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The use of Giant African Snails (*Archachatina maginata*) and (*Achatina folica*) as Bio-Indicators of Heavy Metal Pollution

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ABSTRACT

The bioaccumulation of heavy metals copper, iron, zinc, cobalt, manganese, nickel, cadmium and lead were determined in the shell and flesh of biota, the African giant snails *Archachatina marginata* and *Achatina fulica* purchased in some markets in southern Nigeria. The snail samples were oven dried, digested and analysed for heavy metals using Atomic Absorption Spectrophotometer (AAS). The results from AAS shows that copper for both shell and flesh in all locations exceed the threshold limit (0.06 mgkg^{-1}). Samples from Iheagwa-Owerri, Imo state was observed to have the highest level of iron (608.34 mgkg^{-1}) in shell while samples from Abak market, Akwa-Ibom state had the highest also in iron (25.76 mgkg^{-1}) but in flesh. Some heavy metals like copper, zinc, and manganese had higher values in flesh while the others had higher values in shell. Zinc concentration were recorded very high for both flesh, and shell values were within threshold limit (15 mgkg^{-1}), this might be as a result of Brass production common in the eastern part of the country. Cobalt was not observed in both shell and flesh in all the locations except for the samples from Osogbo market, Osun state, same is the trend for Nickel. The threshold value for lead and cadmium (0.1 mgkg^{-1}and 0.06mgkg^{-1}) were exceeded in majority of the shell and flesh. Samples from Iheagwa-Owerri, Imo state showed the highest value of manganese in the flesh which indicates exceeding pollution due to welding activities. The range for the heavy metals analysed for the shell are 5.96 to 67.24 mgkg^{-1}, 41.09 to 608.34 mgkg^{-1}, 0.00 to 4.48mgkg^{-1}, 0.00 to 1.14 mgkg^{-1}, 1.28 to 23.00mgkg^{-1}, 0.00 to 5.28mgkg^{-1}, 0.00 to 1.14mgkg^{-1}, and 0.00 to 3.60mgkg^{-1} for copper, iron, zinc, cobalt, manganese, nickel, cadmium, and lead respectively. Sample from Iheagwa-Owerri, Imo state showed the highest value of contaminant in the manganese (333.30mgkg^{-1}) which may be an indication of welding activities within that area. Most of the values collected for both shell and flesh samples indicates that there are no significant variations between the values amongst group of shell and flesh. There is also no correlation between all the values. Apart from cobalt and nickel which had recorded nothing for their level in flesh, may be attributed to low level of molten magma from igneous rock as a major source of its pollution and low level of industrial activities/cobalt burning within the areas the samples were picked before taking them to the market. The range of values recorded for copper, iron, zinc, manganese, lead, and cadmium were 43.84 to 93.58 mgkg^{-1}, 25.76 to 327.11mgkg^{-1}, 48.14 to 164.00mgkg^{-1}, 85.47 to 333.30mgkg^{-1}, 0.23 to 12.78mgkg^{-1}, and 0.03 to 14.74mgkg^{-1} respectively. This is a clear indication that the accumulation of metals is higher or more significance in the flesh than it is on the shell. Lead accumulation in the shell is higher with samples from Benin by pass market, Edo, indicating the high traffic or vehicular movement in the area affecting the food chain. It is obvious that the pollution of the environment with heavy metals through improper treatment of waste before discharging into the environment, use of inorganic agrochemicals, and emission from vehicles has increased the level of contamination and thus increases the risk as introduced into the food chain through biota (snail). Therefore, it is highly recommendable that sources of snails must be scrutinized before consumption and snails should possibly be reared in an isolated farm free from pollution so as to reduce the level of contamination and its toxicity owing to its high demand due its medicinal and nutritive value.

Keywords:Giant African Snails, *Archachatina maginata*, *Achatina folica*, Bio-Indicators, Heavy Metal, Pollution.

INTRODUCTION

The pollution of our ecosystem by heavy metals has been recognised as a serious environmental challenge. Heavy metals occur naturally below their toxic threshold in terrestrial habitat [1,2,3]. However, due to their non-degradable nature, they still pose risk of damage to the ecosystem through uptake and subsequent bioaccumulation by organisms that cannot effectively metabolise and excrete the metals already absorbed. Several scientific observations have revealed that heavy metals are bioconcentrated or bioaccumulated in several compartments across food webs [4,5,6]. The terrestrial snails feed on the debris from soil surface which are likely to be contaminated with heavy metals or other organic pollutants; thus, this may accumulate the pollutants to levels that may become harmful [7,8,9,10]. Besides, the contamination of these organisms with heavy metals may have devastating effect on natural ecosystem, its functionality, as well decrease of biodiversity, and extinction of sensitivebiota/taxa[11,12,13,14].Bioaccumulation of these heavy metals can be of importance if viewed from the perspective of public health, especially when the accumulators are consumed by human. This phenomenon is now being explained in the assessment of environmental quality/environmental monitoring, in addition, to Chemical surveys of water and sediments [15,16]. Not much is known of heavy metals concentration and occurrence in terrestrial snails consumed by most tribes in Nigeria [17,18,19,20].

OBJECTIVE OF STUDY

The general objective of this study is to examine the levels heavy metals cadmium (Cd), zinc (Zn), iron (Fe), cobalt (Co), manganese (Mn), lead (Pb), nickel (Ni), and copper (Cu)), present in commonly consumed snails in selected states in southern Nigeria. These elements are believed to bioaccumulate in biota, giant African snails found in southern Nigeria market. Giant African snails live in wet soils, or amphibious, lay eggs in the soil, feed on the soil and moves on the soil with the aid of its foot muscles and secretion of mucus, thus making it an ideal biota to use in the research for the accumulation of heavy metals.

MATERIALS AND METHODS

AREA OF SAMPLING

A total of 46 snail specimens of varying sizes and ages were purchased from selected markets in southern Nigeria. The selected markets are: *Cele Market in Lagos, Ore market in Ondo, Effurun Market in warri, Delta State, Osogbo Market in Osun, Yenagoa Market in Bayelsa, Abak Market in Akwa-Ibom, Nkwegu market in Ebonyi, Benin by-pass market in Edo and Ihiagwa-owerri in Imo State*. Snail samples were purchased and transported with High-Density Polyethylene (HDPE) sample containers (rectangular boxed bowel) between January and February 2014. They were purchased from the l. selected market because they are easily consumed in the area without any religious or cultural restriction. The snail samples habit in these areas because of the availability of the rain forest to swampy vegetation and the favourable temperature as can be seen in figure 12. Snail samples were collected without consideration of age or size. Snail collection and sale due to the demand is on the increase, and considering the level of pollution due to urbanisation, industrialisation and exploration of mineral resources as well as waste disposa

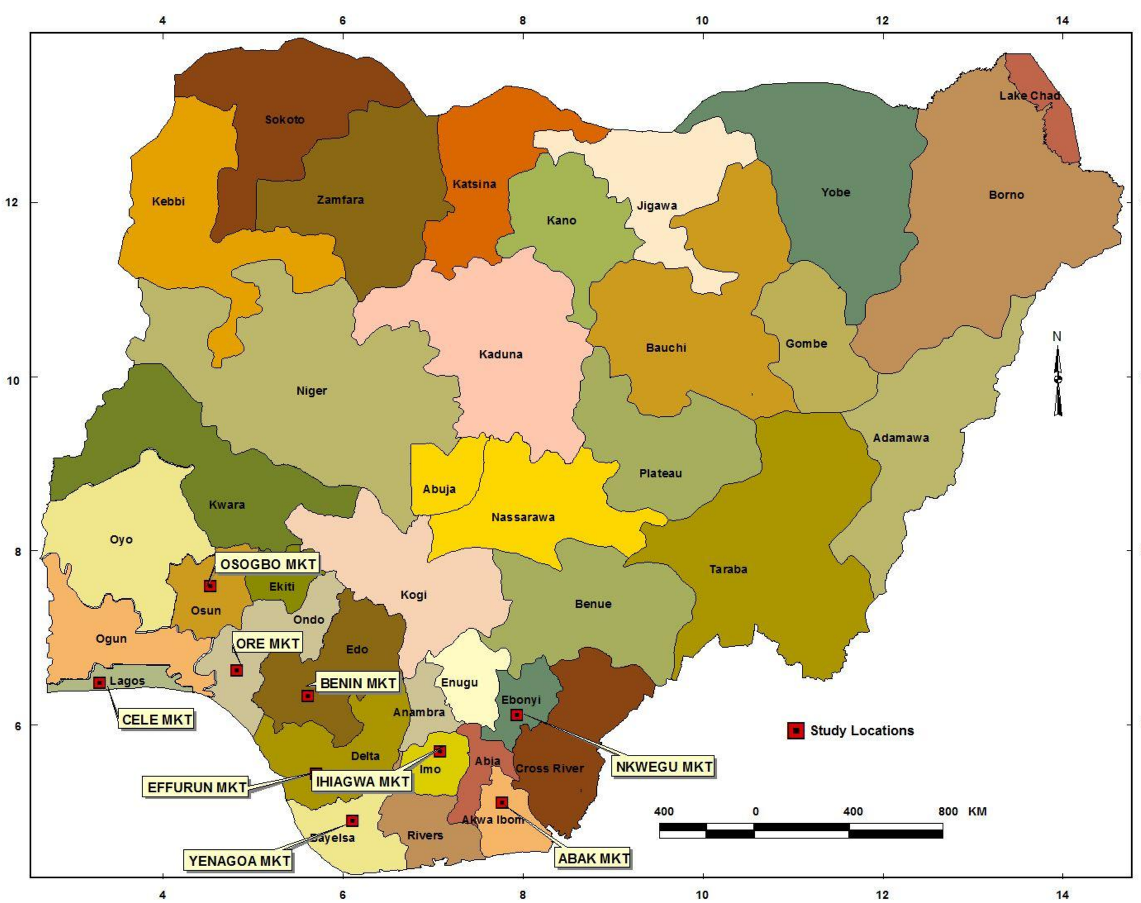

Plate 1:GPS map of Nigeria showing the areas in Southern Nigeria where samples were purchased.

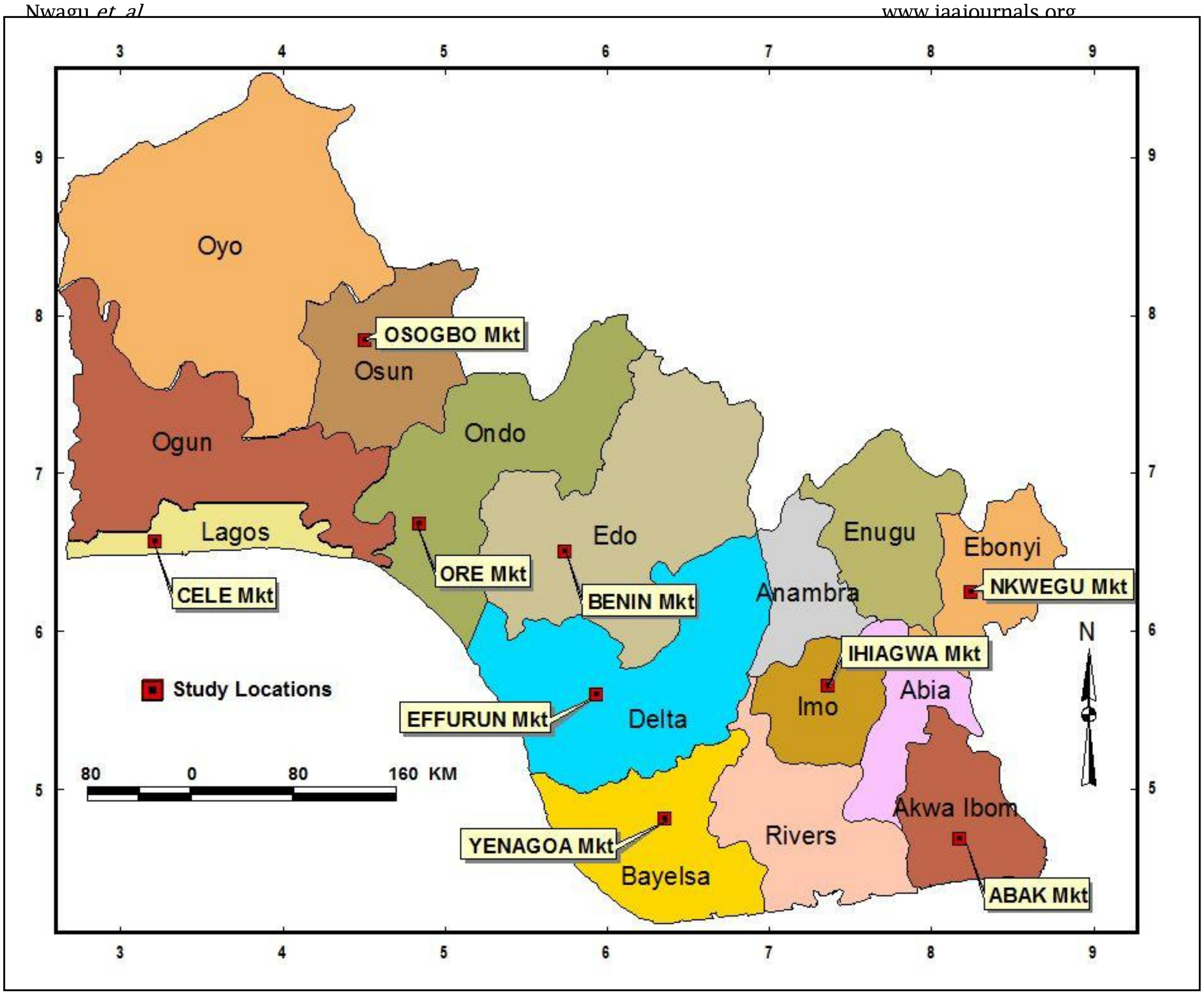

Plate 2:GPS map of Southern Nigeria indicating the points of purchase of the samples and their names.

APPARATUS AND REAGENT

The apparatus used for the entire analysis from the preservation to the analysis are as follows, High-Density Polyethylene (HDPE) containers for the sample collection and transportation, refrigerator, indelible marker, laboratory oven, mortar and pistol, paper tape, glass beakers, conical flasks, foil paper, measuring cylinder, measuring flask, forceps, thermometer, laboratory hot plate, fume cupboard, analytical balance, Atomic Absorption Spectrophotometer (AAS), spatula, hand gloves, nose mask, whatman filter paper, plastic funnels, High-Density Polyethylene (HDPE) sample containers, High-Density Polyethylene(HDPE) sample bottles, and wash bottle.The reagents and solvent used include, distilled water, Sodium chloride (Nacl), concentrated hydrochloric acid (Hcl), and concentrated Nitric acid (HNO_3).

IDENTIFICATION AND PRESERVATION

The specimens were identified as *Archachatina marginata,* and *Achatina fulica,* belonging to the same family, Achatinidae in the Department of Zoology, Federal University of Agriculture, Abeokuta. They were washed with distilled water and preserved in the refrigerator with High-Density Polyethylene(HDPE) sample container to the temperature of -18°c after collection prior to digestion and analysis. The purchased samples were collectively labelled with alphabets according to the source of collection which are as follow: A – Cele market in Lagos State; B – Ore market in Ondo State; C – Iheagwa, Owerri, in Imo State; D – Effurun market, warri in Delta State and E – Osogbo market in Osun state. Other location include: F – Yenagoa market in Bayelsa state; G – Abak market in Akwa-Ibom state; H – Nkwoegu market in Ebonyi state and I – Benin by pass market in Edo state. These locations are clearly shown in the GPS map of southern Nigeria in plate 2.

PRE-ANALYSIS PROCEDURE

The snails were alphabetically labelled according to the sources with the indelible permanent marker on the shells and were placed in an oven to remove water and the lipid content for about 48 hours. When the snail samples became relatively dry, they were broken with piston to separate the content (snail, digestive tract & others) from the shell and were placed back into the oven to further dry for about 36 hours. The shells were separated differently from the content and were labelled accordingly with foil paper and paper tape before placing back to the ovum with forceps to completely drain the lipid content. After drying further for about 28hours at a temperature of about 150°C, the dry flesh were carefully grinded with ceramic mortar and pistol and were labelled with sub letter "F"-meaning flesh, while the shells were crushed with a machine – Fritsch laboratory jaw crusher and disk mill made in Canada, and were labelled with sub letter "S"-meaning shell. During the grinding, the snail samples collected from Warri in Delta State (labelled – D), Yenagoa in Bayelsa State (labelled – F), and Abak in Akwa-Ibom State (labelled – G) were harder than the snails from other areas. Conical flasks used for the sample digestion were washed with soapy water, rinsed with tap water and soaked in 10% nitric acid. The flasks were then rinsed with distilled water and oven dried before use. The dry flasks were labelled in triplicates for the flesh and shell samples collected in nine (9) different locations which are 54 conical flasks in all. The additional three (3) flasks were blank and meant for control (3 blank labelled "X", "F", & "B"); 27 (3 x 9) shells and 27 (3 x 9) flesh. The blank contained nothing. Both the flesh and shell samples were carefully weighed using Ohaus analytical balance made in USA were weighed into 1.000g (±0.002). Aqua agar was formed by mixing/addition of Hydrochloric acid (HCl), and Nitric acid (HNO_3) all concentrated and measured with a glass measuring cylinder at the ratio of 3:1. 15ml of the Aqua agar was carefully measured and added to the samples. Digestion of the samples in the conical flasks was done in a fume cupboard. The samples were heated with laboratory hot plate placed in the fume cupboard to a temperature of 110°c for a period of 2-5mins when it became clear for both the shell and flesh. During digestion, the shells were noticed to produce foam which is an indication of the presence of $CaCO_3$. The blank samples were meant to be a control as they were also digested to achieve same colour change as were the case of the other samples. After the digestion, 5ml of distilled H_2O was added to each of samples to dilute the concentrated sample solution using the wash bottle. These diluted samples were filtered with whatman filter paper into a labelled HDPE sample bottles through a plastic funnel that were soaked in a bath of 1% NaCl for 24hrs before rinsing them with distilled H_2O. The HDPE sample bottles were appropriately labelled. Little quantity of distilled H_2O was used to rinse the conical flask used for specimen digestion. Distilled water was added to each of the sample container before they were finally corked for heavy metal analysis. The flesh samples were observed to be more coloured than the shell samples that is almost completely clear.

ANALYSIS OF METALS

The labelled High-Density Polyethylene (HDPE) bottle were then taken to the Central Laboratory, Biotechnology Unit, Federal University of Agriculture, Abeokuta, Ogun State for metal analysis using Atomic Absorption Spectrophotometer (AAS) equipment made by Thermo Fisher, USA. The following metals were analysed from the samples for both shell and flesh: cadmium (Cd), cobalt (Co), copper (Cu), iron (Fe), manganese (Mn), nickel (Ni), lead (Pb), and zinc (Zn).

RESULTS

The mean values of heavy metals obtained from the analysed snails' Shell (S) are presented in Figures 1 to 8, while that of Flesh (F) are presented in Figures 9 to 16 with AS – IS and AF – IF representing the sampling locations respectively for shell and flesh. Also, variations were measured using Analysis of variance (ANOVA) and presented on Table 2 while relationship between each heavy metal and locations is presented in Table 3 using Pearson's Correlation.

HEAVY METAL CONCENTRATION OF SNAILS' SHELL SAMPLES

Mean values of copper obtained are presented on Figure 1. The highest value of 67.24 $mgkg^{-1}$ and lowest of 5.96 $mgkg^{-1}$ were observed in samples purchased from Osogbo market, Osun state and Abak market, Akwa-Ibom. Figure 2 shows the mean values with respect to Iron. Out of all metals analysed for, Iron recorded the highest value of 608.34 $mgkg^{-1}$ in sample from Iheagwa-Owerri, Imo state, while 41.09 $mgkg^{-1}$ was observed for sample from Osogbo market, Osun state. The range values of Zinc concentration are 0.00 - 4.48 $mgkg^{-1}$ observed for samples from Osogbo market, Osun state and Abak market, Akwa-Ibom state respectively as presented in Figure 3. However, Figure 4 shows Cobalt concentrations for all the locations which were not significant except for samples from Osogbo market, Osun state which recorded 1.14 $mgkg^{-1}$. Meanwhile, mean values of Manganese range between 23.00 $mgkg^{-1}$ for samples from Iheagwa-Owerri, Imo state and 1.28 $mgkg^{-1}$ for samples from Osogbo market, Osun state in Figure 5. Figure 6 show the mean values of Nickel concentration in snail shell samples. Samples from Effurun market, Warri, Delta state, Osogbo market, Osun state and Cele market, Lagos state are 5.28 $mgkg^{-1}$, 2.09 $mgkg^{-1}$ and 2.75 $mgkg^{-1}$. It was observed that other locations recorded 0.00 $mgkg^{-1}$. Meanwhile, the highest value of 1.14 $mgkg^{-1}$ of Cadmium was recorded for samples from Ore market, Ondo state while 0.00 $mgkg^{-1}$ was recorded for both samples from Osogbo market, Osun state and Nkwoegu market, Ebonyi state as shown in Figure 7. Taking a cue from Cadmium concentration shown above, samples from Osogbo market, Osun state and Nkwoegu market, Ebonyi state also recorded 0.00 $mgkg^{-1}$ for Lead while samples from Abak market in Akwa-Ibom state recorded 3.60 $mgkg^{-1}$ as shown in Figure 8.

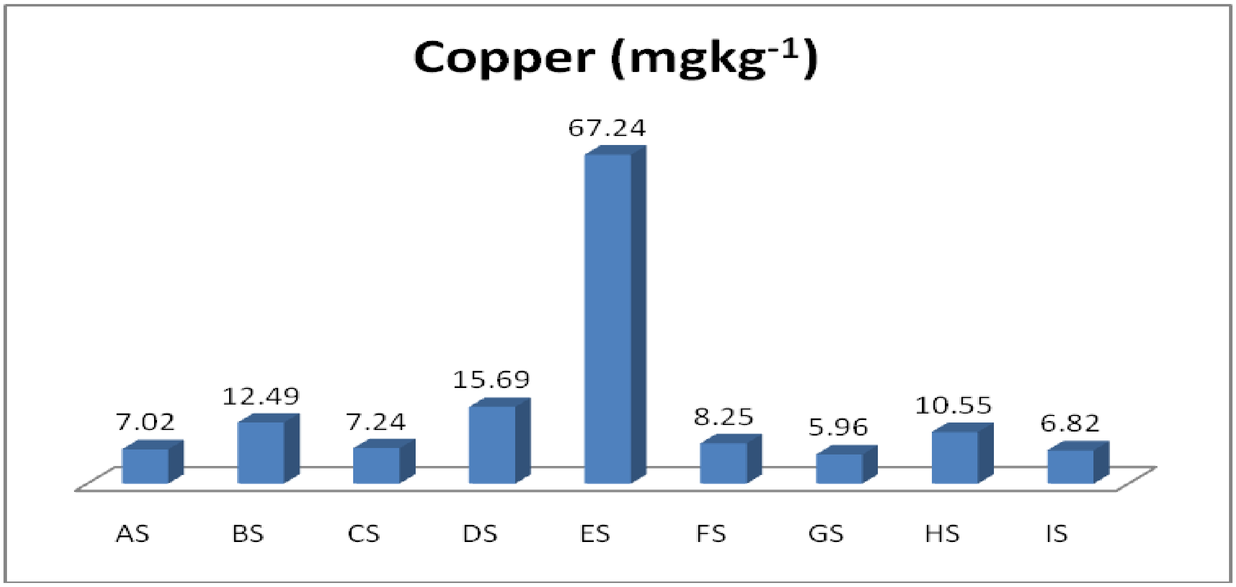

Figure 1: Mean concentration of Copper in snail shell

Legend

AS - Cele Market, Lagos
BS – Ore market, Ondo
CS – Iheagwa-Owerri, Imo
DS – Effurum market, Warri, Delta
ES – Osogbo market, Osun
FS – Yenegoa market, Bayelsa
GS – Abak market, Akwa-Ibom
HS – Nkwoegu market, Ebonyi
IS – Benin by pass market, Edo

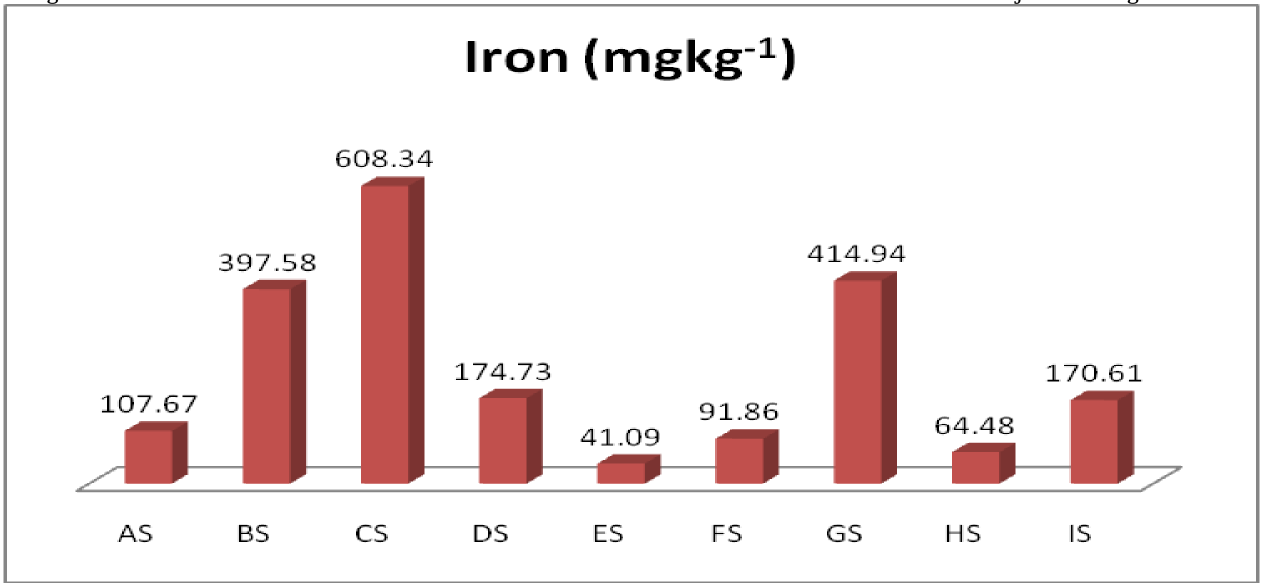

Figure 2: Mean concentration of Iron in snail shell

Legend
AS - Cele Market, Lagos
BS – Ore market, Ondo
CS – Iheagwa-Owerri, Imo
DS – Effurum market, Warri, Delta
ES – Osogbo market, Osun
FS – Yenegoa market, Bayelsa
GS – Abak market, Akwa-Ibom
HS – Nkwoegu market, Ebonyi
IS – Benin by pass market, Edo

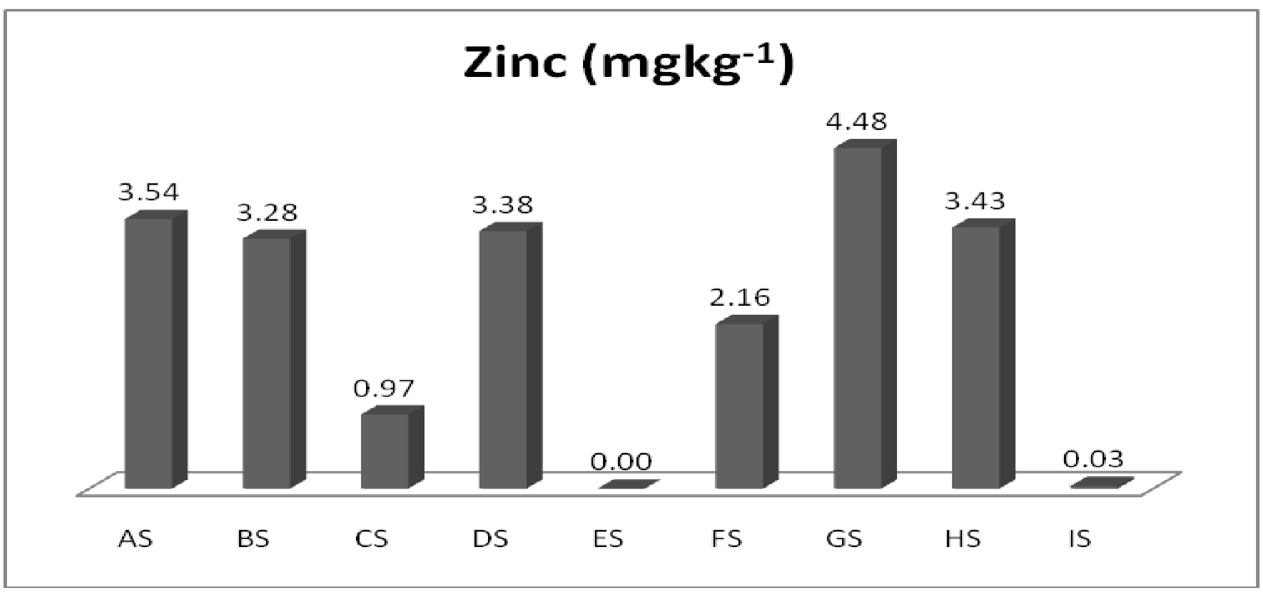

Figure 3: Mean concentration of Zinc in snail shell

Legend
AS - Cele Market, Lagos
BS – Ore market, Ondo
CS – Iheagwa-Owerri, Imo
DS – Effurum market, Warri, Delta

ES – Osogbo market, Osun
FS – Yenegoa market, Bayelsa
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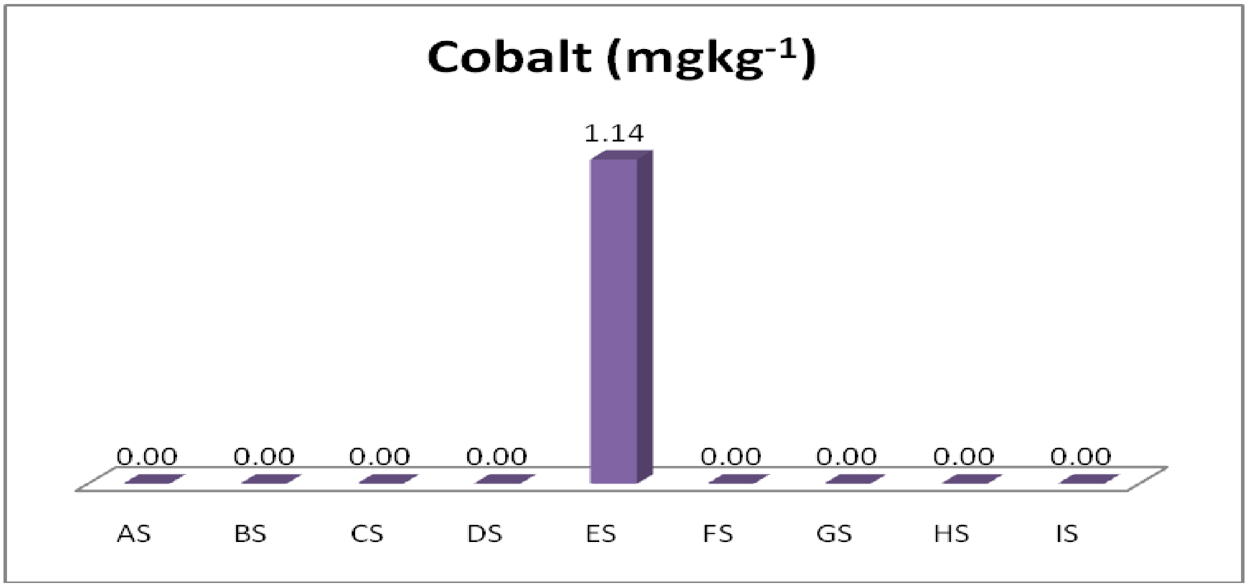

Figure 4: Mean concentration of Cobalt in snail shell

Legend
AS - Cele Market, Lagos
BS – Ore market, Ondo
CS – Iheagwa-Owerri, Imo
DS – Effurum market, Warri, Delta
ES – Osogbo market, Osun
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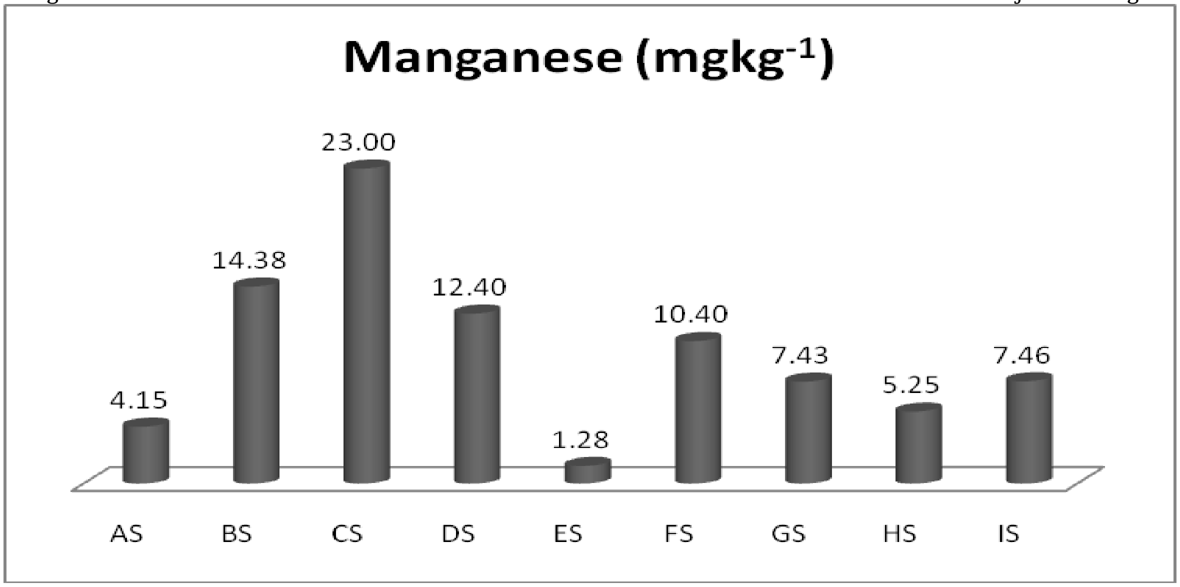

Figure 5: Mean concentration of Manganese in snail shell

Legend

AS - Cele Market, Lagos
BS – Ore market, Ondo
CS – Iheagwa-Owerri, Imo
DS – Effurum market, Warri, Delta
ES – Osogbo market, Osun
FS – Yenegoa market, Bayelsa
GS – Abak market, Akwa-Ibom
HS – Nkwoegu market, Ebonyi
IS – Benin by pass market, Edo

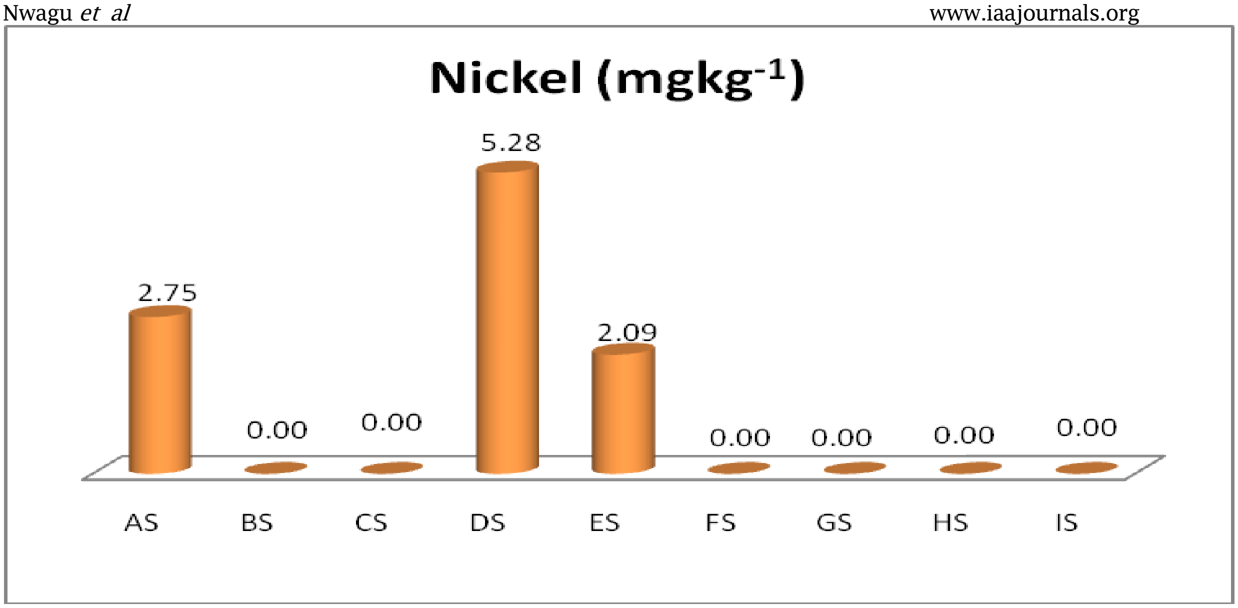

Figure 6: Mean concentration of Nickel in snail shell

Legend

AS - Cele Market, Lagos
BS – Ore market, Ondo
CS – Iheagwa-Owerri, Imo
DS – Effurum market, Warri, Delta
ES – Osogbo market, Osun
FS – Yenegoa market, Bayelsa
GS – Abak market, Akwa-Ibom
HS – Nkwoegu market, Ebonyi
IS – Benin by pass market, Edo

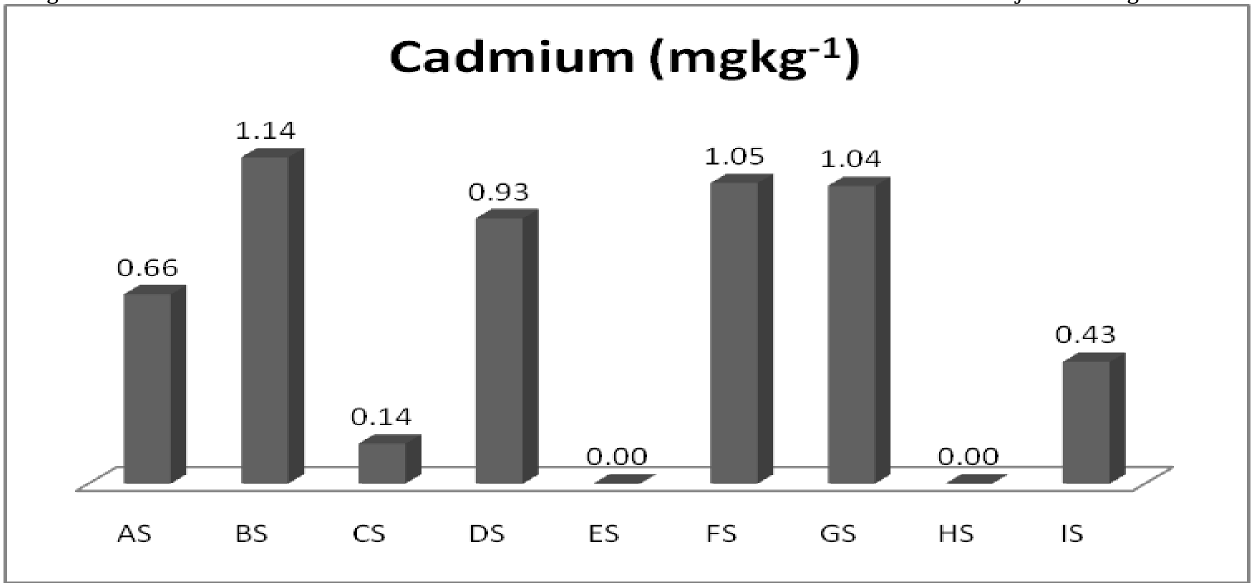

Figure 7: Mean concentration of Cadmium in snail shell

Legend
AS - Cele Market, Lagos
BS – Ore market, Ondo
CS – Iheagwa-Owerri, Imo
DS – Effurum market, Warri, Delta
ES – Osogbo market, Osun
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IS – Benin by pass market, Edo

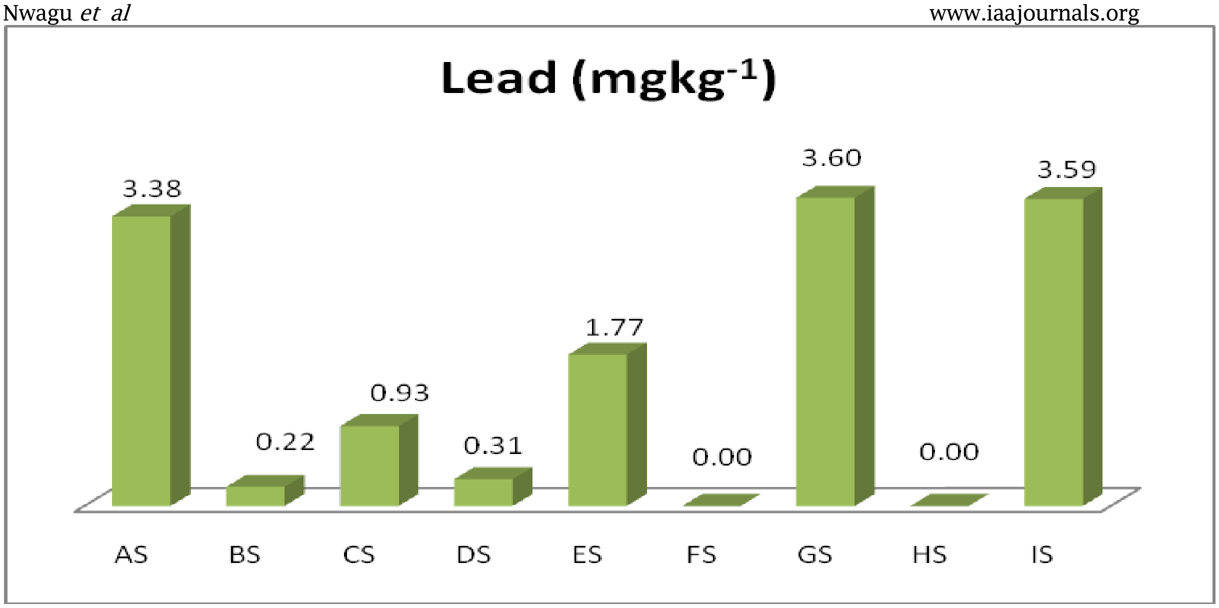

Figure 8: Mean concentration of Lead in snail shell

Legend
AS - Cele Market, Lagos
BS – Ore market, Ondo
CS – Iheagwa-Owerri, Imo
DS – Effurum market, Warri, Delta
ES – Osogbo market, Osun
FS – Yenegoa market, Bayelsa
GS – Abak market, Akwa-Ibom
HS – Nkwoegu market, Ebonyi
IS – Benin by pass market, Edo

HEAVY METAL CONCENTRATION IN SNAILS' FLESH SAMPLES

Mean values of Copper obtained in the flesh samples are presented in Figure 9. The highest value of 93.58 $mgkg^{-1}$ and lowest of 43.84 $mgkg^{-1}$ were observed in samples purchased from Abak market, Akwa-Ibom state and Cele market, Lagos state. Figure 10 shows the mean values recorded for Iron concentration. It can be observed that samples purchased from Yenegoa market, Bayelsa state gave the highest value of 327.11 $mgkg^{-1}$ while that of Abak market, Akwa-Ibom state being the lowest is 25.76 $mgkg^{-1}$.However, for Zinc concentration, samples from Yenegoa market, Bayelsa state yielded least value of 48.14 $mgkg^{-1}$ while the ones from Nkwoegu market, Ebonyi state yielded highest value of 164.00 $mgkg^{-1}$ as shown in Figure 11.All locations flesh samples gave no significant value with respect to Cobalt and Nickel concentration as presented on Figure 12 and 14 respectively. A totally different trend is observed for Manganese as shown on Figure 13. The highest value of 333.30 $mgkg^{-1}$ is recorded from the sample purchased from Iheagwa-Owerri, Imo state and the lowest is 85.47 $mgkg^{-1}$ for samples from Yenegoa market, Bayelsa state.Meanwhile, the range of Lead concentration is 12.78 $mgkg^{-1}$ – 0.23 $mgkg^{-1}$ for Benin by pass market, Edo state and Abak market, Akwa-Ibom state respectively according to Figure 15.

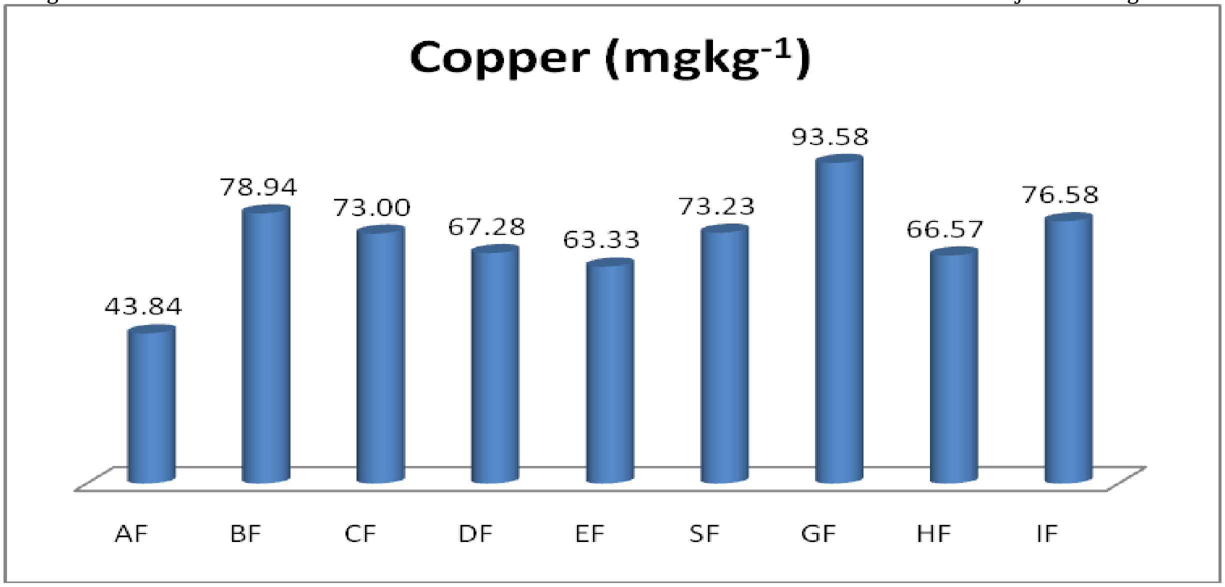

Figure 9: Mean concentration of Copper in snail flesh

Legend

AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo
DF – Effurun market, Warri, Delta
EF – Osogbo market, Osun
FF – Yenegoa market, Bayelsa
GF – Abak market, Akwa-Ibom
HF – Nkwoegu market, Ebonyi
IF – Benin by pass market, Edo

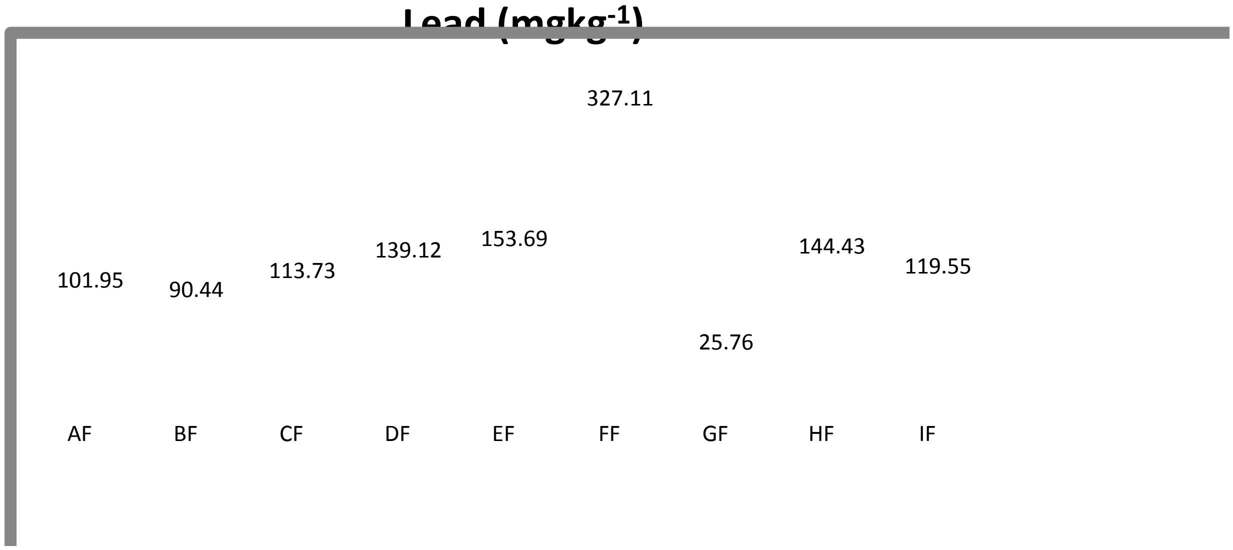

Figure 10: Mean concentration of Iron in snail flesh

Legend

AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo
DF – Effurun market, Warri, Delta
EF – Osogbo market, Osun
FF – Yenegoa market, Bayelsa
GF – Abak market, Akwa-Ibom
HF – Nkwoegu market, Ebonyi
IF – Benin by pass market, Edo

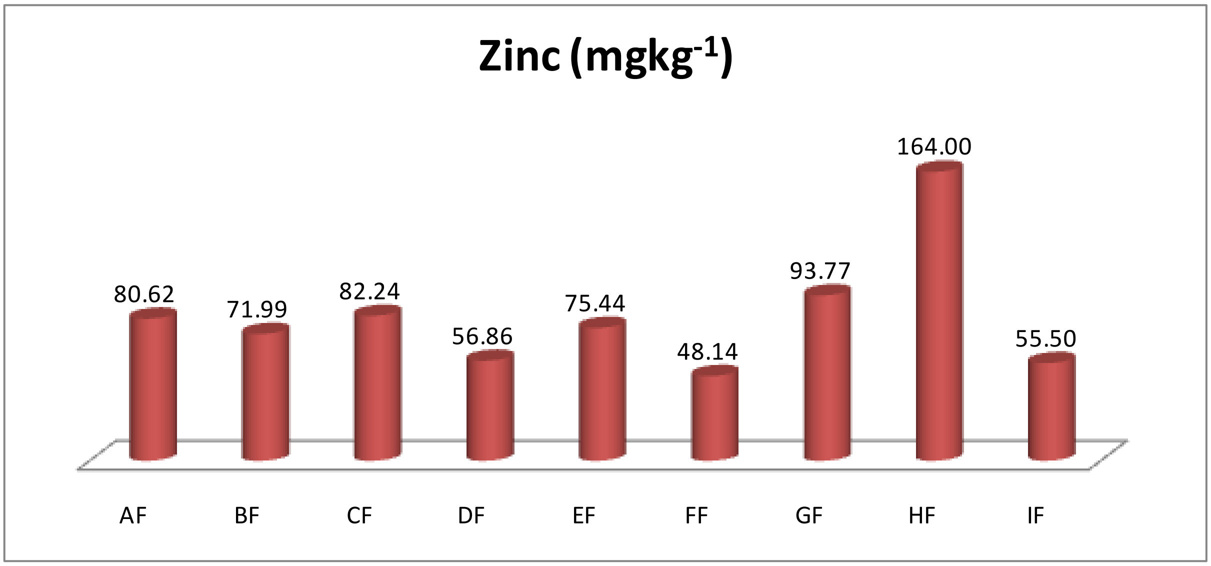

Figure 11: Mean concentration of Zinc in snail flesh

Legend

AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo
DF – Effurun market, Warri, Delta
EF – Osogbo market, Osun
FF – Yenegoa market, Bayelsa
GF – Abak market, Akwa-Ibom
HF – Nkwoegu market, Ebonyi
IF – Benin by pass market, Edo

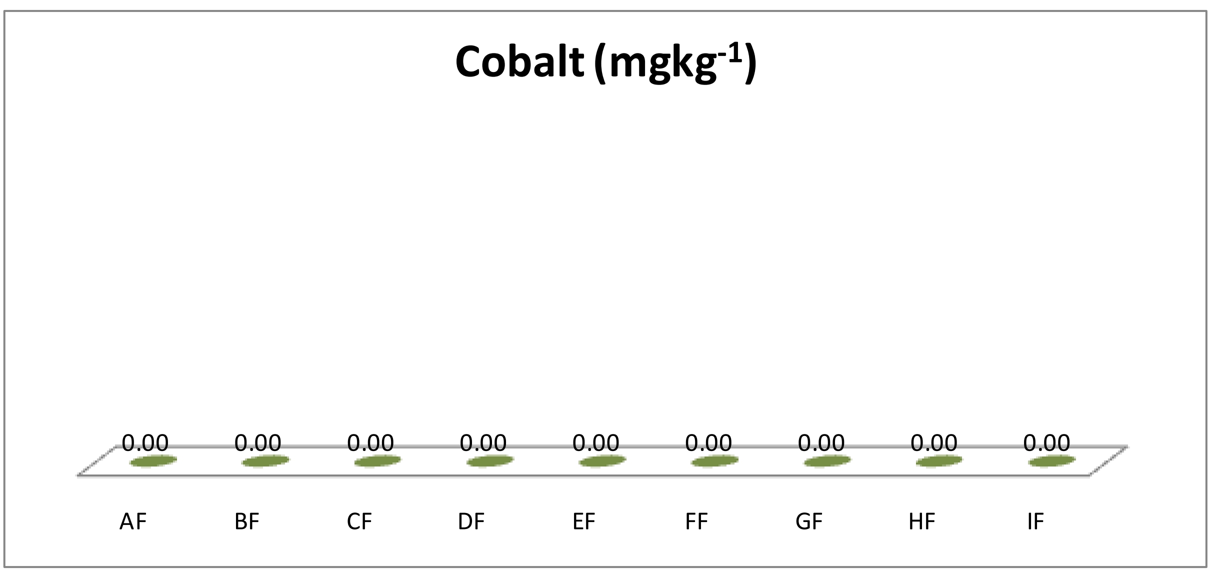

Figure 12: Mean concentration of Cobalt in snail flesh

Legend

AF - Cele Market, Lagos
BF – Ore market, Ondo
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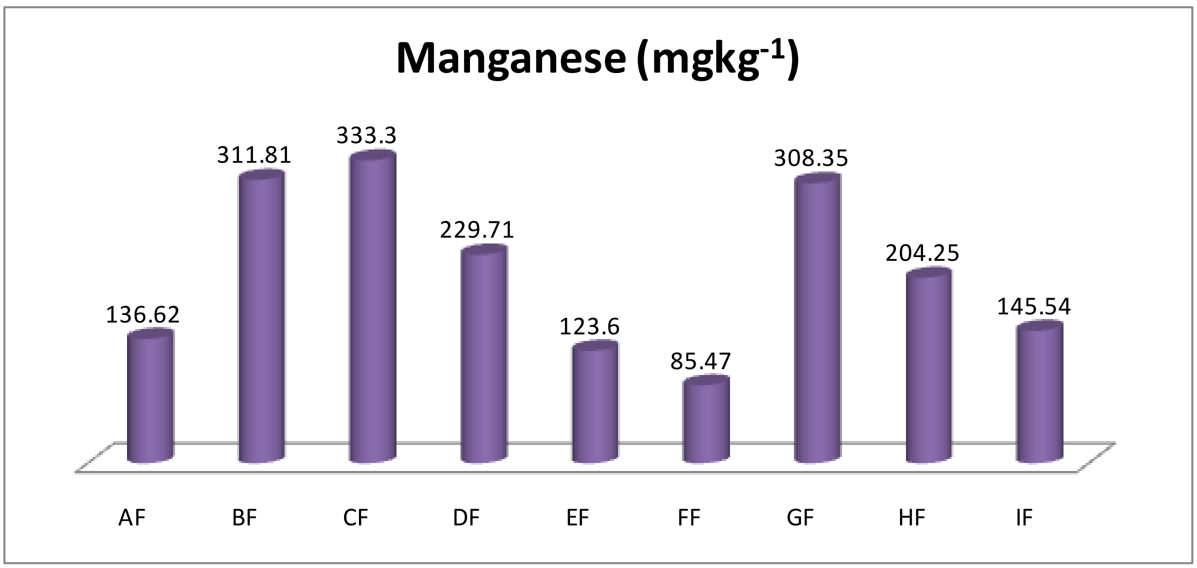

Figure 13: Mean concentration of Manganese in snail flesh

Legend

AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo
DF – Effurun market, Warri, Delta
EF – Osogbo market, Osun
FF – Yenegoa market, Bayelsa
GF – Abak market, Akwa-Ibom
HF – Nkwoegu market, Ebonyi
IF – Benin by pass market, Edo

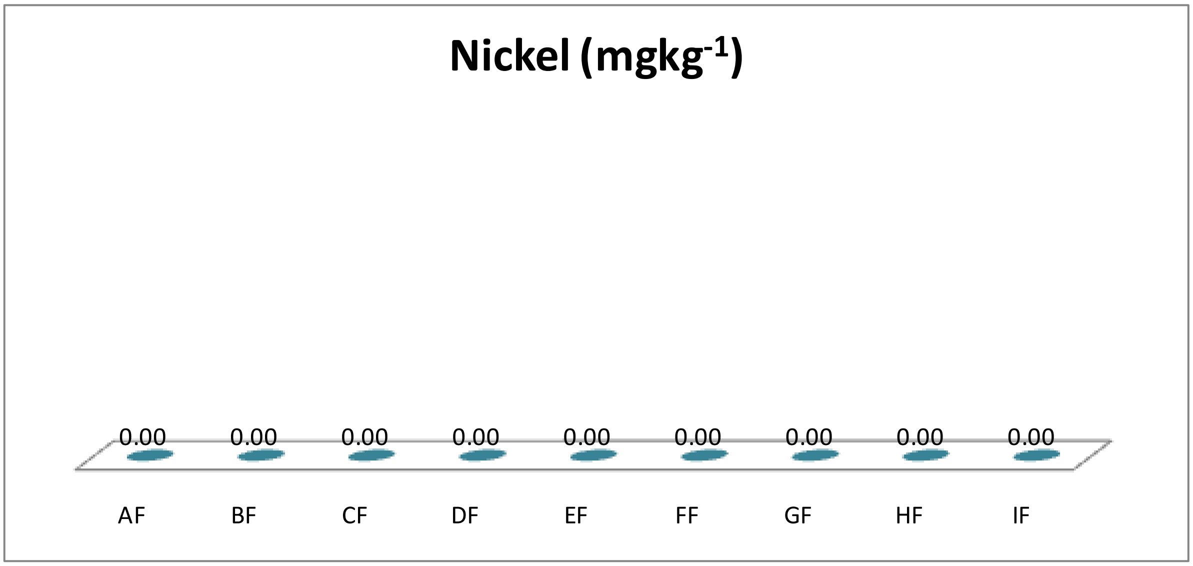

Figure 14: Mean concentration of Nickel in snail flesh

Legend

AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo
DF – Effurun market, Warri, Delta
EF – Osogbo market, Osun
FF – Yenegoa market, Bayelsa
GF – Abak market, Akwa-Ibom
HF – Nkwoegu market, Ebonyi
IF – Benin by pass market, Edo

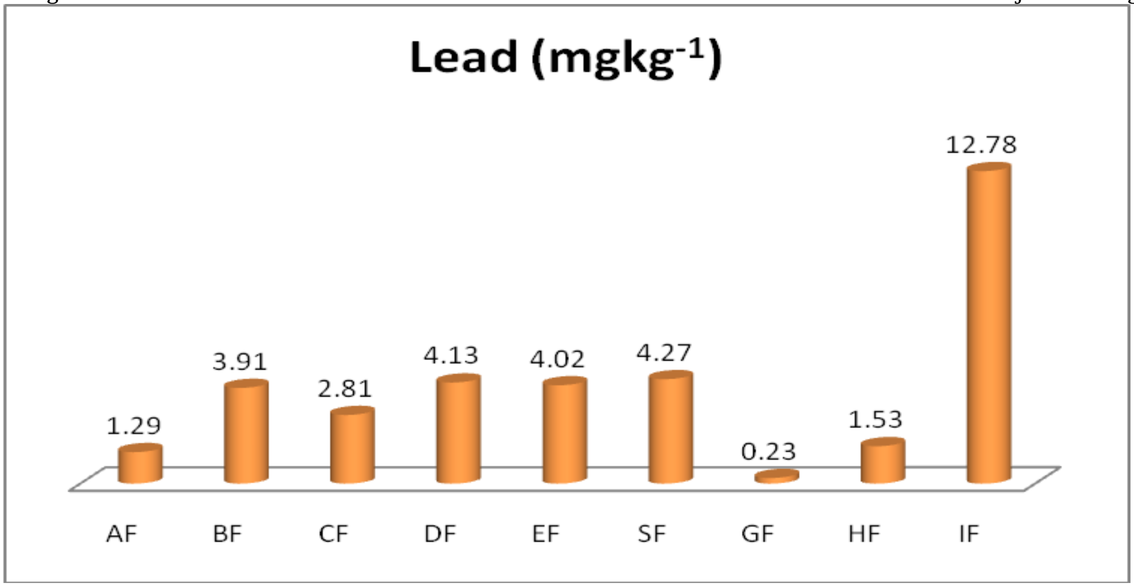

Figure 15: Mean concentration of Lead in snail flesh

Legend

AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo
DF – Effurun market, Warri, Delta
EF – Osogbo market, Osun
FF – Yenegoa market, Bayelsa
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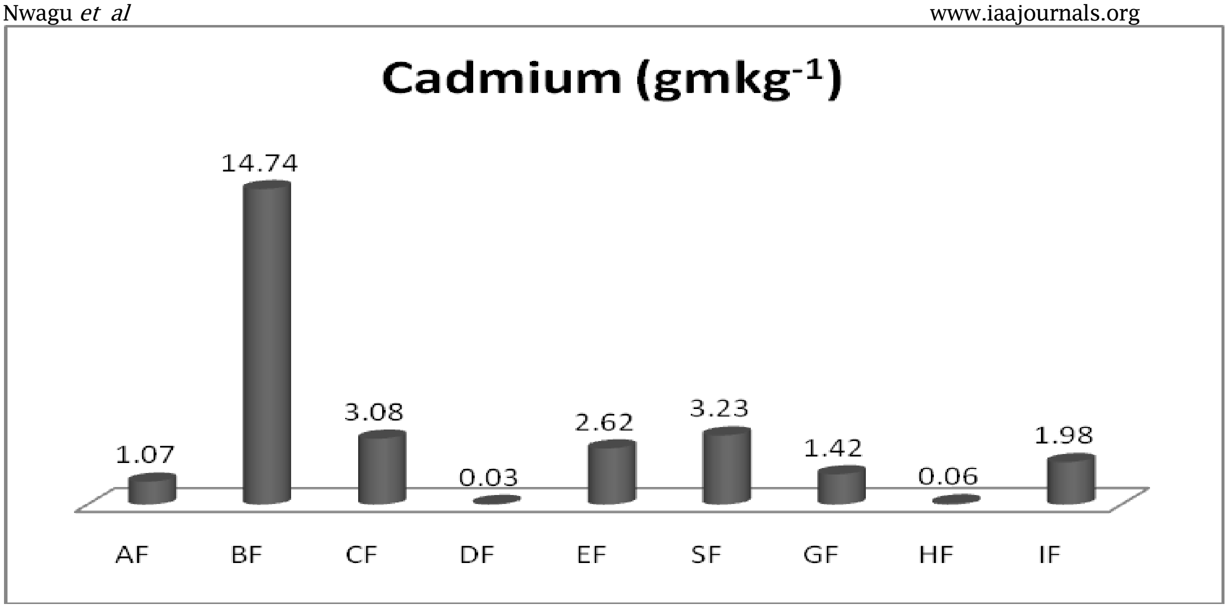

Figure 16:Mean concentration of Cadmium in snail flesh

Legend

AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo
DF – Effurun market, Warri, Delta
EF – Osogbo market, Osun
FF – Yenegoa market, Bayelsa
GF – Abak market, Akwa-Ibom
HF – Nkwoegu market, Ebonyi
IF – Benin by pass market, Edo

VARIATIONS BETWEEN SHELL AND FLESH SAMPLES AND LOCATIONS

The Analysis of variance using Duncan Multiple Rating (DMR) as shown on Table 2 shows that for copper and zinc concentrations varied significantly ($P<0.05$) between shell and flesh samples across the study areas. There was no significant difference ($P>0.05$) between groups of shell and flesh but locations display a little bit of differences for all metals monitored. There was significant difference ($P<0.05$) between other samples except for samples from Cele market, Lagos (Flesh and shell), Effurun market, Warri, Delta (shell and flesh), Oshogbo market, Osun (flesh), Nkwoegu market, Ebonyi (flesh) and Benin by pass market, Edo (flesh) for zinc, and this is the trend for other metals. As presented on Table 3, there exist no significant relationship between locations and within shell and fleshy sample.

Table 2: Variations between heavy metals concentration based on sampling location

Sample	Copper	Iron	Zinc	Cobalt	Manganese	Nickel	Lead	Cadmium
1S	7.02±2.03^{a}	107.67±6.91^{abc}	3.54±6.13a	0±0.00a	4.15±3.60a	2.75±4.76ab	3.38±3.04a	0.66±0.33a
2S	12.49±3.59^{ab}	397.58±382.49^{bcd}	3.28±5.32a	0±0.00a	14.37±2.77a	0±0.00a	0.23±0.39a	1.14±1.50a
3S	7.25±3.77^{a}	608.34±370.77^{d}	0.97±1.69a	0±0.00a	23.00±2.11a	0±0.00a	0.93±1.62a	0.14±0.24a
4S	15.68±3.37^{ab}	174.73±152.00^{abc}	3.38±4.88a	0±0.00a	12.40±10.66a	5.28±9.14b	0.31±0.54a	0.93±1.61a
5S	67.24±53.73^{cd}	41.09±71.18^{a}	0±0.00a	1.14±1.97b	1.28±2.22a	2.09±2.08ab	1.77±1.99a	0±0.00a
6S	8.25±2.72^{a}	91.86±80.55^{abc}	2.16±3.63a	0±0.00a	10.40±1.26a	0±0.00a	0±0.00a	1.05±1.56a
7S	5.96±1.21^{a}	414.94±82.77^{cd}	4.48±7.75a	0±0.00a	7.43±2.31a	0±0.00a	3.6±3.39ab	1.04±1,81a
8S	10.56±1.66^{a}	64.48±54.08^{ab}	3.43±4.91a	0±0.00a	5.25±0.14a	0±0.00a	0±0.00a	0±0.00a
9S	6.81±4.80^{a}	170.61±35.05^{abc}	0.03±0.06a	0±0.00a	7.46±0.62a	0±0.00a	3.58±3.56ab	0.43±0.54a
1F	43.84±16.26^{bc}	101.95±53.95^{abc}	80.62±28.83de	0±0.00a	136.62±19.66bc	0±0.00a	1.29±2.23a	1.07±0.46a
2F	78.94±7.48^{d}	90.43±12.29^{abc}	71.99±7.70bcde	0±0.00a	311.81±64.78ef	0±0.00a	3.91±3,98ab	14.74±23.61b
3F	73.00±8.73^{cd}	113.73±10.54^{abc}	82.24±17.71e	0±0.00a	333.30±39.33f	±0.00a	2.81±2.63a	3.07±1.81a
4F	67.28±9.89^{cd}	139.12±111.83^{abc}	56.86±13.02^{bcd}	0±0.00a	229.71±178.34de	0±0.00a	4.14±2.88ab	0.03±0.06a
5F	63.33±19.10^{cd}	153.69±20.92^{abc}	75.44±16.38^{cde}	0±0.00a	123.60±12.39bc	0±0.00a	4.02±2.51ab	2.610±0.71a
6F	73.23±30.76^{cd}	327.11±422.71^{abcd}	48.14±17.64^{b}	0±0.00a	85.47±40.10ab	0±0.00a	4.27±3.91ab	3.23±1.23a
7F	93.58±27.87^{d}	25.76±28.33^{a}	93.77±15.50^{e}	0±0.00a	308.34±42.86ef	0±0.00a	0.23±0.40a	1.42±0.69a
8F	66.57±7.45^{cd}	144.43±13.33^{abc}	163.99±23.48^{f}	0±0.00a	204.25±37.52cd	0±0.00a	1.53±2.51a	0.06±0.10a
9F	76.58±16.77^{cd}	119.55±47.03^{abc}	55.50±16.62^{bc}	0±0.00a	145.54±10.23bcd	0±0.00a	12.78±18.05b	1.98±0.85a

Means with the same superscript column-wise are not significantly different according to Duncan Multiple Range test

Legend

AS and AF - Cele Market, Lagos
BS and BF – Ore market, Ondo
CS and CF – Iheagwa-Owerri, Imo
DS and DF – Effurn market, Warri, Delta
ES and EF – Osogbo market, Osun
FS and FF – Yenegoa market, Bayelsa
GS and GF– Abak market, Akwa-Ibom
HS and HF – Nkwoegu market, Ebonyi
IS and IF – Benin by pass market, Edo

Table 3: Relationship between heavy metals concentration of snails' shell and flesh

	Copper Shell	Copper Flesh	Iron Shell	Iron Flesh	Zinc Shell	Zinc Flesh	Cobalt Shell	Cobalt Flesh	Manganese Shell	Manganese Flesh	Nickel Shell	Nickel Flesh	Lead Shell	Lead Flesh	Cadmium Shell	Cadmium Flesh
Copper Shell	1	-0.22	-0.38	0.10	-0.49	-0.06	0.99	.(a)	-0.45	-0.32	0.28	.(a)	-0.05	0.01	-0.44	0.00
Copper Flesh		1	0.54	-0.21	0.09	-0.06	-0.21	.(a)	0.32	0.53	-0.53	.(a)	0.01	0.11	0.35	0.27
Iron Shell			1	-0.49	0.10	-0.07	-0.36	.(a)	0.81	0.88	-0.35	.(a)	0.03	-0.17	0.18	0.38
Iron Flesh				1	-0.29	-0.28	0.09	.(a)	-0.02	-0.66	-0.03	.(a)	-0.54	0.18	0.03	-0.09
Zinc Shell					1	0.35	-0.54	.(a)	-0.03	0.36	0.15	.(a)	-0.08	-0.68	0.57	0.04
Zinc Flesh						1	-0.06	.(a)	-0.23	0.23	-0.27	.(a)	-0.15	-0.49	-0.50	-0.22
Cobalt Shell							1	.(a)	-0.48	-0.35	0.19	.(a)	0.06	0.01	-0.48	-0.04
Cobalt Flesh								1	.(a)	. (a)	.(a)	.(a)	.(a)	.(a)	.(a)	.(a)
Manganese Shell									1	0.66	-0.17	.(a)	-0.40	-0.01	0.18	0.35
Manganese Flesh										1	-0.19	.(a)	-0.10	-0.34	0.16	0.37
Nickel Shell											1	.(a)	-0.04	-0.10	0.09	-0.34
Nickel Flesh												.(a)	.(a)	.(a)	.(a)	.(a)
Lead Shell													1	0.20	-0.01	-0.29
Lead Flesh														1	-0.09	0.06
Cadmium Shell															1	0.40
Cadmium Flesh																1

DISCUSSION

The values of copper recorded for both shell and flesh samples in all the locations all exceed the threshold limit of 0.06 $mgkg^{-1}$ and the estimated daily intake for adult is 0.014-0.19$mgkg^{-1}day^{-1}$[21,22,23,24]. However, the values recorded for flesh samples are generally higher than that of shell (Table 2). This is an indication that the snails have accumulated this particular metal for a long time since the snail will live in soils either contaminated or otherwise from egg to adult, hence any ingestion by humans of this snail may result in bioaccumulation of copper [25,26]. There is no metal irrespective of how essential to humans it is, at considerably high concentration that will not be toxic to human health. Considering the concentrations recorded for iron in both shell and flesh samples, Imo state gave the highest value of 608. 34 $mgkg^{-1}$ in shell sample while Akwa-Ibom yielded 25.76 $mgkg^{-1}$ in flesh sample. This trend of high iron concentration was also reported by [27,28,29]. Iron plays an important role as an essential element in all systems from invertebrates to humans but increased iron in the environment may result in the bioaccumulation [30,31]. Generally in a similar research carried out in Bayelsa state by [32,33], the shell samples contained higher values of heavy metals than the flesh. In another study in India by [34] however, the concentration of heavy metals in the tissue was generally more than that of the shell concentration. This is not the trend in this research work. Some heavy metals like copper, zinc and manganese had higher values in flesh samples while the others had higher values in shell samples. A closer look at the zinc concentration for both flesh samples revealed that very high values are recorded while the values of shell samples recorded were within the threshold limit of 15 $mgkg^{-1}$[35]. This high values might be as a result of brass production that is common in the Eastern parts of the country [36]. Except for the shell from Osogbo market, all other locations both for shell and flesh recorded no significant value of cobalt (Figure 4 and 12). Although, the study of [37], did not outrightly conform with this, but they also recorded very low cobalt values. This is also the trend noticed in nickel for all the flesh samples (Figure 14). Also, samples from Iheagwa-Owerri, Imo state showed the highest value of magnesium point with the flesh which may be an indication of excessive pollution due to welding activities [38,39]. The permissible levels of lead and cadmium i.e. 0.1 $mgkg^{-1}$and 0.06$mgkg^{-1}$ (Singh, et al., 2011) were exceeded in majority of both the shell and flesh samples. It is not confusing that most of the values collected for both shell and flesh samples displayed that there is no significant variations between the values between groups of shell and flesh (Table 2). This is because it is expected that if there are accumulations, the flesh samples should have higher concentrations than the shell. Also, there is no correlation between all the values (Table 3). This implies that increase in one doesn't either increase or decrease in the other. However, some studies of the shell material have also been conducted and many authors suggest that shells can provide a more accurate indication of environmental change and pollution; they exhibit less variability than the living organism's tissue and they provide a historical record of metal content throughout the organism's life time [16].

CONCLUSION

There is growing concern on effects of heavy metals on human health and hence this has led to increase in research upon the flora and fauna upon which humans feed. The results of this study have confirmed that:

1. The concentration of heavy metals in flesh samples were higher than in shell samples for copper, zinc, manganese, lead, and cadmium. This implies that the aforementioned heavy metals have bioaccumulate in the snails' flesh while for other heavy metals concentration for shell samples were higher.
2. The highest concentration was found in shell samples from Effurun market, Warri, Delta. This might be as a result of activities taking place in that part of the country. It is to be noted that although, iron is very essential but at this very high concentration it is toxic.
3. There are significant variations between shell and flesh samples. This implies that there is a wide gap between the values recorded for shell and flesh samples.
4. There is no significant relationship between all samples collected.

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