



## Fractionation of Fe, Zn, Cu and Health Risk Appraisal in two Terrestrial and Aquatic Snails Commonly Consumed in Nigeria

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### ABSTRACT

This study consist of chemical fractionation of Fe, Cu and Zn in two commonly consumed terrestrial and aquatic snails, and appraised the risk associated via consumption. The concentrations of the metals in each fraction were analyzed using Flame Atomic Absorption Spectrometer. Results showed that, Fe accumulated higher than Cu and Zn in all the snails. Fe levels were highest in the residual fraction and the values were higher than standard maximum limits of M.A.F.F. and W.H.O./F.A.O. except I.A.E.A., while Cu and Zn levels were below their limits. Risk appraisal study showed that, Fe was a major risk contributor to the H.I. values, accounting over 70%. The study also revealed that, a daily consumption of  $0.025 \text{ mg kg}^{-1} - \text{bw day}^{-1}$  of Fe, Cu and Zn via these snails will not pose non-carcinogenic risk to consumers for now but, in light of metal toxicity and its bio-accumulative nature, moderate intake of these snails is advisable.

**Keywords:** Fractionation, Heavy Metals, Risk Appraisal, Terrestrial and Aquatic Snails.

### INTRODUCTION

Fractionation is a separation process in which analyte(s) of a given matrix are partitioned into smaller fraction according to size, density, solubility, bonding or reactivity<sup>1</sup>. Basically, fractionation studies focus on the chemical nature of the species under investigation which include solubility, affinity, charge, hydrophobicity/hydrophilic, and in some cases, physical separation such as filtration and size-exclusion chromatography may also be involved<sup>2</sup>.

Fractionation can also be viewed as additional methods of speciation analysis. For

instance, a fractionation of Copper in an unpolluted ecosystem can be investigated to ascertain the different chemical forms of copper which could be organic or inorganic. Also, discrete analysis may also be carried out to determine the inorganic fraction by identifying individual inorganic species of Cu in a given sample. However, there are two basic approaches in fractionation techniques. These include, "physical fractionation where the separation of the analyte is based on particle size and density<sup>3</sup> and "chemical fractionation which focused on selective dissolution of the analyte(s) or contaminants in different reagents<sup>2</sup>. Krauss *et al.*,<sup>3</sup> studied the distribution of heavy metals in different



particle sizes and density fraction of municipal refuse. Their study revealed that, the fine dense fractions had the highest heavy metal content and coarse light fraction contained the least amount of each heavy metal.

According to Okoro *et al.*,<sup>2</sup> the environmental fate of metals, that is, their toxicity, bioavailability, mobility and rate of contamination in an organism are correlated with different forms or species of the metal than total concentration because, the former gives further information about the fundamental reaction that governs the behaviour of metals. Therefore, chemical fractionation schemes for partitioning heavy metals via sequential extraction procedures have been found to be the most effective method in evaluating individual fraction of heavy metals<sup>4</sup>. Again, these are based on three (3) to six (6) fractions or more, most of which are derived and modified from Tessier's scheme<sup>5-6</sup>. Basically, in chemical fractionation two different approaches are available; these include single extraction using one reagent only or sequential extraction using different chemical reagents<sup>7</sup>. However, the later approach was employed in this study.

Sequential extraction procedures (SEP) are functionally valued methods of fractionation which is commonly employed to assess metal mobility, bioavailability and toxicity in an environmental matrix<sup>8-10</sup>. The principal advantages of sequential extraction over single extraction procedure are as follows;

- Sequential extraction procedure improves the phased specificity.
- It provides vital knowledge for risk evaluation because, metal concentrations obtained using different chemical reagents can be determined.
- Also, it provides information on the diverse bonding strength of metals in liquid and solid phases and their potential reactivity under different ecological condition<sup>11</sup>.

The fundamental principle of sequential extraction procedures is based on the potency of the reagent or extractant to successfully dissolve the analyte(s) into its component form in a sequential order, liberating the analyte(s) within the matrix or sample without affecting other components<sup>12</sup>.

Generally, fractionation and speciation of heavy metals on environmental samples, provide insight on the elemental bioavailability, mobility and toxicity and this may present a better degree of heavy metal pollution compare to total metal concentration since, heavy metals exist in different chemical forms and only a fraction can be remobilized easily, the later provides no insight to elemental bioavailability and mobility<sup>13</sup>.

Terrestrial and aquatic snails are non-conventional important wildlife protein source. However, recent studies have shown that the consumption of snails has been on the increase due to its nutritional values, such as; high protein content, minerals and vitamins, which are required for normal tissue development and maintenance<sup>14</sup>. Snails are also reported to have low lipid content, saturated fatty acids and low cholesterol content, which help to prevent diabetes and heart related diseases<sup>15-16</sup>.

In Nigeria, edible snails are part of culinary delicacies and the demand has been on the increase. Snail (especially land snails) farming in cities is now gaining importance<sup>17</sup>. While in the rural communities, these snails are collected from the forest and water bodies<sup>18</sup>, and traditionally, they form a single item of animal protein in the diet of consumers<sup>19</sup>. The snails used in this study include; *Achatina achatina*, *Limicolaria flammea* commonly referred to as the giant land and garden snails, and *Pachymeria aurita*, *Tympanotous fuscatus* commonly known as marine periwinkles.

Several studies had revealed the importance of snails (gastropods) as a good bioindicator for monitoring heavy metals pollution in terrestrial and aquatic environments due to their mode of feeding<sup>15,20-22</sup>. Since these snails are filter-feeding organisms, the possibility of accumulating chemical elements or toxicants in their tissues is inevitable. Therefore, consumption of snails may be an important route for human exposure to toxic metals and this may pose risk to human health. In view of this, it is eminent to investigate the fractionation (chemical forms) of Fe, Zn, Cu in two terrestrial (*Achatina achatina*, *Limicolaria flammea*) and aquatic snails (*Pachymeria aurita*, *Tympanotous fuscatus*) and assess the health risks related via consumption of snails.

## MATERIAL AND METHODS

### Materials and Reagents

Terrestrial snails (*Achatina achatina*, *Limicolaria flammea*), aquatic snails (*Pachymeria aurita*, *Tympanotous fuscatus*), 250 mL conical flask, Weighing balance, 250 mL beakers, funnel, spatula, stirring rod, Whatman filter paper (No. 541), aluminium foil, measuring cylinder, hot plate, Flame Atomic Absorption Spectrophotometer (GBC avanta 2.02 model), distilled water, hexane, methanol/DCM (8:2), aqua regia (3: V/V Conc. HCl/HNO<sub>3</sub>) and HClO<sub>4</sub>.

### Sampling and Sample Preparation

Two commonly consumed terrestrial snail species (*Achatina achatina*, *Limicolaria flammea*) and aquatic snails (*Pachymeria aurita*, *Tympanotous fuscatus*) were bought from local farmers in Yenagoa main market, Bayelsa State, Nigeria. Immediately, after collection, the snails were rinsed with cold water, wrapped in bags, labelled accordingly. Upon preparation the snails' shells were cracked open to obtain the whole soft tissues (edible parts). The soft tissues were washed thoroughly with distilled water, oven dried at 105°C and ground to fine powder.

### Fractionation Analysis

Fractionation analyses were determined using three-stage sequential chemical extraction procedures<sup>4</sup>. The chemical extractants employed in this study were, water, hexane and methanol/dichloromethane (8:2), and these gave rise to;

- Water soluble fraction, WSF
- Non-polar (hexane) fraction (NHF)
- Polar (methanol) fraction, PMF

### Determination of Water Soluble Fraction

Approximately, 1.0 g of each snail biomass was weighed into extraction bottles and 15 mL of distilled water was added. The mixtures were placed on an electrical shaker for twenty four (24) hours. Thereafter, the mixtures were removed, filtered and the water soluble fractions (WSF) were obtained. Then, the residues were dried at room temperature for two days.

### Determination of Non-Polar/or Hexane Fraction

The dried residues of the WSF were put into extraction bottles, and 10 mL of hexane was added. All mixtures were placed on electrical shaker for

twenty-four (24) hours. The extraction bottles were removed, then, the mixtures were filtered and the Non-polar or hexane fractions (NHF) were obtained, and the residues were dried at room temperature for two days.

### Determination of Polar/or Methanol Fraction (PMF)

10 mL of methanol/dichloromethane (8:2) was added to the dried residues of NHF and placed on an electrical shaker for twenty four (24) hours. Thereafter, the mixtures were removed, filtered and methanol/or polar extracts were obtained. Again, the residues were dried at room temperature for two days.

### Digestion of Residual Fraction, (RSF)

10 mL of conc. HCl/HNO<sub>3</sub> was added into 250 mL beakers containing the dried residues of the polar (methanol) fractions (PMF), thereafter 1 ml of HClO<sub>4</sub> was also added. The mixtures were heated on a hot plate in a fume chamber until all the residues completely went into solution<sup>23</sup>. Then, the digests were allowed to cool, diluted to 25 mL with distilled water, stirred and filtered to obtain the residual fractions (RSF).

### Analysis of Fe, Cu, Zn in Each Fraction

The concentrations of Fe, Cu; Zn in each fraction were analysed by Flame Atomic Absorption Spectrophotometers, FAAS (GBC Avant aver 2.02 design).

### Quality Assurance/Control

Preparation of reagent blanks, triplicate samples, a certified reference material solutions provided by NRC, Canada and triplicate metals determination were some of the parameters used to check the quality assurance/control in this study. Also, Pearson correlation coefficient > 0.98 was considered acceptable. Again, percentage recoveries of the metals, Fe, Cu, Zn ranged from 90.67% - 99.60%, which indicates accuracy of result as well as good precision.

### Health Risk Appraisal

Health risk appraisements are the fastest growing methods in assessing the pollution standards of metals in the environment and its biota<sup>23,24-26</sup>, because they provide information emanating from metal presence in the environment, biota and other food products. Health risk appraisal methods may be subdivided into two; non-carcinogenic risk (THQ of the

individual metal and the combine hazard indices, HI of all the metals in a given matrix) and carcinogenic risk (TR)<sup>23</sup> as proposed by the United State Environmental Protection Agency (U.S.E.P.A.)<sup>27-29</sup>. However, this study investigated only the non-carcinogenic risk of Fe, Cu, Zn in land and marine snail's fractions. The methods employed to determine the non-carcinogenic risk of these metals were;

- The Estimated Dietary Intake (EDI) expressed mathematically as follows; EDI (mg kg<sup>-1</sup> bw-day<sup>-1</sup>) =  $\frac{MI \times MC(s)}{BW\alpha}$  (1)

EDI: This is the maximum amount of estimated dietary intake of a contaminant or toxicant in which a person can be exposed to per day over a life time without an unacceptable risk of health effects.

- The Target Hazard Quotient (THQ i.e the hazard quotient of the individual metals).

$$= \frac{EF \times ED \times MI(s) \times MC_s}{RfD \times BW\alpha \times ATn} \times 10^{-3} \quad (2)$$

THQ defines the relative magnitudes between exposure to a pollutant and the average reference dose of the pollutant.

- Hazard Index (HI). That is the combined or total sum of THQ effects of the individual metal in the snails. It is expressed as;

$$HI = THQ_{Fe} + THQ_{Cu} + THQ_{Zn} \quad (3)$$

HI: The interpretation of HI values is binary; HI is either > 1 or < 1. When HI is < 1, the exposed population is at safe limits but, when HI > 1, it indicates reason for concern because the exposed population is at risk<sup>28</sup>.

**Table 2: Levels of Fe, Cu, Zn (mg kg<sup>-1</sup> dry wt) in the WSF, NHF, PMF, and RSF of terrestrial and aquatic snails in comparison to standard limits of MAFF, FAO/WHO and IAEA**

Fractions	Snails	Iron (Fe)	Copper (Cu)	Zinc (Zn)
Water soluble fraction (WFS)	<i>Achatina achatina</i>	294.03 ± 18.85	1.75 ± 0.07	7.55 ± 2.22
	<i>Limicolaria flammea</i>	212.30 ± 56.16	1.75 ± 0.70	20.55 ± 1.75
	<i>Pachymeria aurita</i>	33.35 ± 32.80	8.60 ± 0.50	3.50 ± 0.82
	<i>Tympanotous fuscatus</i>	21.00 ± 4.24	5.40 ± 0.68	4.15 ± 0.62
Non-Polar (Hexane) fraction (NHF)	<i>Achatina achatina</i>	12.92 ± 0.06	3.27 ± 2.10	26.20 ± 2.10
	<i>Limicolaria flammea</i>	34.23 ± 0.51	5.63 ± 0.75	28.50 ± 0.41
	<i>Pachymeria aurita</i>	29.60 ± 4.52	2.80 ± 1.44	26.40 ± 0.99
	<i>Tympanotous fuscatus</i>	34.80 ± 2.41	8.30 ± 0.13	27.80 ± 1.80
Polar (methanol) fraction (PMF)	<i>Achatina achatina</i>	6.23 ± 3.45	0.63 ± 0.24	13.73 ± 63
	<i>Limicolaria flammea</i>	12.30 ± 3.91	0.70 ± 0.40	14.20 ± 0.90
	<i>Pachymeria aurita</i>	11.00 ± 6.30	0.97 ± 0.42	14.40 ± 0.90
	<i>Tympanotous fuscatus</i>	10.50 ± 5.60	0.93 ± 0.25	14.20 ± 1.03
Residual fraction (RSF)	<i>Achatina achatina</i>	2036.70 ± 0.91	26.25 ± 0.65	26.42 ± 0.31
	<i>Limicolaria flammea</i>	635.27 ± 0.34	13.53 ± 0.98	63.70 ± 0.12
	<i>Pachymeria aurita</i>	1078.68 ± 0.38	43.38 ± 2.43	14.30 ± 1.50
	<i>Tympanotous fuscatus</i>	1767.02 ± 1.43	16.85 ± 0.25	25.33 ± 0.12
MAFF <sup>32</sup>		-	20	50
FAO/WHO <sup>33</sup>		2	30	75
IAEA <sup>34</sup>		146	3.28	67.1

**Table 1: summary of risk assessment parameters**

Symbol	Description	Unit	Value(s)
MI <sub>s</sub>	Mass of the snail ingested	Kg day <sup>-1</sup>	0.025
MC <sub>s</sub>	Metal Concentration in the snails	mg kg <sup>-1</sup>	Presented in Table 2
EF	Exposure frequency	days year <sup>-1</sup>	365
WD	Exposure duration	Years	51.86
BW <sub>a</sub>	Body weight adult	Kg	60
RfD	Oral Reference Dose	mg kg <sup>-1</sup> -bw day <sup>-1</sup>	Fe=0.7 Cu=0.04 Zn=0.3
AT <sub>n</sub>	Average Time for non-carcinogens	Days	ED X EF=365X51.86=18928.9
10 <sup>-3</sup>	Is the unit conversion factor	-	-

The ingestion rate is derived from the annual consumption rate of fish, shellfish and meat products in towns and cities of Nigeria as proposed by F.A.O. which is averaged 9.0 kg and is equivalent to 0.025 kg per day<sup>30</sup>. Other parameters were derived from similar studies in Nigeria and U.S.E.P.A. risk based assessment Table.<sup>23,26,28,31</sup>.

## RESULT AND DISCUSSION

### Fe, Cu, Zn levels in each fraction of the snails

The mean levels of Fe, Cu, Zn in the snails in various fractions and their comparison to standard limits set by regulatory bodies are presented in Table 2 and Fig. 1, 2 and 3. The result showed that the metals concentrations were unevenly distributed among the snail species studied which depend on the availability of each metal in the snail's environment. Generally, it was observed that Fe accumulated higher concentrations in most of the snail fraction especially the residual fraction.

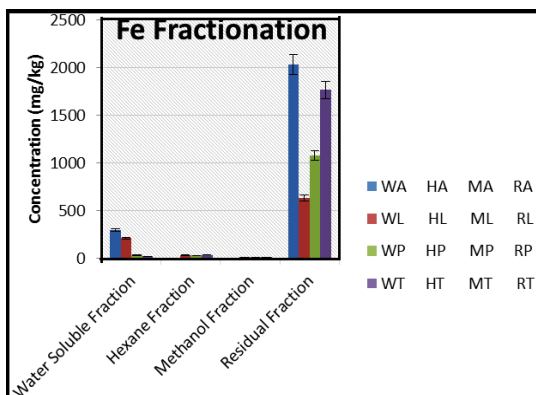


Fig. 1. Mean Concentrations of Iron in the Snail Samples (*Achatina achatina*, *Limicolaria flammea*, *Pachymeria aurita* and *Tympanotous fuscatus*) of different fractions

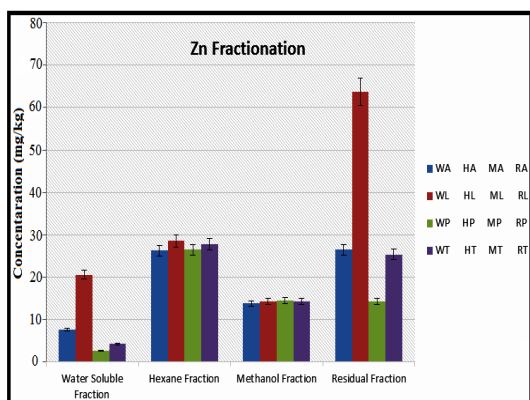


Fig. 2. Mean Concentrations of Copper in the Snail Samples (*Achatina achatina*, *Limicolaria flammea*, *Pachymeria aurita* and *Tympanotous fuscatus*) of different fractions

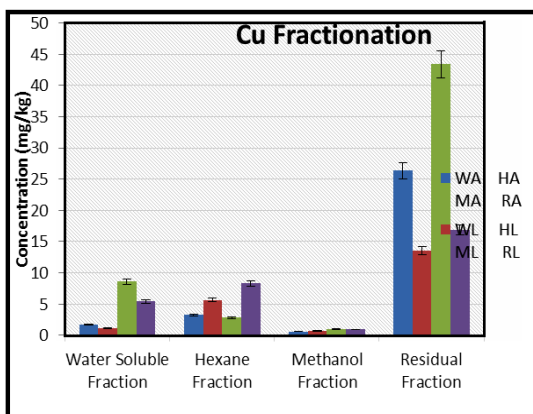


Fig. 3. Mean Concentrations of Zinc, Zn in the Snail Samples (*Achatina achatina*, *Limicolaria flammea*, *Pachymeria aurita* and *Tympanotous fuscatus*) of the different fractions

- **WA, HA, MA, RA** = water soluble, Hexane, Methanol and Residual fractions of *Achatina achatina*.

- **WL, HL, ML & RL** = water soluble, Hexane, Methanol and residual fractions of *Limicolaria flammea*.
- **WP, HP, MP, & RP** = water soluble, Hexane Methanol and residual fractions of *Pachymeria aurita*.
- **WT, HT, MT, RT** = Water soluble, Hexane Methanol and residual fractions of *Tympanotous fuscatus* respectively.

### Concentration of Iron (Fe)

The findings in Table 2 revealed that, Fe concentrations in the different fractions (WSF, NHF, PMF and RSF) of the snails were unevenly distributed depending on the snail's species. This is attributed to the bioavailability of Fe for uptake by these snails because bioavailability depends on the amount of Fe readily available for uptake by these organisms in their environment. It was observed that, the residual fraction (RSF) in all the snail species recorded the highest values which ranged from  $635.27 \text{ mg kg}^{-1}$  -  $2036.70 \text{ mg kg}^{-1}$  compared to other fractions. This implies that, most of the Fe species (especially complexes) were locked up during the extraction process and these bonds were broken during strong acid digestion and the metals were released, while the comparable amount found in the water soluble fraction for each of the snail species may be attributed to highly dissolved water soluble species of Fe in these organism.

Generally, the concentrations of Fe in all the fractions were more than the recommended standard values of M.A.F.F.<sup>32</sup>, F.A.O./W.H.O.<sup>33</sup>, except I.A.E.A.<sup>34</sup>. Also, Fe levels in these snails are higher than the values reported by Ibe *et al.*,<sup>35</sup> which were  $0.950 \text{ mg kg}^{-1}$  and  $2.061 \text{ mg kg}^{-1}$  in catfish and tilapia. However, the Fe levels in some fractions (especially the polar (PMF) and non-polar (NHF) are lower than the range values of  $198.73\text{-}457.11 \text{ mg kg}^{-1}$  reported by Olowoyo,<sup>36</sup> in periwinkles except in the residual fraction (RSF).

Iron (Fe) exists in the form of iron oxides, organic salt, inorganic or organic composites such as heamiron. The effects of toxic doses associated with Fe studies of animal are categorised by initial depression, respiratory failure, coma or cardiac arrest; convulsion etc<sup>37</sup>. However, Fe is an essential mineral in every living cell, and it is imperative for the production of hemoglobin, myoglobin and some



protein cells. Anemia which is one of the commonest nutrient inadequacies in contemporary times is caused by Fe dearth, thereby leaving its victims with weakness, inability to concentrate and susceptibility to infection<sup>38</sup>. Hence, these snails are good sources of Fe to consumers.

### Copper Fractionation

The levels of Cu in the snail species (*Achatina achatina*, *Limicolaria flammea*, *Pachymeria aurita* and *Tympanotous fuscatus*) are presented on Table 2 and Fig. 2. The data obtained follow the pattern of *Pachymeria aurita* > *Tympanotous fuscatus* > *Achatina achatina* > *Limicolaria flammea* with *Pachymeria aurita* recording the highest value of 8.60 mg kg<sup>-1</sup> and *Limicolaria flammea* recording the lowest value of 1.70 mg kg<sup>-1</sup> in the water soluble fraction. For the non-polar or hexane fraction, Cu levels are in the pattern of *Tympanotous fuscatus* > *Limicolaria flammea* > *Achatina achatina* > *Pachymeria aurita*. *Tympanotous fuscatus* recorded the highest value of 8.30 mg kg<sup>-1</sup>, and *Pachymeria aurita* recorded the lowest value of 2.80 mg kg<sup>-1</sup>. In the polar fractions, the results reflected that, *Pachymeria aurita* > *Tympanotous fuscatus* > *Limicolaria flammea* > *Achatina achatina*. *Pachymeria aurita* recorded the highest value of 0.93 mg kg<sup>-1</sup> and *Achatina achatina* recorded the lowest value of 0.63 mg kg<sup>-1</sup>. While the residual fraction of Cu follows the pattern of *Pachymeria aurita* > *Achatina achatina* > *Tympanotous fuscatus* > *Limicolaria flammea* with *Pachymeria aurita* recording the highest value of 43.38 mg kg<sup>-1</sup>, and *Achatina achatina* recording the lowest mean value of 13.53 mg kg<sup>-1</sup>.

Collectively, the findings showed that, Cu mean concentrations in the entire snail samples of the residual fraction were higher than the other fractions. This implies that most of the Cu species in these snail samples are stable complexes that can only be released via strong acid digestion. However, reasonable amount of copper found in the non-polar and water soluble fractions indicate non-polar species (mostly organic) and water soluble species (mostly the cupric, Cu<sup>2+</sup> salt which readily dissolve in water to form an aqua complex, Cu(H<sub>2</sub>O)<sub>4</sub><sup>2+</sup>.) The water molecules can be replaced by variety of organic ligands to form different complexes, and some of these organic complexes are essential to life principally, hemocyanin which constitutes the blood pigment found in mollusks<sup>39</sup>. Thus, the concentrations

of Cu in this study in all the fractions were below the standard limits value of MAFF<sup>32</sup> and FAO/WHO<sup>33</sup> but higher than the standard limits of IAEA<sup>34</sup> except in the polar fraction. Copper is an essential trace metal found in living systems, however, prolonged exposure (especially higher concentration) has been found to reduce production of antibodies which may lead to brain damage<sup>40</sup>. Other adverse health effects include; anemia, major organ damage such as lungs, liver, kidney and sometime stomach as well as intestinal irritation. The main areas Cu is commonly found in the human systems are liver, muscle and bone<sup>41</sup>.

### Zinc Fractionation

The fractionation of zinc in the snail species are presented in Table 1 and Fig. 3. The mean concentrations of zinc in the water soluble fraction (WSF) of the snails were; *Limicolaria flammea* > *Achatina achatina* > *Tympanotous fuscatus* > *Pachymeria aurita*. *Limicolaria flammea* recorded the highest value of 20.55 mg kg<sup>-1</sup>, while *Pachymeria aurita* recorded the lowest value of 3.50 mg kg<sup>-1</sup>. Non-polar or hexane fraction (NHF) were; *Limicolaria flammea* > *Tympanotous fuscatus* > *Pachymeria aurita* > *Achatina achatina*. *Limicolaria flammea* ranked the highest with value of 28.50 mg kg<sup>-1</sup>, and *Achatina achatina* ranked the lowest with 26.20 mg kg<sup>-1</sup> (p < 0.05). The highest mean value of 14.40 mg kg<sup>-1</sup> was found in *Pachymeria aurita* while, *Achatina achatina* recorded lowest mean value of 13.73 mg kg<sup>-1</sup>. Finally, in the residual fraction (RSF) of Zn, *Limicolaria flammea* ranked the highest with value of 63.70 mg kg<sup>-1</sup>, and *Pachymeria aurita* ranked the lowest with value of 14.30 mg kg<sup>-1</sup>.

The concentrations of Zn in all the snail species in each fraction were evenly distributed except in the WSF and RSF where, *Limicolaria flammea* were far higher than the other snails. This implies that, Zn species are readily bioavailable in the snails' environment and are from the same source. Also, the result shows that, the mean value of Zn in the non-polar (hexane) fractions were higher than other fractions, this indicates more non-polar (organic) species of zinc in these snails. However, the mean concentrations of Zn in these snails were lower than the recommended standard limits of regulatory bodies presented in Table 1. These values were higher than the value obtained by Olowoyo,<sup>36</sup> in periwinkles and tilapia, and the value reported by Chindah *et al.*,<sup>20</sup> in periwinkles. Zinc plays ubiquitous

biological roles in the human system. It interfaces with a lot of organic ligands and plays key role in the chemical reaction that occurs in RNA and DNA cells, genetic transfer and genetic expressibility. Also, it regulates the excitability of the brain<sup>42</sup>. Therefore, these snails are healthy sources of Zn to the consumer.

### Non-carcinogenic Risk Appraisalment of Fe, Cu, Zn in Land and Marine Snails

Non-carcinogenic risk appraisements in this study were conducted using the mean Fe, Cu, Zn concentrations of the various fractions (water soluble, non-polar or hexane, polar or methanol and residual fractions). This frame work is essentially useful in understanding the health hazards associated with

individual chemical forms (species) of these metals, rather than total concentration, because metal toxicity, their ecological mobility and bioavailability are related chemical species. For example, Fe (II) is dissolvable under normal physiological states and circulates easily across membranes, while, Fe (III) does not penetrates cells easily and is more susceptible to decomposition in ecological systems<sup>43</sup>. Furthermore, the toxic hazard posed by metals to humans relies on metal(s) concentration in the food in relation to daily consumption over time. The risk assessment models employed in this study were; the estimated dietary intake. EDI, target hazard quotient (THQ) and hazard index proposed by the USEPA<sup>27-29</sup> and the results obtained are presented in Table 3 and 4.

**Table 3: Estimated Dietary, EDI (mg kg<sup>-1</sup>-bw day<sup>-1</sup>) of Fe, Cu, Zn in Terrestrial and Aquatic Snail in the Different Fractions**

Fractions	Snails	Fe	Cu	Zn
Water soluble fraction (WFS)	<i>Achatina achatina</i>	1.23E <sup>-01</sup>	7.29E <sup>-04</sup>	3.15E <sup>-03</sup>
	<i>Limicolaria flammea</i>	8.80E <sup>-02</sup>	7.08E <sup>-04</sup>	8.56E <sup>-03</sup>
	<i>Pachymeria aurita</i>	1.40E <sup>-02</sup>	3.58E <sup>-03</sup>	1.46E <sup>-03</sup>
	<i>Tympanotous fuscatus</i>	9.00E <sup>-03</sup>	2.25E <sup>-03</sup>	1.73E <sup>-03</sup>
Non-Polar (Hexane) fraction (NHF)	<i>Achatina achatina</i>	5.38E <sup>-03</sup>	1.36E <sup>-03</sup>	1.09E <sup>-02</sup>
	<i>Limicolaria flammea</i>	1.43E <sup>-02</sup>	2.35E <sup>-03</sup>	1.19E <sup>-02</sup>
	<i>Pachymeria aurita</i>	1.23E <sup>-02</sup>	1.17E <sup>-03</sup>	1.10E <sup>-02</sup>
	<i>Tympanotous fuscatus</i>	1.45E <sup>-03</sup>	3.46E <sup>-03</sup>	1.16E <sup>-02</sup>
Polar (methanol) fraction (PMF)	<i>Achatina achatina</i>	2.59E <sup>-03</sup>	2.63E <sup>-04</sup>	5.72E <sup>-03</sup>
	<i>Limicolaria flammea</i>	5.13E <sup>-03</sup>	2.92E <sup>-04</sup>	5.92E <sup>-03</sup>
	<i>Pachymeria aurita</i>	4.58E <sup>-03</sup>	4.04E <sup>-04</sup>	6.00E <sup>-03</sup>
	<i>Tympanotous fuscatus</i>	4.38E <sup>-03</sup>	3.88E <sup>-04</sup>	5.92E <sup>-03</sup>
Residual fraction (RSF)	<i>Achatina achatina</i>	8.48E <sup>-01</sup>	1.09E <sup>-02</sup>	1.10E <sup>-02</sup>
	<i>Limicolaria flammea</i>	2.65E <sup>-01</sup>	5.60E <sup>-03</sup>	2.65E <sup>-02</sup>
	<i>Pachymeria aurita</i>	4.49E <sup>-01</sup>	1.81E <sup>-02</sup>	5.96E <sup>-03</sup>
	<i>Tympanotous fuscatus</i>	7.36E <sup>-01</sup>	7.02E <sup>-03</sup>	1.06E <sup>-02</sup>

**Table 4: Non-Carcinogenic Risk (THQ&HI) Appraisals, and the Percentage Hazard Index (%HI) of Cu, and Zn in Terrestrial and Aquatic Snail**

Fractions	Snails	THQ			HI (ΣTHQ)% Contribution of each metal to HI			
		Fe	Cu	Zn	Fe	Cu	Ze	
Water soluble fraction (WFS)	<i>Achatina achatina</i>	1.75E <sup>-04</sup>	1.82E <sup>-05</sup>	1.05E <sup>-05</sup>	2.04E <sup>-04</sup>	85.91	8.94	5.15
	<i>Limicolaria flammea</i>	1.26E <sup>-04</sup>	1.77E <sup>-05</sup>	2.85E <sup>-05</sup>	1.73E <sup>-04</sup>	73.21	10.26	16.53
	<i>Pachymeria aurita</i>	1.99E <sup>-05</sup>	8.96E <sup>-05</sup>	4.86E <sup>-05</sup>	1.14E <sup>-04</sup>	17.37	78.38	4.25
	<i>Tympanotous fuscatus</i>	1.25E <sup>-05</sup>	5.63E <sup>-05</sup>	5.76E <sup>-06</sup>	7.45E <sup>-04</sup>	16.78	75.49	7.74
Non-Polar (Hexane) fraction (NHF)	<i>Achatina achatina</i>	7.69E <sup>-06</sup>	3.41E <sup>-05</sup>	3.64E <sup>-06</sup>	7.81E <sup>-05</sup>	9.84	43.59	46.57
	<i>Limicolaria flammea</i>	2.04E <sup>-05</sup>	5.86E <sup>-05</sup>	3.96E <sup>-05</sup>	1.19E <sup>-05</sup>	17.18	49.45	33.37
	<i>Pachymeria aurita</i>	1.76E <sup>-05</sup>	2.92E <sup>-05</sup>	3.67E <sup>-05</sup>	8.35E <sup>-05</sup>	21.11	34.95	43.94
	<i>Tympanotous fuscatus</i>	2.07E <sup>-05</sup>	8.65E <sup>-05</sup>	3.86E <sup>-05</sup>	1.46E <sup>-05</sup>	14.63	59.31	26.49
Polar (methanol) fraction (PMF)	<i>Achatina achatina</i>	3.71E <sup>-06</sup>	6.56E <sup>-05</sup>	1.91E <sup>-05</sup>	2.93E <sup>-05</sup>	21.23	22.37	64.99
	<i>Limicolaria flammea</i>	7.32E <sup>-06</sup>	7.29E <sup>-05</sup>	1.97E <sup>-05</sup>	3.43E <sup>-05</sup>	17.86	21.24	57.44
	<i>Pachymeria aurita</i>	6.55E <sup>-06</sup>	1.01E <sup>-05</sup>	2.00E <sup>-05</sup>	3.67E <sup>-05</sup>	17.53	27.57	54.57
	<i>Tympanotous fuscatus</i>	6.25E <sup>-06</sup>	9.69E <sup>-06</sup>	1.97E <sup>-05</sup>	3.57E <sup>-05</sup>	-	27.17	55.31
Residual fraction (RSF)	<i>Achatina achatina</i>	1.21E <sup>-03</sup>	2.74E <sup>-04</sup>	3.67E <sup>-05</sup>	1.52E <sup>-03</sup>	79.57	18.02	2.41
	<i>Limicolaria flammea</i>	3.78E <sup>-04</sup>	1.41E <sup>-04</sup>	8.85E <sup>-05</sup>	6.08E <sup>-04</sup>	62.24	23.19	14.56
	<i>Pachymeria aurita</i>	6.42E <sup>-04</sup>	4.52E <sup>-04</sup>	1.99E <sup>-05</sup>	1.11E <sup>-03</sup>	57.65	40.57	1.78
	<i>Tympanotous fuscatus</i>	1.05E <sup>-03</sup>	1.76E <sup>-04</sup>	3.52E <sup>-05</sup>	1.26E <sup>-03</sup>	83.31	13.9	2.79

### Estimated Dietary Intake

The Estimated Dietary Intake (EDI) is an estimated daily intake of a nutriment considered adequate for daily prerequisites of almost 97-98% healthful persons in a life-time. In this study, a daily intake of  $0.025 \text{ mg kg}^{-1}\text{-bw day}^{-1}$  was considered safe based on the annual intake of fish and shell fish in Nigeria for meat and fish products which is averaged  $9.0 \text{ kg}^{30}$ . Nevertheless, the required intakes depend on the concentration of the metal(s) in the snails, the amount of snails consumed daily ( $0.025 \text{ mg kg}^{-1}\text{-bw day}^{-1}$ ). The body weight of an adult-individual was averaged 60 kg. The results showed that, the estimated daily intake of metals, Fe, Cu, Zn in each fraction of the snails were below the recommended daily allowances set by the Institute of Medicine<sup>44</sup> and WHO,<sup>45</sup> at  $0.3 \text{ mg kg}^{-1}\text{-bw day}^{-1}$  for Zn, Cu;  $0.5 \text{ mg kg}^{-1}\text{-bw day}^{-1}$  and Fe;  $10\text{-}19 \text{ mg kg}^{-1}\text{-bw day}^{-1}$  respectively. Therefore, the snails were considered safe for daily intake of  $0.025 \text{ mg kg}^{-1}\text{-bw day}^{-1}$  at the moment.

### Target Hazard Quotient, THQ and Hazard Index, HI

The THQ of the individual metals (Fe, Cu, Zn) and the combined hazard indices of all the three metals are shown in Table 4. Results showed that, the THQ of Fe, Cu, Zn in each fraction of all the snails were less than their oral reference dose. The New York State Department of Health, NYSDOH<sup>46</sup>, reported that, the risk of a contaminant becomes minimal if the ratio of the THQ to its RfD is equivalent or  $>$  RfD values of the contaminant. Also, the aggregated effects of the metals under investigation were lower than maximum tolerable limits of I (HI  $>$  I) in each snails' fraction. This indicates that, these metals (Fe, Cu, Zn) will not pose non-carcinogenic risk via consumption of these snail species. However, studies had revealed that a given matrix may contain more than two or three pollutant or toxicant and often suffer combined or interactive effects<sup>47</sup>. Therefore, considering the combined interactive effects of

other pollutants and the bio-accumulative innate characteristics of metals, moderate consumption of these snails is recommended.

### CONCLUSION

This study provided data on fractionation of Fe, Cu, Zn in different snail species obtained from Bayelsa State Nigeria. The results showed variability of the metals in each fraction of the snails. Fe accumulated higher than Cu, Zn in all the snail species and Fe levels were highest in the residual fraction contributing over 70% to the H.I. values especially in *Achatina achatina* and *Tympanotus fuscatus*. Again, the Fe levels in these snails were more than the maximum recommended values of M.A.F.F., F.A.O./W.H.O., I.A.E.A. while, Zn and Cu levels were below the limits set by these regulatory bodies. The present study also revealed that, a daily consumption of  $0.025 \text{ mg kg}^{-1}\text{-bw/day}^{-1}$  of Fe, Cu, Zn in these snail species will not pose non carcinogenic risk at the moment. However, more research should be carried out on other pollutants such as persistent organic pollutant (POPS) of health concern (e.g chlorinated pesticides, dichlorodiphenyltrichloroethane, DDT, polychlorinated biphenyl (PCBs) etc.

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### Conflict of interest

No conflict of interest

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