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Assessment of Non-carcinogenic Human Health Risk of some Heavy Metals in Land Snails commonly consumed in Bayelsa State, Nigeria

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Abstract

The body burden of Pb, Cu, Zn and Fe in two edible species of land snails (*A. achatina*and *L. flammea*) obtained from the wild in Bayelsa State Nigeria and the non-carcinogenic health risk consequent upon the consumption of these snails was investigated in this study. The concentrations (mg/kg) on dry weight basis of the metals were determined using flame atomic absorption spectrometer, FAAS (GBC Avanta Ver 2.02 model) and found to be ($\bar{x}$ ± SD)Pb (29.5 ± 4.42), Cu (33.8 ± 6.25), Zn (75.3 ± 4.87) and Fe (2358.3 ± 35.67) for *A. achatina* and Pb (8.0 ± 082), Cu (23.7 ± 2.62), Zn (138.7 ± 4.49) and Fe (900 ± 303.56) for *L. flammea*. The target hazard quotients (THQs) and hazard index (HI) for evaluating the non-carcinogenic health risk was obtained from the individual and combined heavy metals due to dietary intake. THQ for the individual metals in *A. achatina* and*L. flammea* are in the decreasing order of Fe > Cu > Zn > Pb with risk value of 1.39, 0.34, 0.10, 0.00 and 0.53, 0.24, 0.19 and 0.00 respectively. Fe is the major contributor of the risk value which accounted for 55 - 75% of the THQs. The hazard index (HI) value obtained for *A. achatina* and *L. flemmea* were 1.83 and 0.96 respectively. The HI values indicates the presence of potential adverse health risk through the consumption of these two species of land snails. Therefore, moderate consumption of *A. achatina* and *L. flemmea* may be advisable considering the bio-accumulative nature of metals and to avoid human health risks to consumers in the future.

Keyword: Heavy Metals, Bioaccumulation, Biomonitoring, Land Snails, Human Health Risk, Target Hazard Quotient (THQ), Hazard Index (HI), *A. achatina, L. flemmea*.

Introduction

Land snails are important sources of animal protein for many coastal communities in the Niger Delta region of Nigeria. Many consumers in Nigeria obtain their snail from the conventional wildlife source[1].

Several authors[2-5] have reported the importance of mollusks (gastropods) as good indicator for monitoring heavy metal pollution as well as the environment they live. Since they are filter feeders, they have the tendency to accumulate substantial amount of chemical elements or toxic compounds in their tissues and thus present a major dietary source of these elements or compounds to human. Therefore, consumption of different species of land snails may be an important route for human exposure to trace metals.

Despite the high concentrations of heavy metals in mollusks[6], several people continue to consume different species of mollusks as a major source of protein.

Heavy metals enter the human body by two major routes; namely inhalation (air) and ingestion (food and drinking water). It has been reported that some of these metals are commonly found naturally in food stuffs, fruits, vegetable and commercially available multivitamins products and have normal physiological regulatory function in the body[5,7]. At lower concentrations, some heavy metals (e.g. iron, copper, cobalt and manganese) are nutritionally essential to maintain the metabolism of the human body, but at higher concentration they have various toxic effects such as reduced growth and development, cancer, with major organ damage to the kidney, liver and lung; they can also cause damages to the nervous system and in extreme cases death[8]. Metals such as mercury, cadmium, arsenic and lead have no beneficial effects on human health but are omnipresent in nature, so that some level of exposure is not readily preventable. Among these heavy metals only lead, copper, zinc and iron were investigated in this study.

Theintent of this paper is to report the concentration and non-carcinogenic health risk of Pb, Cu, Zn and Fe in two land snails (*A. achatina and L. flemmea*) obtained from Bayelsa State, Nigeria.

Material and Methods

*Site Description***:** Bayelsa State with an area of about 21110 square kilometers is located within Latitude $04^{O}15''$ North, 05^{O} $23''$South and Longitude 05^{O} $22''$ West arid 06^{O} $45''$ East. The State lies in the tropical rain forest belt and have the heaviest rainfall in Nigeria with a short dry season (from November to March).The State shares boundaries with Delta State in the

North, Rivers State in the East and the Atlantic Ocean in the West and South. More than three quarters of the state is covered by water with a moderately low land stretching from Ekeremor to Nembe. The major occupations in the State are fishing, farming, palm wine tapping, local gin, trading, carving and weaving. However, Bayelsa State is major oil and gas producing area and it contribute over 30% of Nigeria's oil production. There are hundreds of oil wells and flow Station across the State.

Sample Collection and Preparation: The land snails:A. achatina and L. flemmea where purchased from the Swali Market in the state capital, Yenagoa City. The whole soft tissue which is the edible part of the land snails were obtained by cracking the shells. The tissue were thoroughly washed several times and rinsed with distilled water stored in polythene bags, labeled accordingly and kept in the freezer.

In the laboratory, samples were thawed and oven dried at $105^{O}C$ to constant weight. The oven-dried samples were ground with a manual grinder (Oronal 3D Landers) and sieved with 0.15 mm mesh size to obtain uniform particle size.

Sample Digestion: 0.5gram sample was weighed into conical flash and digested with 10ml of 3:1 (v/v) conc. HCl/ HNO_3 (aqua regia), followed by addition of 1ml $HClO_4$ to the mixture. The mixture was placed on a hot plate and heated to near dryness. The digest was removed from the hot plate, allowed to cool to room temperature and diluted with 25ml distilled water and filtered [9-10].

The concentrations of Pb, Cu, Zn and Fe were analyzed by flame atomic absorption Spectrophotometer, FAAS (GBC Avanta Ver 2.02 model). The fuel used was acetylene gas, while the oxidant is compressed air. Concentration of the metals were calculated with reference to a standard curve and calibration of the instrument was done by preparing standard solutions of the metals of interest by diluting their certified reference material (CRM) with samples to determine the accuracy of the method.Samples were analyzed in triplicate.

Human Health Risk Assessment for Land Snail Consumption: The human health risks assessment was prepared using estimated dietary intakes (EDI/EWI), target hazard quotient (THQ) and hazard index (HI).

Estimation of Dietary intake: The estimated daily intakes (EDI) of Pb, Cu, Zn and Fe from consumption of land snails (A. achatina and L. flemmea were assessed using the formula[11].

$$\text{EDI}(\text{mg/kg} - \text{bw/day/week}) = \frac{\text{MI}_s \text{x} \text{CM}_s}{\text{BW}}$$

Where MI_s = mass of snail ingested per day; CM_S = concentration of metal in snail; BW = body weight (60kg for adult). The per capital consumption of fish and shellfish in Nigeria for human food is 9.0kg[12], which is equivalent to 24.7kg per day.

Target Hazard Quotient (THQ): Target hazard quotient (THQ) is calculated by formulation established by United States Environmental Protection Agency, Region III Risk-Based concentration [13,14]. The equation used for estimating THQ was:

$$\text{THQ} = \frac{\text{EF x ED x MI x CM}}{\text{ORD x BW x AT}} \text{x} 10^{-3}$$

Where; THQ is the target hazard quotient, EF = exposure frequency (365 days/year); ED is the exposure duration (51.86 years)[15], which corresponded to average life expectancy of a Nigerian; AT = average exposure time for non-carcinogens (365 days/year x ED). The oral reference dose (ORD) is an estimate of daily exposure to human population (including sensitive sub-group) that is likely to be without an appreciable risk of deleterious effect during life time. The oral reference dose (ORD) (mg/kg/day) used were Pb(1.5), Cu (0.04), Zn (0.3), Fe (0.7)[13]. 10^{-3} is the unit conversion factor.

Hazard Index (HI): The hazard index (HI) from the consumption of land snails (A. achatina and L. flemmea) obtained from Bayelsa State was calculated as the sum of THQs of all the metals in the snail samples[16] and was expressed as follows;

$HI = THQ_{Pb} + THQ_{Cu} + THQ_{Zn} + THQ_{Fe}$

Where HI is the hazard index; THQ_{Pb} = the target hazard quotient for Pb intake THQ_{Cu} = the target hazard quotient for Cu intake THQ_{Zn} = the target hazard quotient for Zn intake; and THQ_{Fe} = the target hazard quotient for Fe intake respectively.

Acceptable Risk Distribution: THQ is the ratio between the exposure and the reference dose (RFD) and it is used to express the risk of non-carcinogenic effects. Ratio of less than 1 signifies non-obvious risk. Conversely, an exposed population of concern will experience health risk if the dose is equal to or greater than the RFD[9].

Results and Discussion

Total Heavy Metals Concentrations in Land Snails: The concentrations of Pb, Cu, Zn and Fe in land snails (*A. achatina* and *L. flemmea*) obtained from Bayelsa State of the Niger Delta Region of Nigeria are represented in table-1.

The data showed that, the concentration of Pb in *A. achatina* ranged from 23.5 – 34.0mg/kg with a mean and standard deviation of 29.5 ± 4.42mg/kg; concentration of Cu ranged from 25 – 38.5 (mean ± SD: 33.8 + 6.25mg/kg), Zn level ranged from 68.5 – 79.5mg/kg with a mean and standard deviation of 75.3 ± 4.87 and Fe concentration ranged from 2310 – 2395 (mean ± SD: 2358.3 ± 35.67mg/kg) respectively. Fe has the highest mean value of 2358 mg/kg while Pb has the lowest mean value of 29.5mg/kg in *A. achatina.* While for *L. flemmea*, the

concentrations of the metals are as follows; Pb value ranged from 7.0 – 9.0 and mean ± SD of 8.0 ± 0.82 mg/kg, Cu value ranged from 20 – 26 mg/kg (with mean ± SD of 23.7 ± 2.62 mg/kg), Zn value ranged from 635 – 1325mg/kg (with mean ± SD: 900 ± 303.56 mg/kg) respectively. Also, for *L. flemmea* Fe has the highest mean concentration, while Pb showed the lowest mean concentration.

Table-1
Total Heavy metals Concentrations (mg/kg dry weight) of *A. achatina* (giant land snails) and *L. flemmea* (garden snail) obtained from Bayelsa State, Nigeria

Heavy Metals	Statistics	Land Snails	
		A. achatina	***L. flammea***
Pb	Range	23.5 -34.0	7.0 – 9.0
	Mean ± SD	29.5 ± 4.42	8.0 ± 0.82
Cu	Range	25.0 – 38.5	20.0 – 26.0
	Mean ± SD	33.8 ± 6.25	23.7 ± 2.62
Zn	Range	68.5 – 79.5	135 – 145
	Mean ± SD	75.3 ± 4.87	138.7 ± 4.49
Fe	Range	2310 – 2395	635 – 1325
	Mean ± SD	2358.3 ± 35.67	900 ± 303.56

Therefore, the mean total heavy metals concentrations in the snail samples are in the decreasing order of Fe > Zn > Cu > Pb respectively. This suggests the difference in the bioavailability of metals to terrestrial organisms. Among the heavy metals studied, Fe showed the highest level of accumulation in the snail samples. A similar situation was observed in studied by Osakwe *et al*[18] and Ijeomah *et al*[19]. The reason is that, the non-vertebrate fauna species have high affinity for Fe, Zn and Cu intake. This could be attributed to the role these heavy metals play as essential elements for the organisms. According to Chien[22], Fe, Zn and Cu are integral parts of important physiological compounds such as enzymes and protein, especially metalloenzymes which as a Co factors of larger number of enzymes. These metals also function in replacement of cells, and activation of a low immune system. However excess of these metals in the human systems are known to cause adverse health effects ranging from reduced immune body function to series of illness, they cause damages in major organs in the body (lung, liver, kidney, nervous system etc.) and in extreme causes it can lead to death[20,21].

The concentration of heavy metals in the land snail obtained from this study was compared to FAO/WHO, FEPA and international standards as shown in table-2. The data showed that, the concentration of the metals in the two species of land snails investigated in this study were higher than the limits set by WHO/FEPA and international criterion. Since Pb has no beneficial role in the human system[22,23], the high level of lead found in snails from this study should be a source of concern to regulatory agencies and consumers. These results lead us to evaluate the non-carcinogenic health risk of Pb, Cu, Zn and Fe in the two land snails (*A. achatina and L. flemmea*) obtained from Bayelsa State, Nigeria.

Human Health Risk in Land Snails (A. achatina and L. flammea): The human health risk assessment models which includes carcinogenic and non-carcinogenic risk proposed by the United States Environmental Protection Agency (USEPA) have proved successful and has been adopted worldwide. In Nigeria at the moment, there are no approved limits for acceptable maximum carcinogenic and non-carcinogenic risk levels. Therefore, the USEPA model was employed in this study. The threshold values set by WHO and USEPA were used to assess the potential human health risks posed by heavy metals (Pb, Cu, Zn and Fe) in the consumption of land snails (*A. achatina* and *L. flammea*) obtained from Bayelsa State, Nigeria.

Table-2
Mean metals concentrations(mg/kg) in Land snails of Bayelsa State in comparison with the limits set by other regulatory bodies for heavy metals

Samples	Heavy Metals				Reference
	Pb	**Cu**	**Zn**	**Fe**	
A. achatina	29.50	33.80	75.30	2358.8	This study
L. flammea	8.00	23.70	138.70	900	This study
FAO/WHO	2.00	3.0	10–75.00	0.5	9
FEPA	2.00	1–3.00	75.00	2.00	27
International criterion	0.1	15	60	0.3	10

Food products having toxic metals could present a toxic hazard for the consumer and that is dependent on the metal concentration in the food and the amount of food consumed. Hazard consists of determining the toxicological properties related to a specific substance[23]. The tolerable intake is widely used to describe safe levels of intake of a toxicant and can be expressed as the Estimated Daily Intake (EDI) or the Estimated Weekly Intake (EWI). The EDI and EWI are set by the Food and Agriculture Organization/ World Health Organization (FAO/WHO) Joint Expert Committee on Food Additives (JECFA). The EDI and EWI are the maximum amount of a contaminant to which a person can be exposed per day/ week over a lifetime without and unacceptable risk of health effects.

While the THQ is a ratio of determined dose of a pollutant or contaminant to a reference dose level. The interpretation of the THQ value is binary: THQ is either ≥ 1 or < 1, where THQ > 1 indicates a reason for health concern. The hazard index (HI) in this study is treated as the arithmetic sum of the individual metal THQ values derived by the method of Chien *et al*[22]. Because it has been reported that exposure to two or more pollutant may result in additive and/ or interactive effects [22]. The results of the non-carcinogenic (hazard quotient) risks or the heavy metals through edible tissue exposure route are shown in table-3.

The provisional daily/ weekly intakes of the metals are in the decreasing order of Fe > Zn > Cu > Pb. While the target hazard quotient of the metals are in the decreasing order of Fe > Cu > Zn > Pb and their risk values are 1.39, 0.34, 0.10 and 0.00. The diet pathway which accounted for 95% was the dominant exposure route of all the metals to the local consumers. The THQ values obtained from this study due to this primary exposure route for the heavy metals investigated were less than 1, except Fe which was equal to 1. While the total non-carcinogenic hazard index (HI) for the consumption of *A. achatina* (giant snail) is equals to 1. This indicates no potential health risk hazard to the exposed population at the moment. However, due to bio-accumulative nature of these metals in the human body, moderate amount of intake of this snail is advisable.

The non-carcinogenic risk of four heavy metals exposure to consumption of *L. flammea* from Bayelsa State are presented in table 4. The provisional daily and weekly intakes (EDI/EWI) for the metals are in the decreasing order of Fe > Zn > Cu > Pb respectively. The Target Hazard Quotient of the individual metal are in the decreasing order of Fe > Cu > Zn > Pb with risk values of 0.53, 0.24, 0.19 and 0.00 respectively. While the total non-carcinogenic hazard index (HI) for *L. flammea* is 0.96, this value is less than the threshold limit of 1 recommended by United State Environmental Protection Agency and FAO/ WHO.

Table-3
Estimated Dietary Intake (mg/kg/ bw/ day/week), Targets Hazard Quotient (THQ), and Hazard Index (HI) for the intake of Pb, Cu, Zn and Fe in *A. achatina*

Heavy Metals	Risk Model			Percent contribution of each metal to THQ
	EDI	EWI	THQ	
Pb	12.44	87.08	0.00	0.00
Cu	13.91	97.37	0.34	18.58
Zn	30.99	216.93	0.10	5.46
Fe	970.83	6795.81	1.39	75.96
HI	-	-	1.83	-

Table-4
Estimated Dietary Intake (mg/kg/bw/day/week), Target Hazard Quotient (THQ), and Hazard Index (HI) for the intake of Pb, cu, Zn and Fe in *L. flammea* (garden snail)

Heavy Metals	Risk Model			Percentcontribution of each metal to THQ
	EDI	EWI	THQ	
Pb	3.29	23.03	0.00	0.00
Cu	9.76	68.32	0.24	25.00
Zn	57.10	399.7	0.19	19.79
Fe	370.50	2593.5	0.53	55.21
HI	-	-	0.96	-

The highest THQ value in both *A. achatina* and *L. flammea* belong to Fe and was higher than comparable values for Cu, Zn and Pb. This research found out that Fe was a major risk contributor for the general population exposed to these snails, accounting up to 55 – 75% of the total THQ. However, the THQ of each metal studied in this research is generally less than 1, this implies that the consumers would not experience significant health risk from the consumption of individual metals through contaminated snails (*A. achatina* and *L. flammea*) from Bayelsa State.

Conclusion

The concentrations of the metals (Pb, Cu, Zn and Fe) in *A. achatina* (giant land snail) and *L. flammea* (garden snail) from Bayelsa State were all higher than the limits set by WHO [24-26] and FEPA[27], except Zn. This call for concern considering the fact that these metal especially Pb are toxic and their accumulation may lead to serious health issues. Among the metals studied Fe showed the highest level of accumulation in the two snail samples. While the non-carcinogenic risk (THQs) conducted in this study showed that adverse health effects may not occur when considering the individual metals exposed through the snail samples by consumer. However, considering the bio-accumulative nature of the metals, moderate amount of intake of *A. achatina* and *L. flammea* is strongly recommended to avoid adverse health risk to consumers in future.

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