IAA Journal of Biological Sciences 8(1):74-84, 2022. ISSN: 2636-7254
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Evalution of Heavy Metal Concentration in Snails' Flesh Samples of *Archachatina marginata* and *Achatina fulica*

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ABSTRACT

The bioaccumulation of heavy metals copper, iron, zinc, cobalt, manganese, nickel, cadmium and lead were determined in the 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 level for flesh in all locations exceeded the threshold limit (0.06 $mgkg^{-1}$). Samples from Abak market, Akwa-Ibom state had the highest iron level (25.76 $mgkg^{-1}$). Some heavy metals like copper, zinc, and manganese had higher values in flesh. Zinc concentration was recorded very high in flesh. Cobalt was not found in 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 0.06$mgkg^{-1}$) were exceeded in majority of the flesh analysed. Samples from Iheagwa-Owerri, Imo state showed the highest value of manganese in the flesh which indicates exceeding pollution due to welding activities. The concentration of heavy metals in flesh samples were high. This implies that the aforementioned heavy metals have bioaccumulate in the snails' flesh.

Keywords: Heavy Metal, Concentration, Snails, Flesh, *Archachatina marginata* and *Achatina fulica.*

INTRODUCTION

Snails are widely consumed by most of the ethnic groups in Nigeria and rejected by few due to ethnic or traditional belief [1,2]. Snail farming is yet to become popular in Nigeria, most snails consumed are usually collected in forests and transported to nearby markets. The rapid industrialization and other technological activities within the last 20 – 30 years have resulted in heavy pollution of the environment [3,4]. Heavy metals contained in the soil find their way into organisms of various trophic levels through detritivores or plants. Although, their accumulation in predatory vertebrates has been confirmed [5], the levels of accumulation for vertebrates do not depend directly on the trophic level or the body size [6,7,8]. The metal level is believed to probably be associated with the physiological properties of the species rather than with the trophic level [9,10]. Terrestrial snails found in the trophic region of Nigeria with the scientific nomenclature *Archachatina marginata, Achatina fulica* are air breathing pulmate gastropods of the phylum molluska [11]. Snails are among the gastropod that experience "Torsion" (anatomic twisting or rotation of the visceral mass, mantle and shell to 180°, thus bringing the mantle cavity and anus to anterior position above the head) [12]. During snail's early growth or development in life, it is protected by the shell that is a form of exoskeleton for protection from predators, sun, mechanical damage and muscle attachment [13]. Consequently, snails deposit its excretory products inside the looped shell due to the torsion unlike how it was released outside during the early growth before torsion [14,15,16]. The shell of the snails like other gastropods is typically made of calcium carbonate which is secreted by the snail's body part known as

mantle [17]. They are known to grow up to 20 cm long and can live to about 10 years. Terrestrial snails are hermaphrodites that hibernate during dry season or drought by covering its body with a dry mucus layer called epiphragm and may estivate during rainy season [18,19]. Snails that are unable to hibernate during an unfavourable condition can die or its species may even go extinct [20]."Congo meat" as it is popularly called is a delicacy in high demand in Southern Nigeria and in some West African States because of its rich in protein and iron [11]. Little value was attached to snail before now as a good source of proteinous meat, until it was discovered to have low cholesterol [9].The low fat and cholesterol content makes snail meat good for use as an antidote for vascular diseases, such as heart attack, cardiac arrest, stroke, and hypertension including whooping cough. Other uses include, elongation of life expectancy, increase in sperm count and testicles size as well as being useful as anti-ageing creams. Mineral composition in other meats like beef, broiler, goat meat, pork, etc have been found to be lower than its occurrence in snails and as such recommended for reduction of constipation, labour pain, blood loss and for diabetic treatment [9].

OBJECTIVE OF STUDY

The objective of this study is to evalute Heavy Metal Concentration in Snails'

MATERIALS AND METHODS

AREA OF SAMPLING

Flesh Samples

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 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 disposal.

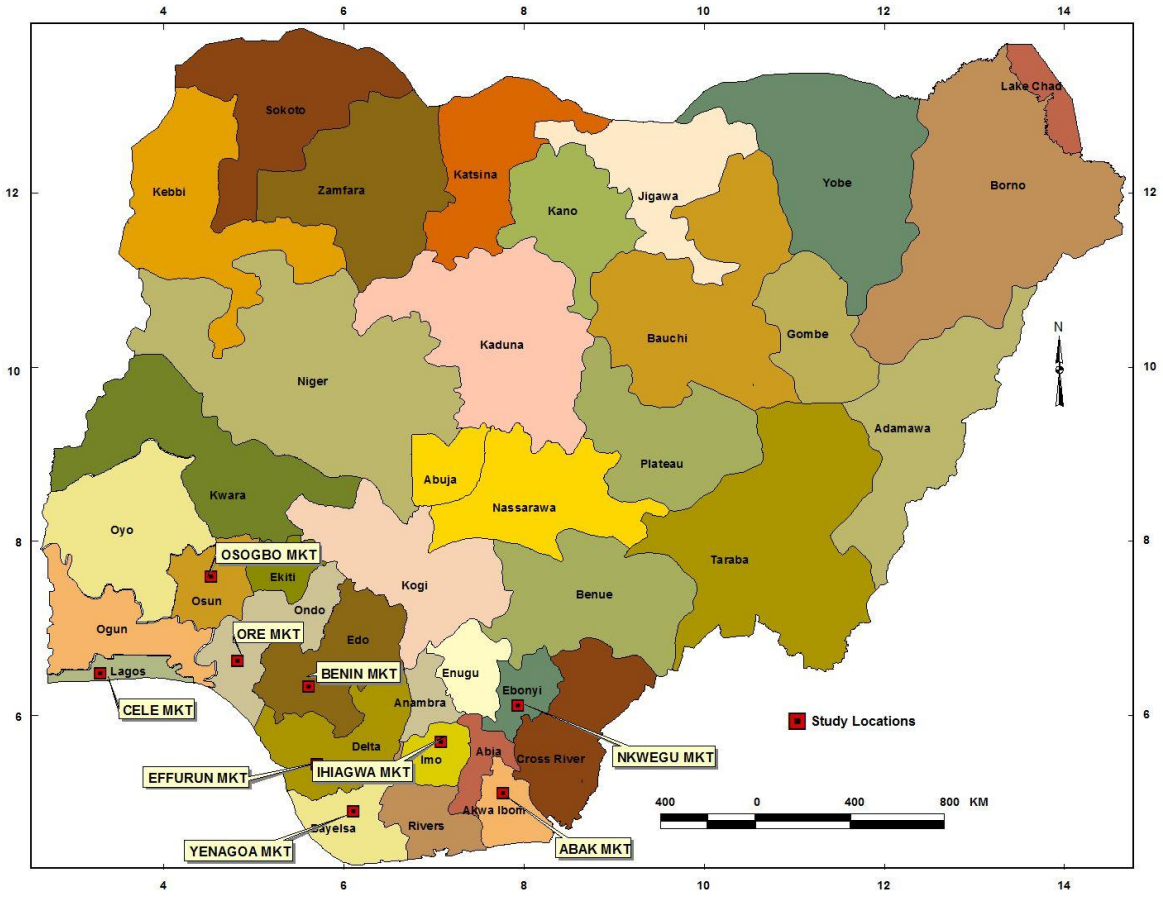

Plate 1 GPS map of Nigeria showing the areas in Southern Nigeria where samples were purchased.

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 13.

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 completelyclear.

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 AtomicAbsorption 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

HEAVY METAL CONCENTRATION IN SNAILS' FLESH SAMPLES

Mean values of Copper obtained in the flesh samples are presented in Figure 1. 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 2 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 $mgkg^{-1}$. All locations flesh samples gave no significant value with respect to Cobalt and Nickel concentration as presented on Figure 4 and 6 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 5.

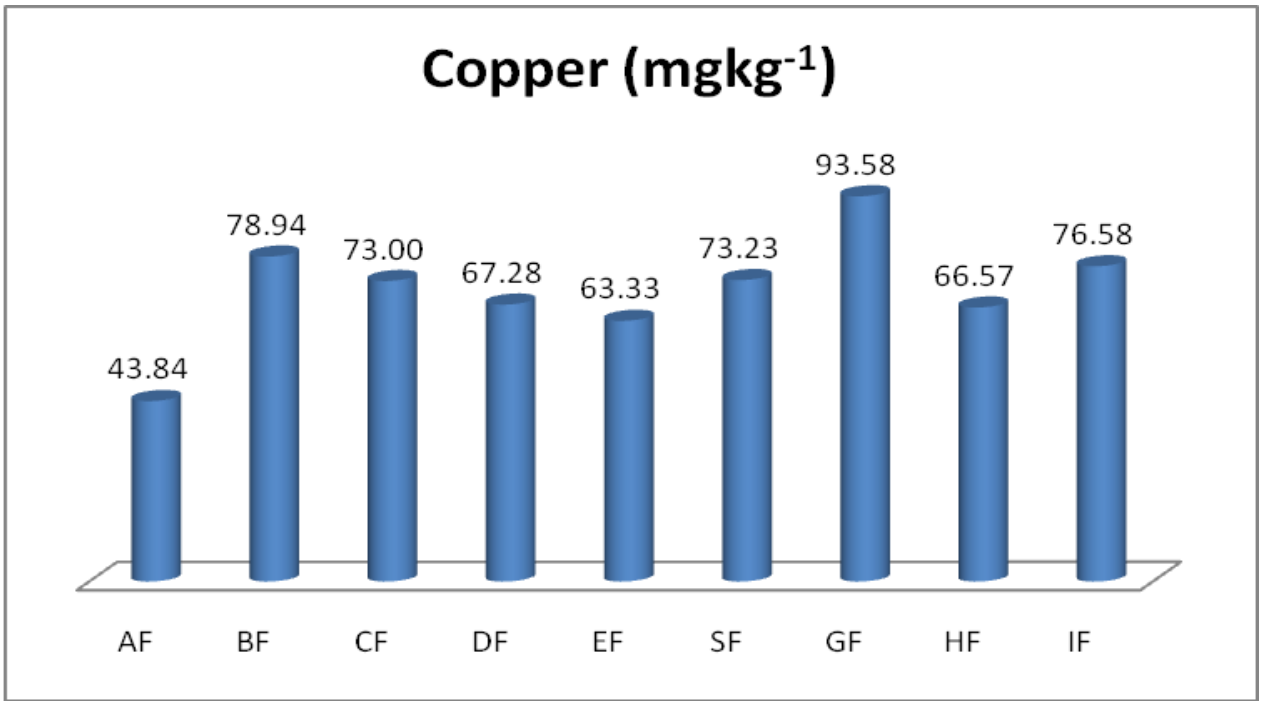

Figure 1: Mean concentration of Copper in snail flesh

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

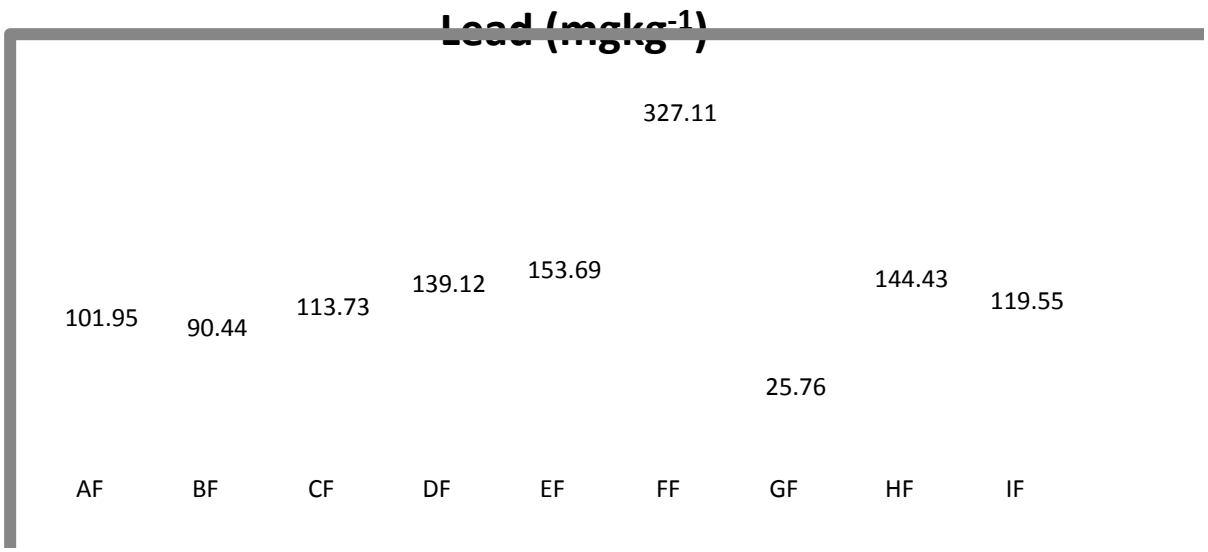

Figure 2: Mean concentration of Iron in snail flesh
Legend
AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo
DF – Effurun market, Warri, Delta
EF – Osogbo market, Osun
FF – Yenegoa market, Bayelsa
GF – Abak market, Akwa-Ibom
HF – Nkwoegu market, Ebonyi
IF – Benin by pass market, Edo

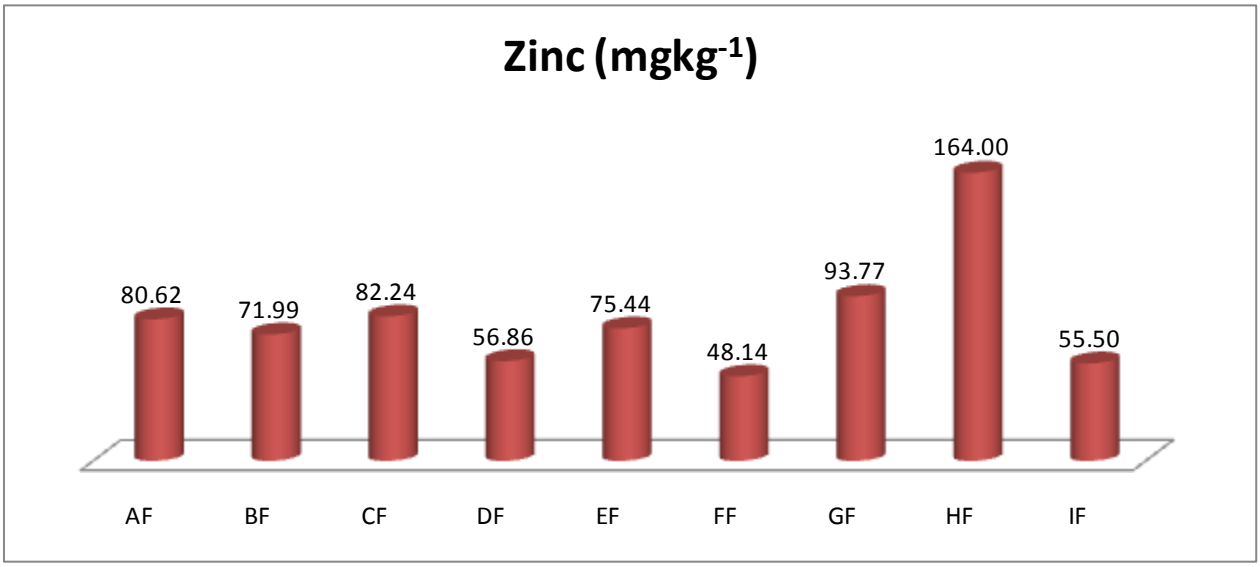

Figure 3: Mean concentration of Zinc in snail flesh
Legend
AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo

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

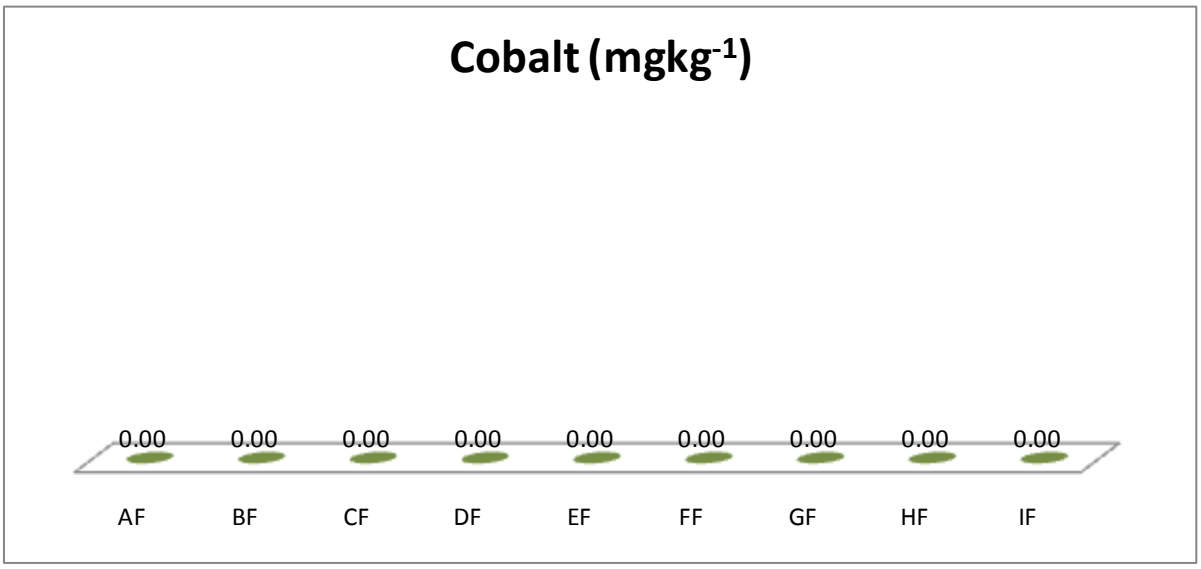

Figure 4: Mean concentration of Cobalt in snail flesh
Legend
AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo
DF – Effurun market, Warri, Delta
EF – Osogbo market, Osun
FF – Yenegoa market, Bayelsa
GF – Abak market, Akwa-Ibom
HF – Nkwoegu market, Ebonyi
IF – Benin by pass market, Edo

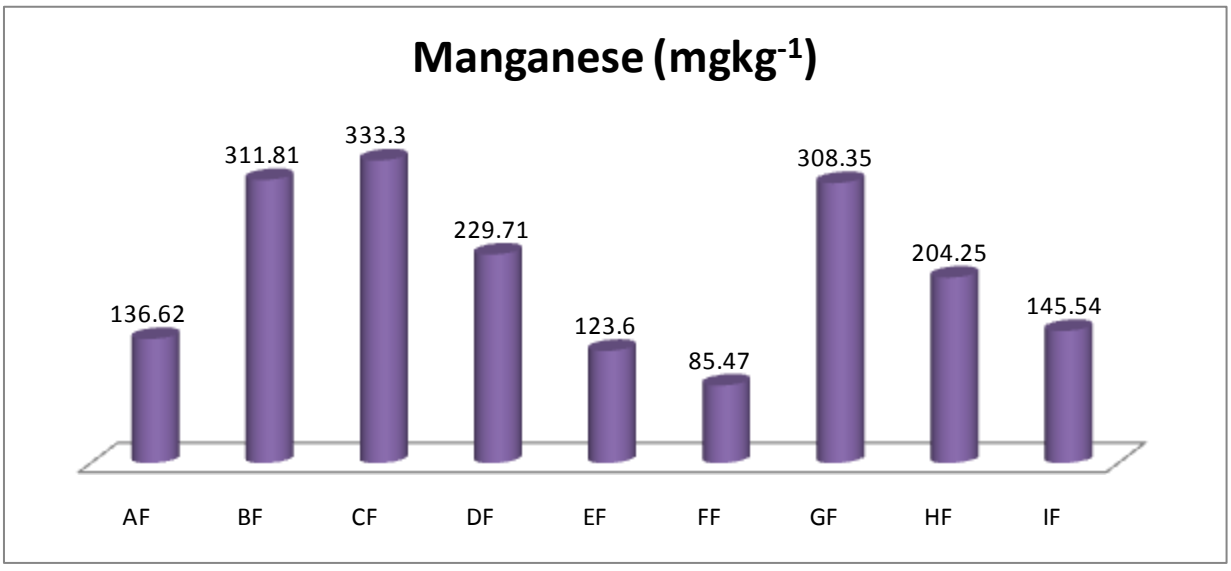

Figure 5: Mean concentration of Manganese in snail flesh

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

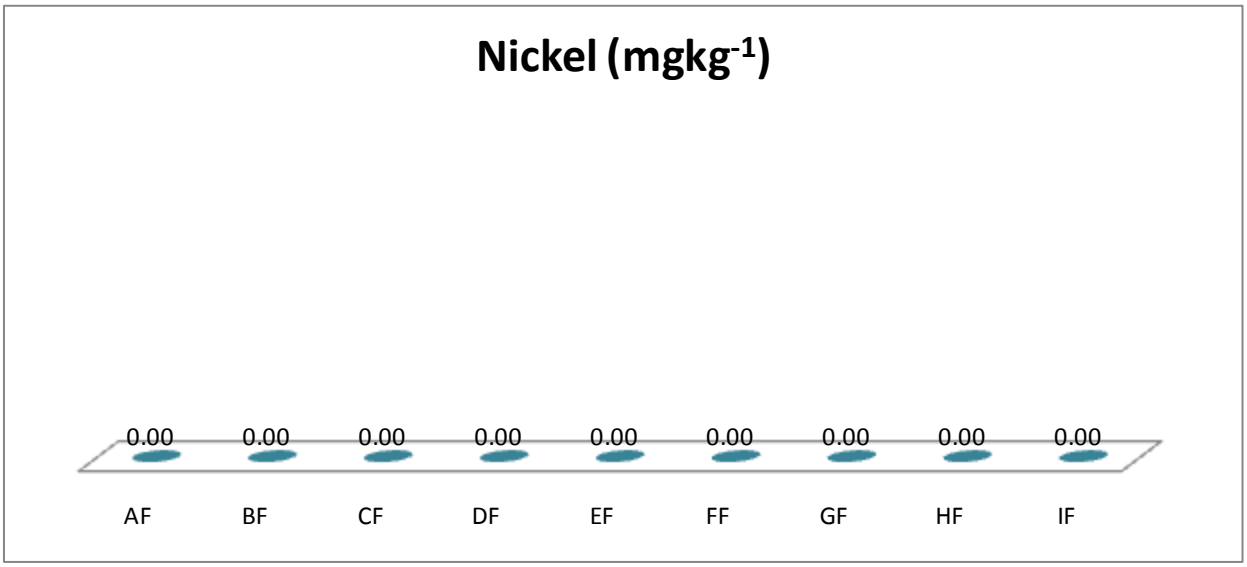

Figure 6: Mean concentration of Nickel in snail flesh

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

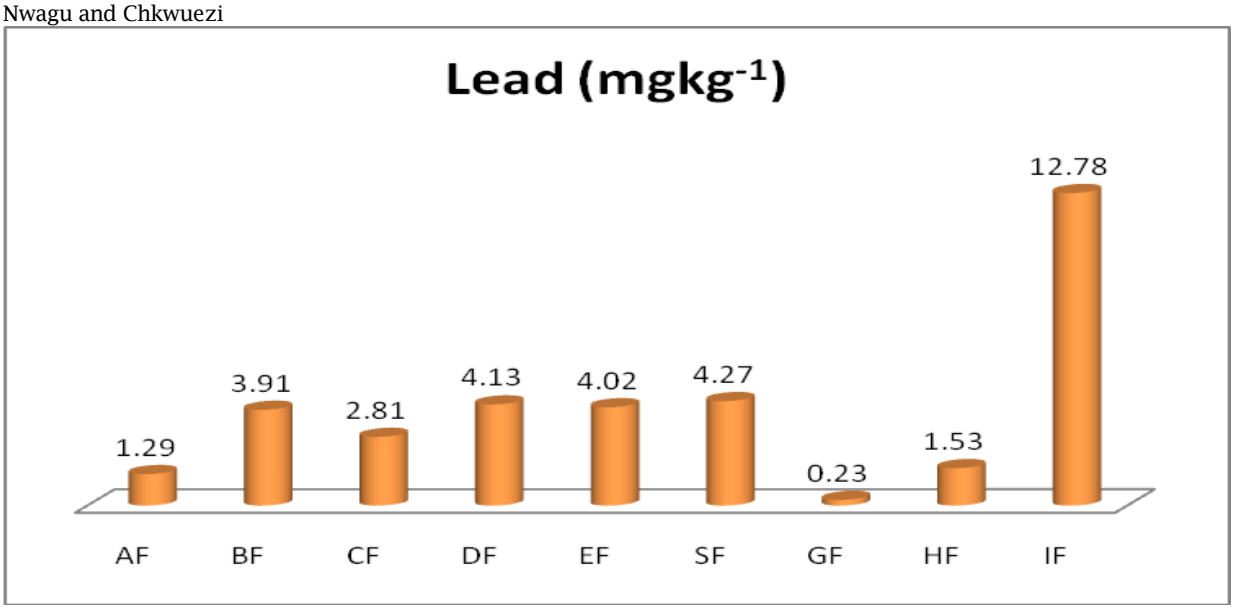

Figure 7: Mean concentration of Lead in snail flesh
Legend
AF - Cele Market, Lagos
BF – Ore market, Ondo
CF – Iheagwa-Owerri, Imo
DF – Effurun market, Warri, Delta
EF – Osogbo market, Osun
FF – Yenegoa market, Bayelsa
GF – Abak market, Akwa-Ibom
HF – Nkwoegu market, Ebonyi
IF – Benin by pass market, Edo

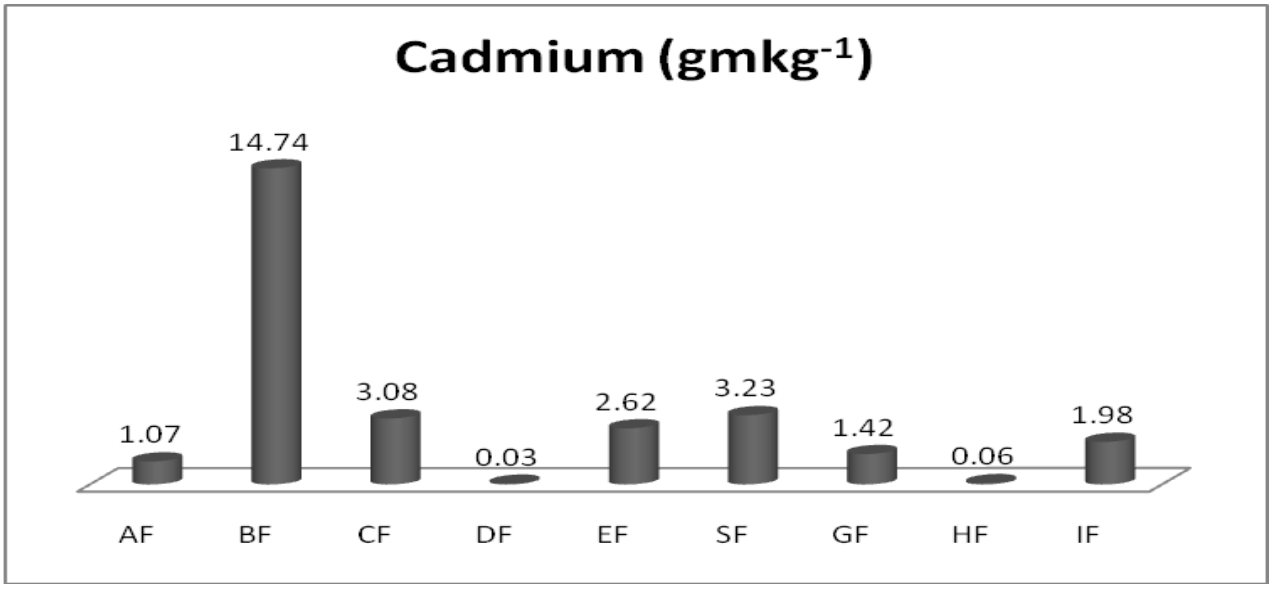

Figure 8:Mean concentration of Cadmium in snail flesh
Legend
AF - Cele Market, Lagos

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

DISCUSSION

The values of copper recorded for 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$mgkg^{-1}day^{-1}$[12]. 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 [8,9]. 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 flesh samples, Akwa-Ibom yielded 25.76 $mgkg^{-1}$ in flesh sample. This trend of high iron concentration was also reported by [15,18]. 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 [8,12,14].

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:The concentration of heavy metals in flesh samples were high. This implies that the aforementioned heavy metals have bioaccumulate in the snails' flesh.

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