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Research Article
Biomarkers in *Achatina achatina* as Ecological Risk Assessment Models of Mining Activities

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Abstract
Background and Objective: Artisanal mining have led to the exposure of heavy metals in the earth crust to the outer layer of the earth. These have led to the accumulation of heavy metals in soils and in burrowing organisms like snails which can further affect its metabolic activity. The objective of this study was to assess quantitatively and qualitatively the levels of heavy metals (Arsenic (As), Lead (Pb), Chromium (Cr), Copper (Cu) and Zinc (Zn)) in land snails from mining site and the use of anti-oxidant defense system as biomarkers (Enzyme activities of Catalase, Superoxide dismutase (SOD), Acetylcholinesterase (ACHE), indices of Lipid Peroxidation and Reduced Glutathione) to monitor the selected area. Results obtained were used to conduct an Ecological Risk Assessment. **Materials and Methods:** Soils and Snails were collected from Snail farm, Leru and Nkalagu mining sites and were denoted as A1, B1 and C1 for soil and A2, B2 and C2 for snails respectively. Heavy metal analysis was determined in both soils and snails while selected anti-oxidant enzyme activities were carried out snails. Heavy metal values and ecological risk assessment values were compared to the United State Environment Protection Agency (USEPA) and other standard values for toxicity. **Results:** Soils from the Leru and Nkalagu mining sites showed significant increases ($p<0.05$) in the levels of heavy metals (As, Pb, Cu, Ni, , Cr, Zn) as compared to soil samples from the Snail farm. Catalase and superoxide dismutase activities of snails from both Leru and Nkalagu mining regions were significantly higher ($p<0.05$) than snails from snail farm. Lipid peroxidation, reduced glutathione and acetylcholinesterase activities of snails also showed significant variations. Ecological risk assessment was estimated in two planes: Heavy metal pollution Index (HMPI) and Bioaccumulation Factor. Estimations of heavy metal pollution index for the Snails from Leru mining site showed index values (HMPI>1.00) with net HMPI (4.056) indicating severe pollution. Heavy metal pollution Index (HMPI) estimations for the Snails from Nkalagu mining site showed index values (HMPI>1.00) except for Cu which fell below the range of pollution (HMPI>1.00) but rather, contamination. **Conclusion:** The research results and analysis suggested that artisanal mining and snail consumption is positively correlated to ecological hazards as such there is a need to call for tough regulations that would restrict picking of snails from mining sites and subsequent consumption for public health safety.

Key words: Mining, snails, biomarkers, risk assessment, heavy metal pollution index, united state environment protection agency and bioaccumulation factor

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In a world where mining activities are almost indispensible and there is increased drive for use and exploration of natural mineral resources, the ecosystem and all biospheres is left to the direct and mostly indirect effect of anthropogenic activities[1]. While many heavy metals are naturally present in the Earth's crust and atmosphere, humans may promote heavy metal pollution through activities such as mining, smelting, transportation, military operations and industrial manufacturing as well as applying metal-containing pesticides and fertilizers in commercial agriculture. These activities release metals into the environment through waste disposal, runoff and application of heavy metal-laden chemical products, which then may enter terrestrial systems via aerial deposition, surface waters or soil[2,3].

Unlike organic pollutants, heavy metals cannot be degraded. As a result, heavy metals persist in the environment for years, well after point sources of pollution have been removed[4]. Heavy metal can be categorized into essential and non-essential groups. Essential heavy metals (i.e., micronutrients), includes copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn), these are required by organisms in small amounts. Although organisms are generally able to regulate small amounts of essential metals, in excess these metals may become toxic. Contrastingly, non-essential metals such as aluminium (Al), arsenic (As), cadmium (Cd), lead (Pb) and mercury (Hg) are not required for normal biological function and may quickly lead to toxicity[5].

These metals are able to bio-accumulate in the systems of most organisms (like snails) and with time increase to levels that are deleterious to the organism, a process called bio-magnification[6]. These may interact directly with biomolecules, disrupting critical biological processes and resulting in toxicity and the concomitant transfer of these metals through the food chain could ultimately pose risk to human life. Consumptions of snails picked up from these mining-polluted regions are capable of eliciting reasons for public health concern. Snails as burrowing organisms are capable of taking up these heavy metals, adapting to the harsh conditions of the mining environment and even reproducing[7]. While the human body may have evolved to combat these heavy metals, frequent/subsequent exposure may hamper the human system either by stimulating a disease condition or exacerbating some salient health risk conditions. With the crusade for environmental sustainability, mining emerges as an opposition to the tenets of salvaging the ecosystem, thus, there is an estimable ecological risk that such activities could portend[7]. Snails are 'large sinks' or reservoirs of heavy metals accumulated over time. According to Hamlet *et al.*[8] these accumulations may alternate the anti-oxidant enzyme properties impairing their ability to function maximally.

Biomarkers in environmental monitoring confer significant advantages over traditional chemical measurements because measured biological effects can be meaningfully linked to environmental consequences so that environmental concerns can be directly addressed. Although the activity of antioxidant enzymes may be increased or inhibited under chemical stress, there is, however, no general rule for the different enzymes. The anti-oxidant enzymes are often measured together to indicate the total oxy-radical scavenging capacity and this has been observed to provide greater indicating value[9].

For reliable environmental risk assessment of pollutants, knowledge on the effects at different levels of biological organization is needed. Biochemical biomarkers are molecules, genes and characteristics which are prone to change depending on the physiology and metabolic rate of the organisms[10]. This 'change' could be due to a positive or a negative induction of a substance, maybe toxic materials into the habitat of the organism. Because enzymes are the biocatalyst of living organisms and are at most times functional to maintain metabolic processes, an appreciable decline or increase in their respective activities may be indicative of stressors of oxidation as can be seen in most anti-oxidant enzymes which could be due to environmental pollutants or in drug quality assessment as to ascertain its exact level of toxicity, as such enzymes as biomarker is yet considered as one of the most promising tool, for detection of real changes that may have metabolic explanations[11].

Ecological risk is the likelihood that a given activity or series of activity may have damaged or will damage the habitat, ecosystem or part of the environment immediately or over a given period of time[12]. Natural disasters such as earthquake, volcanic eruptions and Tsunami most time put the environment at risk; they are capable of shifting tectonic plates, disrupting the natural biosphere and even releasing harmful molten-like substances into the ecosystem and though this could be nature's way of maintaining balance, human anthropogenic activities tends to tilt the balance, as such there is an imbalance evident by the shift in ecological factors such as heavy metals and bioaccumulation factors that would in a long run affect agricultural produce and become direct threats to human life. Metal pollution index is a value that attempts to quantify (numerically) the level of contamination and pollution on a given expanse of soil or substance under scientific investigation[13], the difference between soil contamination range and soil pollution range is given by the metal contamination/pollution index. The index value represents the ratio between the heavy metal content effectively measured in soil by chemical analysis and reference value obtained from the control soil[14].

The aim of the study was to access quantitatively and qualitatively the levels of heavy metals in land snail and the use of anti-oxidant defence system as biochemical biomarkers (enzyme activities which includes Cholinesterase, catalase, Super oxide Dismutase, Reduced Glutathione etc.) to monitor the selected area. More importantly it was to conduct a health and ecological risk assessment using probabilistic risk assessment models, as to determine the extent of risk in which the inhabitants of this region may be exposed to.

MATERIALS AND METHODS

Studied area: Two major mining sites in the South-East were chosen: Leru mining site of Ummuneochi LGA of Abia State with coordinates (6°01'46.7 N, 7°23'11.1 E) and Nkalagu mining site of Ishelu LGA of Ebonyi state with coordinates (6°28'45.1 N, 7°46'32.4 E). Etana Snail farms, Nsukka Enugu state (A non-mining site was also taken into considerations) with coordinates (6°52'25.1 N, 7°22'10.0 E) (Fig. 1).

Sample collection: Soil and snail samples were collected at mining regions of Nkalagu, Ebonyi state and Leru, Abia State. Snail samples were also collected from Etana snail farms at Nsukka, Enugu state. Snails collected were divided into three groups: Group A: Snails from snail farms, Group B: Snails from mining site at Nkalagu, Group C: Snails from mining site at Leru.

Soil and snail samples: Soil and snail samples were major test materials used in this study. Soils were the topmost soil ranging from 0-30 cm from the top soil on the three sites while snails were collected from the top soil or by burrowing between 0-30 cm from the topsoil.

Methods

Experimental design: For the probabilistic model experiment, a total of about 60 snails were used, in the experiment there were four phases containing 3 groups with 3 snails or 3 soil samples per group.

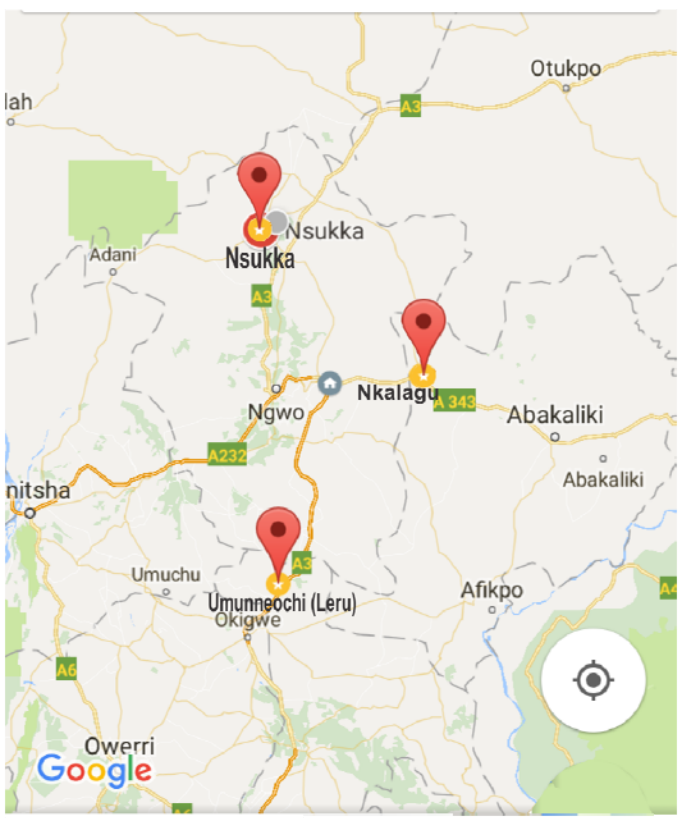

Fig. 1: Map of mining regions considered for the study (Retrieved from Google map Nigeria)

Phase 1: A total of 9 soil samples from the different sites were used in the heavy metal analysis:

- Group A1 were soil samples from the snail farm
- Group B1 were soil samples from Leru mining site
- Group C1 were soil samples from Nkalagu Mining site

Phase 2: Homogenates from 9 different snail samples were used in the analysis of enzymatic (catalase, superoxide dismutase and acetyl cholinesterase) and non-enzymatic (reduced glutathione and lipid peroxidation) anti-oxidants including total protein content.

Phase 3a: Values from heavy metals in soils from Phase I were used in estimating the heavy metal pollution index (HMPI).

Phase 3b: Values from heavy metals in snails from phase II were used in estimating the Bioaccumulation factors (BAF) in snails.

Preparation of snail homogenate: The snails were de-shelled and 3 g each of the snail were weighed into the digestion flask and 30 cm^3 of aqua regia (a mixture of HNO_3 and HCl in the ratio of 1:3) was added and digested in the fume cupboard for the evaporation of HCl until a clear solution was obtained, it was cooled, filtered and then made up to 100 mL mark in a standard volumetric flask with de-ionized water. The digested samples were analyzed for arsenic (As), chromium (Cr), nickel (Ni), copper, lead (Pb), zinc (Zn) and mercury (Hg) using atomic absorption spectrophotometer (AAS)[15].

Preparation of soil samples for heavy metal analysis: The soil samples were air-dried and then sieved to <0.25 mm, 3 g of each of the soil samples were also weighed into the digestion flask and 30 cm^3 of aqua regia was added and digested in the fume cupboard, for the evaporation of HCl until a clear solution was obtained, it was cooled, filtered and then made up to 100 mL mark in a standard volumetric flask with de-ionised water. The digested samples were analyzed for Arsenic (As), chromium (Cr), Nickel (Ni), copper, Lead (Pb), Zinc (Zn) and Mercury (Hg) using atomic absorption spectrophotometer (AAS)[15].

Preparation of snail homogenates for determination of enzymatic/Non-enzymatic antioxidant activities of snail: Six whole snails from each experimental group were pooled and homogenized in (5X weight of soft tissue) milliliter of homogenization buffer (0.1 M potassium phosphate pH 7.4). The homogenates were centrifuged at 10,000xg for 10 min and the resultant supernatant (S-10) fraction stored at -80°C until analyzed. All enzymes assays described below were performed on S-10 fractions of the whole snail homogenates.

Determination of snail catalase activity: The activity of catalase was assayed spectrophotometrically according to the methods of Aebi[16].

Determination of snail superoxide dismutase (SOD) activity: The SOD activity was assayed by the methods of Sun *et al.*[17].

Determination of snail reduced glutathione (GSH): The reduced glutathione level was determined by the method of Exner *et al.*[18].

Estimation of extent of lipid peroxidation (Malondialdehyde): Lipid peroxidation was estimated by measuring spectrophotometrically the level of the lipid peroxidation product, malondialdehyde (MDA) as described by Wallin *et al.*[19].

Determination of acetylcholinesterase (ACHE) activity of snail: Acetylcholinesterase activity was measured according to the method described by Ellman *et al.*[20].

Determination of total protein content: Protein concentration of the homogenised snail fraction was measured according to the methods of Lowry *et al.*[21] using bovine serum albumin as standard.

Ecological risk assessment

Metal Pollution Index (MPI): Metal Pollution Index was derived by adopting contamination/pollution Index as defined by Hong *et al.*[22] as shown in Table 1.

Table 1: Metal pollution index showing levels of index significance

Metal pollution	Index (MPI) Significance	Remark
<0.10	Very slight contamination	---
0.10-0.25	Slight contamination	----
0.26-0.50	Moderate contamination	---
0.50-0.75	Severe contamination	--
0.76-1.00	Very severe contamination	-
1.10-2.00	Slight pollution	+
2.10-4.00	Moderate pollution	++
4.10-8.00	Severe pollution	+++
8.10-16.0	Very severe pollution	++++
>16.00	Excessive pollution	+++++

Source: As described by Hong *et al.*[22], -: "No negative effect on plants, soil and environment", +: "Will pose negative effect soil, plant and environment", Increases in the number of sign depict severity of situation

$$\text{MPI} = \frac{\text{Concentration of metal in soil}}{\text{Reference soil (Control)}}$$

Bioaccumulation factor (BAF): Bioaccumulation factor was estimated according to the models of Lokeshappa *et al.*[23].

$$\text{BAF} = \frac{\text{Concentration of metal in snails}}{\text{Concentration of metal in soil}}$$

All values for different model parameters estimated were compared to the standards and permissible limits of the updated versions of the United States Environmental Protection Agency[12].

Statistical analysis: The SPSS software version 17 (SPSS Inc., Chicago) was used to carry out the statistical analysis. A one-way analysis of variance was carried out at $\alpha = 0.05$ and Duncan's multiple range test was used to discern the source of the observed differences.

RESULTS

Heavy metal levels of soils: From Table 2 it was evident that soil samples from the two mining sites generally contain larger proportions of heavy metal as compared to the soil from snail farms. From statistical analysis, soils from the Leru and Nkalagu mining sites showed a significant increase in the levels of heavy metals ($p<0.05$) as compared to soil samples from the snail farm. Soil from both mining sites in comparison showed no significant difference ($p>0.05$) (specifically Arsenic) and however, showed significant difference within mining sites ($p<0.05$) for other heavy metals (Cr, Ni, Pb and Zn) assayed.

Enzyme activities and total protein content: From graphs of Fig. 2 and 3, catalase activity of snails from both Leru and Nkalagu mining regions were significantly higher ($p<0.05$) as compared to that from the snail farm, likewise, the Super Oxide Dismutase (SOD) activity of snails from both Leru and Nkalagu mining regions were also significantly higher ($p<0.05$) as compared to that from the snail farm. While the indices of lipid peroxidation, reduced glutathione showed higher values for both snail from Leru and Nkalagu Mining site more than those of the snail farm, its difference were not significant ($p>0.05$). However, the acetylcholinesterase activities of snails from Leru were significantly higher than those of both the

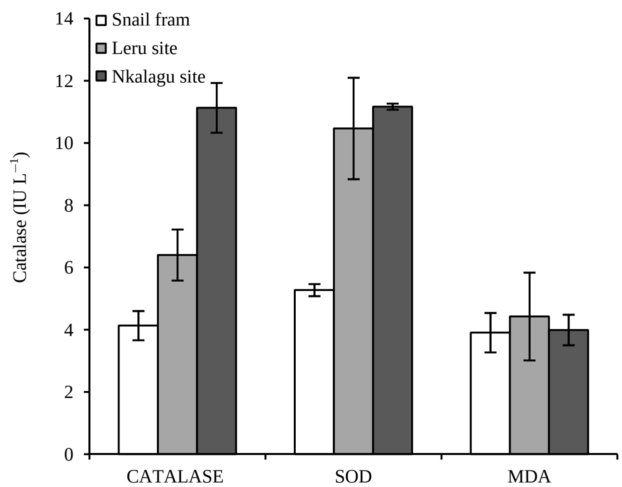

Fig. 2: Enzyme Activities of *Achatina achatina* from the snail farm, Leru and Nkalagu Mining site (Catalase activity, Super Oxide Dismutase Activity and Malondialdehyde) CATALASE (IU L^{-1}), SOD (IU L^{-1}), MDA (mg mL^{-1}) Units of Enzyme Activities

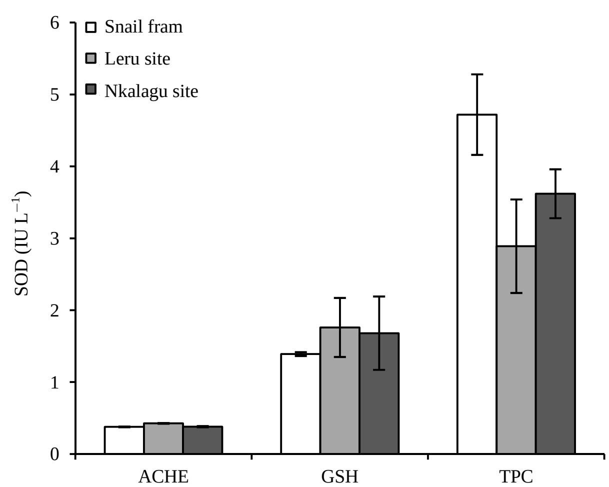

Fig. 3: Enzyme Activities of Achatina achatina and Total Protein Content of of Achatina achatina from snail farms, Leru and Nkalagu mining site, ACHE (IU L^{-1}), GSH (mg dL^{-1}), TPC (mg mL^{-1})

Table 2: Heavy metal content of soil samples from mining sites and snail farm (Mean+SD)

Groups	As (mg kg^{-1})	Cr (mg kg^{-1})	Ni (mg kg^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Zn (mg kg^{-1})
A1	9.409+3.14^{a}	128.434+2.098^{a}	12.821+1.42^{a}	6.95+0.409^{a}	70.124+1.17^{a}	16.982+1.30^{a}
B1	31.068+0.51^{b}	374.413+6.97^{b}	136.488+2.45^{b}	16.918+4.921^{b}	83.045+0.50^{b}	65.502+2.59^{b}
C1	28.225+3.14^{b}	442.511+35.48^{c}	29.821+1.43^{c}	4.676+1.88^{a}	111.065+11.94^{c}	24.928+2.37^{c}

Values are mean of three (n = 3) replicates+standard deviation, A1: Soil from snail farm, Enugu, B1: Soil from Leru Mining site, C1: Soil from Nkalagu Mining site

Table 3: Metal pollution index (MPI) for soil from Leru Mining sites

Heavy metals	Soil from Leru Mining site (Mean) (mg kg^{-1})	Snail farm soil (reference soil) (mg kg^{-1})	Metal pollution index (Leru)	Sign inference
As	31.068	9.409	3.302	++
Cr	374.413	128.434	2.915	++
Ni	136.488	12.821	10.646	++++
Cu	16.918	6.95	2.43	++
Zn	65.502	16.982	3.857	++
Pb	83.045	70.124	1.184	+
Average metal index			4.056	

*Signs and higher number of signs indicate pollution and the severity of the pollution as shown in Table 1

Table 4: Metal Pollution Index (MPI) of soil from Nkalagu Mining sites

Heavy metals	Soil from Nkalagu Mining site (Mean) (mg kg^{-1})	Snail farm soil (reference soil) (mg kg^{-1})	Metal pollution index (Nkalagu)	Sign inference
As	28.225	9.409	3.000	++
Cr	442.511	128.434	3.445	++
Ni	29.821	12.821	2.326	++
Cu	4.676	6.95	0.680	--
Zn	24.928	16.982	1.468	+
Pb	111.065	70.124	1.584	+
Average Metal Index			2.084	

*Signs and higher number of signs indicate pollution and the severity of the pollution as shown in Table 1

Table 5: Bioaccumulation Factor (BAF) of snails from Leru Mining site

Heavy metals	Snails from Leru Mining site (Mean) (mg kg^{-1})	Soil from Leru Mining site (Mean) (mg kg^{-1})	Bioaccumulation factor (BAF) (Leru)
As	43.024	31.068	1.385
Cr	21.315	374.413	0.057
Ni	11.897	136.488	0.0872
Cu	9.848	16.918	0.582
Zn	7.395	65.502	0.113
Pb	114.559	83.045	1.380

Within ranges of 0<BAF<1

snail farm and those from Nkalagu Mining site, although acetylcholinesterase activity of snails from Nkalagu were still higher than those from snail farms, it was not significant (p>0.05) . Results for Total Protein Content were in reverse, Snails from snail farms showed a significantly higher (p<0.05) level of protein content as compared to both snails from Leru and Nkalagu Mining site.

Ecological risk assessment

Metal pollution index: Heavy metal pollution index results as shown in Table 3 for the Snails from Leru Mining site showed index values for respective heavy metals ranged from 1-10, falling in the range of pollution, even severe pollution but not contamination. Nickel showed the highest metal pollution index (10.646) while Lead showed the lowest metal pollution index (1.184). Average culmination of Indexes showed a value of 4.056 indicating moderate pollution.

Heavy metal pollution Index results as shown in Table 4 for the Snails from Nkalagu Mining site showed index values for respective heavy metals ranged from 1-10, except for Cu which falls below the range of pollution but rather, contamination. Arsenic and Chromium showed higher level of pollution with indexes of 3.000 and 3.445, respectively. Average metal index showed values of 2.084 signifying slight pollution.

Bioaccumulation factor: Bioaccumulation Factor (BAF) in Table 5 for snails from Leru Mining site had As and Pb estimated to be above allowable range (0<BAF<1) while other heavy metals (Cr, Ni, Cu, Zn) were estimated to be within range (0<BAF<1).

Bioaccumulation Factor (BAF) Table 6 for snails from Nkalagu Mining site had As , Cu and Pb estimated to be above allowable range (0<BAF<1) while other heavy metals (Cr, Ni, Cu, Zn) were estimated to be within range (0<BAF<1).

Table 6: Bioaccumulation Factor (BAF) of snails from Nkalagu Mining site

Heavy metals	Snail from Nkalagu site (Mean) (mg kg^{-1})	Soil from Nkalagu Mining site (Mean) (mg kg^{-1})	Bioaccumulation factor (BAF) (Nkalagu)
As	33.051	28.225	1.171
Cr	37.901	442.511	0.086
Ni	12.821	29.821	0.430
Cu	12.817	4.676	2.741
Zn	6.265	24.928	0.251
Pb	111.066	111.065	1.000

Within ranges of 0<BAF<1

DISCUSSION

This study was primarily an investigation into mining sites, how it affects the environment and how *Achatina achatina* consumed from such sites could affect human health. Moreover it is majorly the cause-and-effect of human anthropological activities that pose danger to both human and environmental sustainability[24].

Results from soil heavy metal analysis showed that soil samples from both Leru (As: 31.068, Cr: 374.413, Ni: 136.488, Pb: 83.045, Cu: 16.918, Zn: 65.502) (mg kg^{-1}) and Nkalagu (As: 28.225, Cr: 442.511, Ni: 29.821, Pb: 111.065, Cu: 4.676, Zn: 24.928) (Mg kg^{-1}) mining sites generally contain larger proportions of heavy metal as compared to the soil from snail farms (As: 9.409, Cr: 128.434, Ni: 12.821, Pb: 70.124, Cu: 6.95, Zn: 16.982) (Mg kg^{-1}). From statistical analysis, soils from Leru and Nkalagu mining sites showed a significant increase in the levels of heavy metals ($p<0.05$) as compared to soil samples from the snail farm. These results agreed with those of Wang *et al.*[25] and Oti *et al.*[14]. Most heavy metals are deeply seated in the earth crust; human activities such as mining that excavate or permeate into earth crust are able to expose copious amounts of these heavy metals onto the topsoil, hence the increased levels of heavy metals in the mining site. Though the levels of these heavy metals still fall within maximum permissible limits of the USEPA, it is more of what happens to the plants and organisms that inhabit such soil that could be most detrimental to human well-being.

Enzyme activities together with other specific indices form the basis of a reliable environmental risk assessment of pollutants, as the sum total of their effects could shed light on their role as biomarkers of chemical stressors. Catalase activity of snails from both Leru and Nkalagu Mining regions were significantly higher ($p<0.05$) as compared to those from the snail farm. This result agreed with Vlahogianni *et al.*[26], Saglam *et al.*[27] and Bhangale and Mahajan[28] who also recorded significant increases in Catalase activity when acqua-organisms or molluscs were challenged with pollutants. The catalase enzyme system is built up to combat hydrogen radicals and other hydroxyl groups, protecting the organismal tissues, combined heavy metal impact on the snails increases the generation of free radicals including superoxide anion radical (O_2), hydrogen peroxide (H_2O_2), hydroxyl radical (OH^-) and singlet oxygen leading to an overall increase in net oxidative stress, this would lead to the increased activity of catalase in a bid to combat the oxidative stress[29], hence, the significant increase ($p<0.05$).

The Super Oxide Dismutase (SOD) activity of snails from both Leru and Nkalagu mining regions were also significantly higher ($p<0.05$) as compared to that from the snail farm. Diaconescu *et al.*[30] also recorded increases SOD activities with similar organism in varying concentrations of pollutants. SOD works in *sine qua non* to catalase, in the dismutation of superoxide radical also produced as a result of increased heavy metal challenge to either molecular oxygen or hydrogen peroxide. Like catalase, increases in the net oxidative stress would also result to increases in the SOD activities, whose enzyme becomes increasingly produced due to the need to combat the deleterious effect of the oxidative stress. It is also important to note that the net action of groups of anti-oxidant systems gives a better representation of the impact of environmental stressors on an organism by Naderi *et al.*[31].

While the indices of Lipid peroxidation (MDA) and reduced glutathione showed higher values for both snails from Leru and Nkalagu Mining site than those of the snail farm, its difference were not significant ($p>0.05$). Reduced glutathione exerting its function as enzymatic and non-enzymatic antioxidant system carryout both processes of chelating free radicals to protect cells and the need to salvage the overall oxidative stress system could tilt the anti-oxidant system to increased proliferations[32], hence the increased values of reduced glutathione in snails from the mining sites, moreover heavy metal values from snail farm are not absolutely zero.

Similarly, the oxidation of lipids to yield Malondialdehyde still further strengthens the proposition of an increased oxidative stress of snails, according to Nagamani *et al.*[33] the increased presence of MDA in an organism signifies the an increase in the index of its redox state, such that increased free radicals in the organism system would tilt its homeostatic balance, further initiating cell vulnerability leading to an increase in lipid peroxidation and the concomitant rise in the level of Malondialdehyde.

However, the acetylcholinesterase activities of snails from Leru were significantly higher ($p<0.05$) than those of both the snail farm and those from Nkalagu Mining site, although acetylcholinesterase activity of snails from Nkalagu were still higher than those from snail farms, it was not significant ($p>0.05$). This results agrees with those of Franciscato *et al.*[34] who reported that high doses of zinc and copper did not alters the levels of cerebral metal but did increase acetyl cholinesterase activity in suckling rats. Heavy metals from high levels of toxicity and pollution can affect the neural impulses of nerves and nerve endings; this is either by alternating the production of serine protease enzymes e.g., cholinesterase or further inducing increased production of neurochemicals such that the organism would react to its environment in a different light, in a bid to find adaptability[35]. Other toxicants such as insecticides and pesticides whose primary function is the elimination of pest and insect are made to alternate such neuronal pathways either increasing all the activities or inhibiting specific enzyme activities altogether[36].

Results for total protein content were in reverse, snails from snail farms showed a significantly higher ($p<0.05$) level of protein content as compared to both snails from Leru and Nkalagu Mining site. This observation also agrees with Bislimi *et al.*[37] who reported a significant decrease in the total protein content of *Helix pomatai* (Garden Snail) when exposed for year in power plants, Kamble and Kamble[38] also reported a decline in total protein content when freshwater snail *Bellamya bengalensis* was exposed to copper sulphate and pod extract of *Acacia sinuate.* This may suggested that toxicity and prevalence of heavy metals in an organism would reduce the total protein content of the organism, since enzyme activities can be varied as a result of heavy metal toxicity, variations may affect the genetic component of the organism in a way as to repress the transcription and translation of specific coding sequences of proteins necessary for a specific anti-oxidant enzyme production[39]. These alterations in genomes and enzyme activities would alter the total protein content and that would also mean the nutritional value of the snail would be reduced[40].

Ecological risk assessment including Heavy Metal Pollution Index (HMPI) and Bioaccumulation factor (BAF) evaluates how likely it is that the environment may be impacted as a result of exposure to one or more environmental stressors such as chemicals, land change, disease, invasive species and climate change. Heavy metal pollution index estimations for the snails from Leru Mining site showed index values for respective heavy metals ranged from 1-10, falling in the range of pollution, even severe pollution. This indicated that the level of metal pollution in the Leru mining site, is not a contamination but a moderate pollution (for As: 3.302, Cr: 2.915, Cu: 2.43, Zn: 3.857, Pb: 1.184) and a severe pollution (for Ni: 10.646). According to the USEPA, once a value transcends above contamination (HMPI>1.00) to pollution, then there is viable reason to contain the pollution and prevent the public from any impending danger. In like manner, heavy metal pollution index estimations for the snails from Nkalagu Mining site showed index values for respective heavy metals ranged from 1-10, except for Cu (0.680) which falls below the range of pollution rather, contamination. Indications still infer to a great extent a high level of pollution (As: 3.00, Cr: 3.445, Ni: 2.326, Zn: 1.468 and Pb: 1.584) in Nkalagu Mining site and connoting a viable reason to contain the pollution (HMPI>1.00), abating any gross effects.

Bioaccumulation Factor (BAF) for snails from Leru Mining site had As and Pb (1.385, 1.380) estimated to be above allowable range (0<BAF<1) while other heavy metals Cr, Ni, Cu, Zn (0.057, 0.0872, 0.582, 0.113) were estimated to be within range (0<BAF<1), this would suggest that a greater extent of arsenic and lead were more accumulated into the snails at Leru mining site, as such snails of such region with the known deleterious effect of arsenic and lead to human may be disastrous for human consumption. Similarly, Bioaccumulation Factor (BAF) for snails from Nkalagu Mining site had As, Cu and Pb (1.171, 2.741 and 1.000) estimated to be above allowable range (0<BAF<1) while other heavy metals; Cr, Ni, Zn (0.086, 0.430 and 0.251) were estimated to be within range (0<BAF<1). Reiterating the proposition, that snails from Nkalagu Mining site have accumulated arsenic and lead to deleterious levels and thus may not be fit for human consumption.

CONCLUSION

This study indicates that artisanal mining exposes higher levels of heavy metals to the earth crust which are further assimilated into burrowing animals like snails and build-up to toxic level and imposes a lot of stress on burrowing organism. While it may be impossible to stop mining, it is expedient to manage mining practices to avoid a total eradication of organismal biota.

SIGNIFICANCE STATEMENT

This study discovered high anthropogenic input of As, Cu, Pb, Zn, Ni and Cr in soil within the Mining Vicinities of Leru and

Nkalagu area of South-East, Nigeria. This study also revealed that snails in the region were under increased levels of oxidative stress as shown by the biomarkers and by extension most burrowing organism. Thus, the ecological effect of these heavy metals around this area can be estimated. The findings of this study will help policy makers and environmentalist in putting forward and enforcing legislations guiding the mining practices in the South-East, Nigeria.

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