration of potentially toxic metals into the adjoining terrestrial and aquatic environment through runoffs has been documented (Ezemonye and Kadiri, 2000). Human activities have continuously increased the levels of heavy metals circulation in the environment (Ezemonye, 1992). Manufacturing industries, mining, rural agricultural cultivation and the use of fertilizers are typical sources of metal contamination in the environment (Egborge, 1991).

Increasing recycling of sludge on land, combustion of fossil fuels and atmospheric deposition also increases the metal concentration in soil (Neuhauser *et al*., 1995; Spiegel, 2002). Consequently, heavy metals are released from a great number of sources which contribute to metal load in the environment with special implication on the food chains.

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The effects of these processes may result in bioaccumulation of metals in soil biota and metal enrichment along trophic levels (Ezemonye, 1992; Neuhauser *et al*., 1995). Some heavy metals may be important trace elements in the nutrition of plants, animals or humans (e.g. Zn, Mn, Ni, Cu) while others are not known to have positive nutritional effects (e.g. Pb, Cd). These metals may also cause toxic effects if they occur in excess, or even at low concentrations (Spiegel, 2002). For example, low-level chronic exposure to Cd can cause adverse health effects which include gastrointestinal, haematological, renal, neurological and reproductive effects (Le Coultre, 2001).

Significant progress has been made in determining the chemical species of toxic metals available for uptake, but defining bioavailability for a range of plant and animals has been problematic (Gundacker, 2000). It is measured as the fraction solubilized by a variety of extractants, but ecotoxicologists are interested in quantifying the proportion assimilated into the tissues or, in some cases, the concentration that has a toxic impact on plants or animals.

Snails are known to be a major component of soil biota and wildlife food chain. They have also been reported to be potential metal monitors and accumulators in full contact with the substrate they consume (Gomot de Vaufleury and Pihan, 2000). Metals are incorporated in the tissues when they are in a form that allows them to cross cellular membranes primarily by being ionized (Simkiss and Taylor, 1989). Evidence from a variety of sources suggest that free metal species are more likely to be assimilated than dissolved forms (Sauve *et al*, 1997; Ge *et al*, 2000; Ritchie *et al*, 2001). On the other hand, plant vegetation, which is the source of food to soil organisms, has been known to act as a measure of recent dietary exposure (Beeby and Richmond, 2003).

Beeby and Richmond (2003) observed that soil concentrations provide a better long-term measure of metal exposure and expresses site differences more reliably. Posthuma *et al*. (1998) also noted that soil acidity and total metal concentration were the most important predictors of the partitioning of metals between the solid and liquid phases of soils.

The present study is a preliminary assessment of the levels of heavy metals (Zn, Pb and Cd) in the snail (*Limicolaria aurora*), plant leaf (*Zanchezia nobilis*) and soil samples from Urban (polluted) and rural (semi-pristine) environment.

Materials and Methods

The snail, plant leaf and soil samples were collected from site 1 (rural and semi-pristine) and site 2 (urban and contaminated) locations in Benin metropolis, Nigeria (Fig. 1). The method of collection is as described by Beeby and Richmond (2003). All samples were analysed at the Nigerian Institute for Oceanography and Marine Research for a period of four months.

Snail Sample Collection

The snails were collected by hand-searching low vegetation, taking individuals of related large sizes. Only adults were used and although they may be of different ages, most were likely to have been in similar physiological state. Being hermaphrodite, there is no variation due to sex (though they may have been in different reproductive conditions).

The snails were washed with tap water and allowed to clear their guts for two days. After thawing and removal from the shell, the soft tissues were dissected into two components, the kidneys and the rest.

Sample Identification

Snail identification was done according to Taylor and Sohl (1962). *Limicolaria aurora* is narrow with cusps showing traces of tridentation. The ecotone and endocone are prominent with a less prominent mesocone. The basal is triangular in shape from the anterior to the posterior end. It is about 8.1 μm wide and 51.3 μm long for a shell length of about 43 mm. The size ranges from 54.9 μm long and 43.2 μm wide to 35.2 μm long and 32.4 μm wide in an individual of 43 mm shell length. Their teeth are sharp and rough. They are common garden snails, having both ecological and economic importance (Figs. 2 and 3).

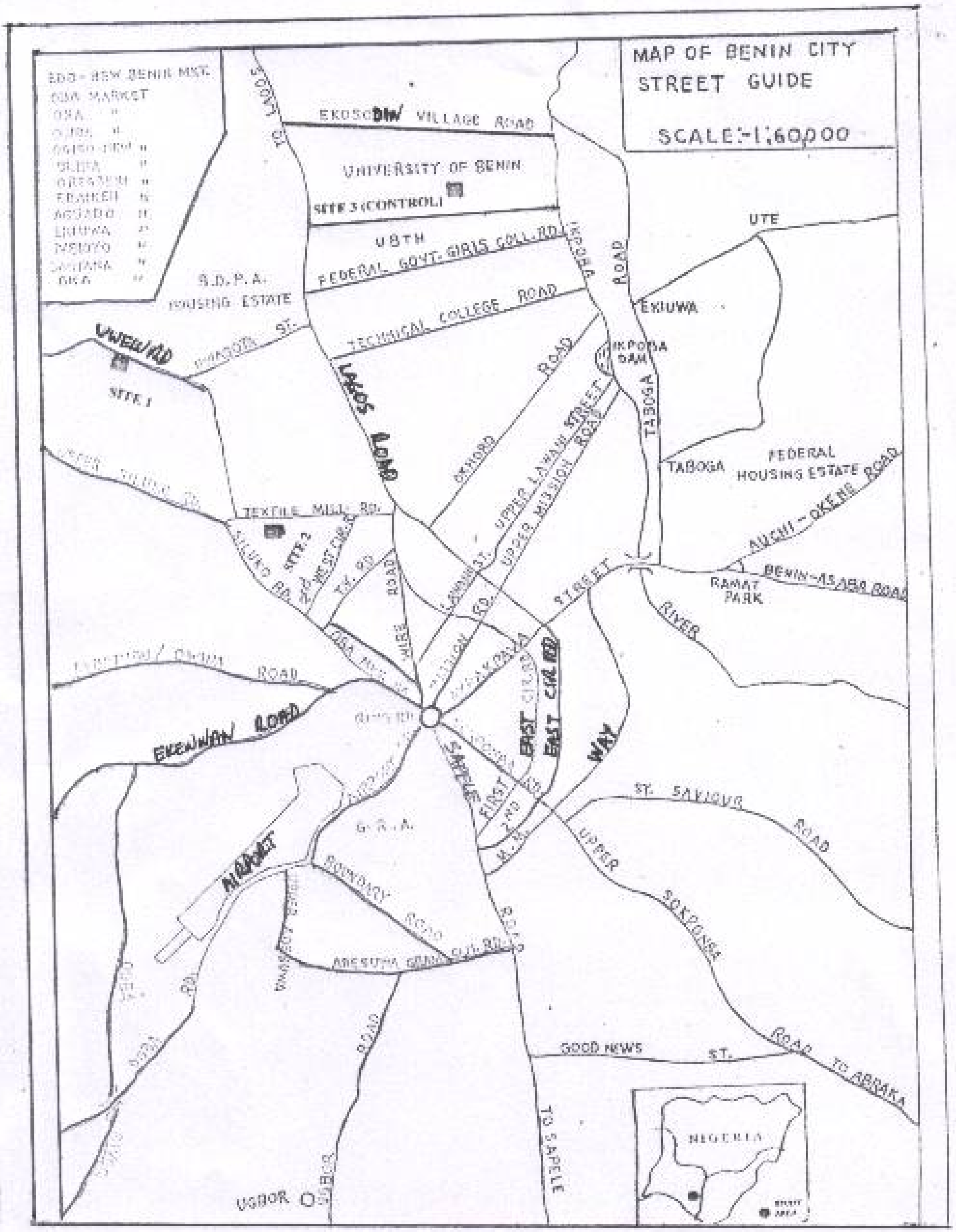

FIG. 1: MAP OF BENIN – CITY SHOWING THE SAMPLING STATIONS
(MAP OF NIGERIA IS INSERTED)

Figure 2: External features of *Limicolaria aurora* (Garden Snail)

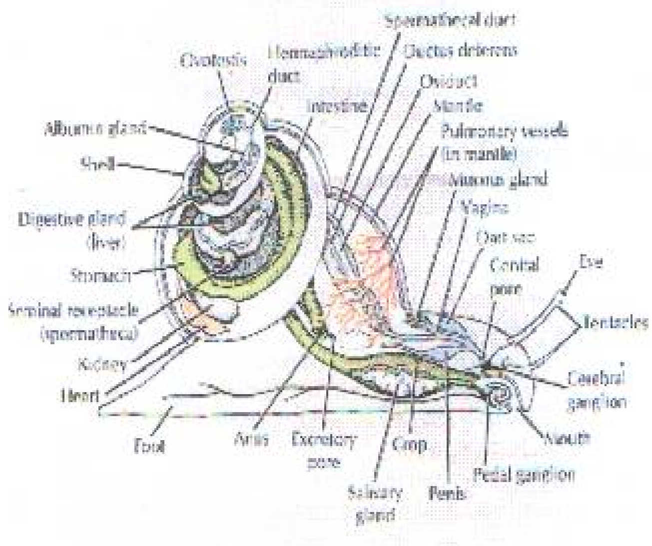

Figure 3: Internal features of Garden Snail

Metal Analysis in Snail

After washing with tap water, the snails were allowed to clear their guts for 48 hours. Thawed soft tissues were removed from the shell and dissected into two components – the kidney and the entire rest. These were dried for 18h at 80^oC before weighing. Subsequent digestion in 10 ml of boiling 50% HNO_3 for one hour was carried out. The cooled and filtered solution was made up to 25 ml with distilled water and analysed for heavy metals using Atomic Absorption Spectrophotometry (AAS).

Vegetation and Soil Samples

The plant leaf (*Zanchezia nobilis*) on which the snails fed were collected for analysis on a monthly basis for four months. The plant leaf samples were oven dried at 80^oC for 12 hours before being crushed and 1g of the sample was taken for digestion and analysis for the same metals (Zn, Pb and Cd) using Atomic Absorption Spectrophotometry (AAS).

Soil samples were collected from the top 3cm of the mineral layer (after removal of the litter) from which the snails were found in the diverse sites. Soil samples were analysed by drying for 24 hours at 80^oC and then milled to pass a 2 mm screen to remove rocks and plant debris. One gram of sieved soil sample was weighed and 10 ml of 50% nitric acid was added to the soil sample in a beaker after which it was evaporated to dryness by heating and making it up to a volume of 25 ml with distilled deionized water and filtered. This was followed by determination of the heavy metals by AAS.

Results

The results of the concentrations of heavy metals in the snail, plant leaf and soil samples from site 1 (Rural) and site 2 (Urban) are presented in Tables 1 – 4.

Mean Metal Concentration in Biota and Soil

Generally, the result of the mean metal concentrations in both sites (rural and urban) showed that heavy metal levels were highest in the snail and least in the leaf (Tables 1 and 2).

Monthly Observation

The monthly trend of the levels of heavy metals showed that the month of May had the highest concentrations of the heavy metals and the least level was observed during the month of August in both sites 1 and 2 as shown in Tables 3 and 4.

Metal Specificity

Zinc was observed to have the highest mean concentration in the matrices at both sites. The major variation occurred in toxic metals (Pb and Cd) where they had their highest levels in the snail and the least in leaf.

Comparison of metal concentrations between sites 1 and 2

The inter-specific comparison of metal concentrations between site 1 (Rural Site) and site 2 (Urban Site) showed that the concentrations of all the metals (Zn, Pb and Cd) were higher in site 2 that in site 1 in all the matrices as shown in Figs. 4 – 6.

Comparison of the patterns of uptake in snail tissues (kidney and entire rest) showed that uptake of heavy metals was higher in the kidney than in the entire rest at both sites 1 and 2 as shown in Figs. 7 and 8. The student paired t-test applied to test the validity of the null hypothesis showed a significant difference at $P < 0.05$ for all the metals in all matrices.

Table 1: Mean metal concentration in biota and soil from site 1 (Rural).

Matrix	Mean	S.D.	S.E.	Range
Zinc (Zn)				
Soil	2.26	3.91	1.96	1.72 – 2.92
Kidney	1.85	2.26	1.13	1.03 – 1.62
Entire Rest	1.55	1.06	0.53	0.18 – 1.16
Leaf	0.18	0.02	0.01	0.12 – 0.33
Lead (Pb)				
Soil	1.35	2.33	1.17	0.51 – 1.71
Kidney	1.21	2.10	1.05	0.64 – 1.61
Entire Rest	0.62	1.07	0.54	0.32 – 0.92
Leaf	0.33	0.56	0.28	0.13 – 0.64
Cadmium (Cd)				
Soil	1.08	1.88	0.94	0.13 – 1.90
Kidney	0.75	1.28	0.64	0.65 – 1.30
Entire Rest	0.45	0.78	0.39	0.31 – 0.91
Leaf	0.23	0.39	0.19	0.20 – 0.25

Table 2: Mean metal concentration in biota and soil from site 2 (Urban).

Matrix	Mean	S.D.	S.E.	Range
Zinc (Zn)				
Soil	3.67	6.36	3.18	3.24 – 4.25
Kidney	3.64	6.29	3.15	3.25 – 3.90
Entire Rest	1.65	2.85	1.43	1.50 – 1.91
Leaf	0.92	1.59	0.79	0.69 – 1.18
Lead (Pb)				
Soil	1.65	2.85	1.43	1.00 – 2.51
Kidney	1.23	2.12	1.06	0.75 – 1.91
Entire Rest	0.65	1.13	0.57	0.50 – 1.00
Leaf	0.40	0.69	0.35	0.24 – 0.51
Cadmium (Cd)				
Soil	1.09	1.89	0.95	0.64 – 1.65
Kidney	1.09	1.90	0.95	1.00 – 1.34
Entire Rest	0.55	0.95	0.48	0.51 – 0.94
Leaf	0.38	0.65	0.33	0.25 – 0.75

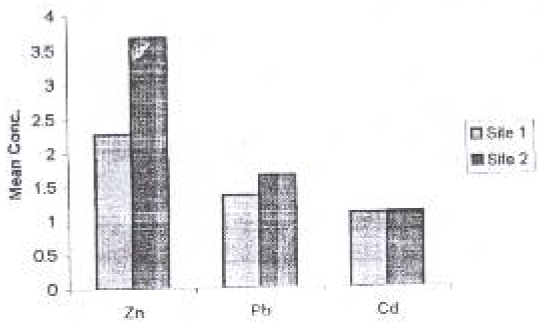

Figure 4: Concentrations of Metals in Soil from Site 1 and 2

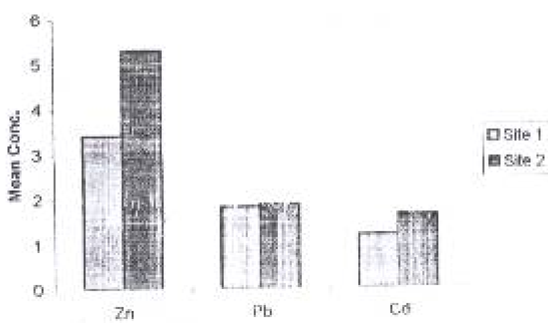

Figure 5: Concentrations of Metals in Snail from site 1 and 2

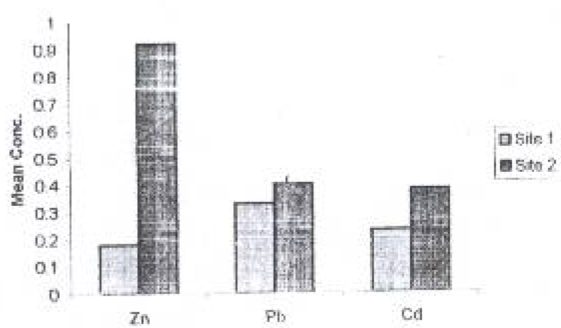

Figure 6: Concentrations of Metals in Plant Leaf from site 1 and 2

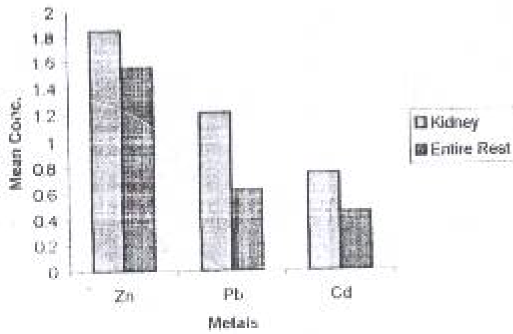

Figure 7: Metals Uptake Pattern in Snails from Site 1

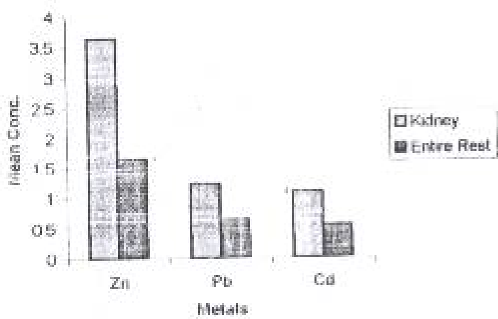

Figure 8: Metals Uptake Pattern in Snails from Site 2

Table 3: Monthly mean concentration of heavy metals in site 1 (Rural)

Matrices	Zinc (Zn)				Lead (Pb)				Cadmium (Cd)			
	May	Jun	Jul	Aug	May	Jun	Jul	Aug	May	Jun	Jul	Aug
Soil	2.92	2.40	1.99	1.72	1.71	1.95	1.21	0.51	1.50	1.90	0.80	0.13
Kidney	2.62	1.69	1.69	1.43	1.61	1.60	0.64	1.00	1.30	0.91	0.65	0.65
Entire Rest	1.69	1.41	1.41	1.18	0.92	0.75	0.48	0.32	0.91	0.58	ND	0.31
Leaf	0.33	0.15	0.15	0.12	0.64	0.40	0.13	0.13	0.20	0.45	0.25	ND

Table 4: Monthly mean concentration of heavy metals in site 2 (Urban)

Matrices	Zinc (Zn)				Lead (Pb)				Cadmium (Cd)			
	May	Jun	Jul	Aug	May	Jun	Jul	Aug	May	Jun	Jul	Aug
Soil	4.25	3.73	3.47	3.24	2.51	1.86	1.22	1.00	1.65	1.17	0.91	0.64
Kidney	3.90	3.58	3.25	3.82	1.91	1.50	0.75	0.75	1.00	1.04	1.31	1.34
Entire Rest	1.67	1.91	1.50	1.51	1.00	0.50	0.60	0.50	0.94	0.75	0.51	ND
Leaf	1.18	1.00	0.69	0.81	0.51	0.24	0.44	0.42	0.75	0.50	ND	0.25

Discussion

Bioavailability refers to the degree to which an element or compound is available fro assimilation by an organism (Ezemonye and Enete, 2004). Only bioavailable metals are of toxicological significance. Diverse biological, physical and chemical activities determine this availability (Van Roon, 1999; Zauke *et al*., 2003). Kennish (1992) also observed that some relevant chemical processes (dissolution, redox reaction, sorption-desorption) could alter the orm and bioavailability of heavy metals. Similarly, in the present stud, the acidic nature of the soil may have influenced the desorption of complexed metals into ionic forms, thereby leading to enhanced bioavailability of metals observed (Graney *et al*. 1983).

The general observation in this study showed that heavy metal levels were higher in the snail than in the soil at both sites studied. This provides an evidence for the bioaccumulation of the heavy metals. Soil pH is widely accepted as one of the key factors contributing to desorption, uptake, potential and environmental hazards of heavy metals (McGeer *et al*., 2003). To produce adverse effects, metals must bioaccumulate (where uptake exceeds excretion) in excess of a threshold concentration at the specific site of action.

Beeby and Richmond (2003) have used a common garden snail as a sentinel species for mapping environmental concentrations of heavy metals because they accumulate very high levels of metals in their soft tissues. Iglesias and Castillejo (1990) also reported that the gastropod genus *Helix* is also known to assimilate several toxic metals to high levels with some impunity. This is similar to the uptake pattern of heavy metals in *L. aurora* as observed in the present study.

Low levels of heavy metals that occurred in the leaf may be due to the specific soil properties which can have a significant effect on the amount of heavy metals assimilated by the plants. An increased level of Cd can lead to a decrease in the amount of heavy metals that can be assimilated by plants. Higher affinity for the essential trace metal (Cu) can also result in the decreased uptake of Cd in the plants (Lecoultre, 2001).

The monthly trend of the levels of heavy metals showed that the month of May had the highest concentration of metals and the least level was observed in August at both site 1 (semi-pristine) and site 2 (urban or polluted site). This may be due to a leaching which usually takes place during the rainy season. Leaching is a factor which is known to affect the concentration and distribution of the heavy metals in top soil (Lecoultre, 2001). Since samples were taken from the top 3 cm of the soil after removal of the litter, it is possible that leaching may have affected the metal concentration during the rainy season months of June to August. This is similar to the observation of Beeby (2001) where concentrations of heavy metals were lower during the rainy seasons. Other factors which may influence the content and distribution of heavy metals include parent material, organic matter, particle size distribution, drainage, pH, type of vegetation, amount of vegetation and aerosol deposition.

At both sites, the concentration of the essential metal (Zn) was observed to be highest throughout the study period and in all matrices. The major variation was observed in the concentrations of the toxic metals (Pb and Cd). This is similar to the observation of Ezemonye and Enete (2004) where the essential metals Mn, Zn, Fe and Cu were accumulated by earthworms more than the non-essential metals Pb, Cd and Cr. A similar observation was also reported by Anderson and Larsen (1982) who worked on *Lumbricus terrestris* in polluted soil.

Bamgbose *et al*. (1999) working on *L. violaceous* reported that only metals essential for biological processes may be regulated at a desired concentration within the body tissues. For example, Cu and Zn, both essential metals, are used as cofactors by enzymes. Vijver (2001) also reported a possible reason for their elevated levels in these organisms.

Due to these physiological attributes, the biotas are able to regulate these essential metals. It usually requires a large concentration to exceed the window of essentiality. These essential metals are also required to maintain the proper functioning of the immune system and are thus regulated leading to t higher concentration in the snail (ATSDR, 1994). Conversely, non-essential metals like Pb and Cd differ from essential ones by the absence of regulated use. Here, the "window of essentiality" is approximately zero, depending on the metal species (Vijver, 2001).

The inter-specific comparison between site 1 (Rural and non-polluted site) and site 2 (Urban and polluted site) showed that the concentrations of all the metals (Zn, Pb and Cd) were higher in site 2 than in site 1 in all the matrices. Similar investigations have shown that metal concentrations in sentinels are higher in polluted sites than non-polluted sites. Morgan and Morgan (1993) and Weigmann (1991) reported higher concentrations of metals in sentinels from polluted sites, irrespective of the degree of pollution.

Comparing biological patterns of uptakes in snail tissues (kidney and entire rest), it was observed that the levels of heavy metals were higher in the kidney that the entire rest at both sites. According to Dallinger and Wieser (1984), Beeby and Richmond (1991) different parts of the tissue fractions are known to accumulate metals at different rates. Gundacker (2001) observed that snails storage organs which include liver, hepatopancreas and kidney are known to accumulate very high levels of heavy metals when compared to their other soft tissues. Similarly, Beeby (2001) partitioned the snail into two fractions (hepatopancreas and the rest) and he observed that of the two fractions, the hepatopancreas appeared to be the most sensitive indicator.

The student paired t-test applied to test the validity of the null hypothesis showed significant differences at $P < 0.05$ for all the metals (Zn, Pb and Cd) in all the matrices. This is similar to the observation of Dallinger and Wieser (1984) who reported that both the essential metals (Zn, Cu, Fe) and non-essential metals (Pb, Cd) that were bioaccumulated by *H. pormatia* soft tissue showed a significant difference at $P < 0.05$.

In conclusion, metal concentrations varied among the sites in all the matrices studied. Variations in the soil metal concentrations may have accounted for the substantial amount of variability observed among metal concentrations in snail, not excluding the role of adaptive mechanisms of the snail and the "window of essentiality" of the metals (Vijver, 2001). The ability to bioaccumulate heavy metals and evidence of a linear correlation between the tissue and soil metal concentrations observed in this study support the use of *L. aurora* as sentinel for mapping soil metal contamination in the Nigerian environment.

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