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Heavy Metal Accumulation and Ecological Risks Assessment of Water Snail (*Lymnaea auricularis*) Harvested from Swamps in Kolo Creek, Bayelsa State, Nigeria.

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Abstract

This study investigates heavy metal accumulation and ecological risk assessment of water snail (*Lymnaea auricularis*) harvested from swamps in Kolo Creek, Bayelsa State, Nigeria. *L. auricularis* (n=5) were harvested from three water bodies (Site 1= Imiringi 1; Site 2=Imiringi 2, and Site 3= Emeyal) around Kolo Creek. Collected water and *L. auricularis* samples were taken to the laboratory for heavy metal (Fe, Zn, Hg, Mn, Cu, Pb) analyses employing standard operating procedures. Results indicated that *L. auricularis* accumulated Fe, Cu and Mn in site 1 as compared to the two other sites (2 and 3). Pb was not accumulated in the water snail. Analysis of heavy metal index (MI) and heavy metal evaluation index (HEI) showed that site 1 is contaminated with Fe, while site 2 is moderately polluted with Fe. The three sites (1, 2 and 3) under investigation showed that they are moderately polluted with Mn. In conclusion, long–term exposure of *L.auricularis* to Fe and Mn may pose a serious ecological risk; hence, it was recommended that regular assessment of heavy metals in water bodies and *L. auricularis* around Kolo Creek be carried out in order to ascertain the environmental health.

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Introduction

Petroleum exploration activities in the Niger Delta of Nigeria often lead to environmental pollution and ecological devastation, and this has been a challenge, particularly in the freshwater habitat (Ukhurebor *et al*., 2021; Olukaejire *et al*., 2024), which is a major sink for large concentrations of these hazardous chemicals and heavy metals present in crude oil and may be harmful to the organisms (Nabebe *et al*., 2024; Priyadarshee *et al*., 2022). The presence of heavy metals in Kolo Creek due to crude oil exploration activities has been reported (Inengite *et al*., 2010; Ogamba and Ebere, 2017; Osioma and Hamilton–Amachree, 2019). These heavy metals, according to Sanyaolu *et al*. (2022) and Briff *et al*. (2020), are non – non-biodegradable pollutants and may accumulate in tissues of the aquatic organism, and at a high concentration may be toxic. However, some heavy metals are essential for biological functions (Briff *et al*., 2020). Heavy metals are persistent pollutants that bioaccumulate in the aquatic environment and pose serious ecological risks, including neurological, developmental and carcinogenic effects (Ali and Khan, 2018; Almashhadamy *et al*., 2024).

Bio-indicator organisms can warn of potential impacts of industrial processes and effectively reflect the nature /or magnitude of environmental changes (El–Khayat *et al*., 2015; Holt and Miller, 2011). *L. auricularis*, a freshwater snail found in Kolo Creek, may not only accumulate these heavy metals but also may be valuable in ecological risk assessment due to its sensitivity to various pollutants, well-understood biology and its importance to many food webs both as consumers and decomposers (Maher *et al*., 2016).

The extent of heavy metal contamination in *L. auricularis* inhabiting the water bodies around Kolo Creek in Bayelsa State remains understudied. To our knowledge, there is no available data on the bioaccumulation of heavy metals and ecological risk assessment of *L. auricularis* in Kolo Creek. Hence, this research investigates the ecological risk associated with heavy metal contamination of swamp water around Kolo Creek employing *Lymnaea auricularis* as – bio-indicator organism.

Materials and Methods

Study Sites: The samples for this study were collected from three different sites around Kolo Creek in Ogbia Local Government Area, Bayelsa State, and were labelled accordingly as 1 = Imiringi 1, 2 = Imiringi 2, and 3 = Emeyal. Crude oil exploration activities are going on around these communities.

Collection of Water Samples: Triplicate samples of water were collected from the three experimental sites: designated as 1 = Imiringi 1, 2 = Imiringi 2, and 3 = Emeyal. Water samples were collected at 0.5m below the water surface into pre-cleaned 1 L plastic

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containers and transported to the laboratory for analysis.

Sample Collection and Analysis: Samples of *L. auricularis* were collected from the swamp water around the sampling sites into a clean pre-labelled polythene bag and immediately transported to the laboratory for the experiment. A total of five snails were collected from three different points in a site (i.e., n = 5).

Digestion of the Snail's Samples: Exactly 2.00g of *L. auricularis* was weighed into a conical flask, and 20 mL of aqua regia and 10 mL of $HClO_4$ were added, and a dish was used to cover the flask. The mixture was heated in a thermostat block at 150 ºC until the sample dissolved completely. After heating, the mixture was allowed to attain ambient temperature. A Whatman No. 4 filter paper was used for filtering the mixture in a 100 mL volumetric flask, and a 1M HNO_3 solution was used to make up the mixture to mark (Csuros and Csuros, 2020).

Digestion of Water Samples: Digestion was carried out as follows: 100 mL of water samples was placed in a 250 mL conical flask, 10 mL of 1M HNO_3 was added, placed in a digestion block and heated at 150°C until the mixture reduced to about 25mL. The mixture was removed from the block, cooled, filtered using a Whatman No. 40 filter paper, transferred to a 100 mL volumetric flask and made up to mark with 1M of HNO_3 (Csuros and Csuros, 2002).

Instrumental Analyses: The filtrates obtained from the digestion of water, soil, earthworm and snail were analysed using an Atomic Absorption spectrophotometer (Varian spectrAA100, USA) for the following metals: Fe, Cu, Mn, Pb and Zn. After appropriate dilution of the stock solution for each metal to be analysed, a calibration graph was prepared using five different concentrations.

Water Pollution Assessment Methods

Bioaccumulation Factor (BAF): The BAF was evaluated as the concentration of contaminant in the soft tissues of *Lymnaea auricularis* using the method of Cortet *et al*. (1999) as indicated in the equations below:

$$BAF_{(Toxic\ metals)} = C_{HME} / C_{HMW} \quad (1)$$

Where; $BAF_{(Toxic\ metals)}$ = Bioaccumulation factor for toxic metals analysed;

C_{HME} = Heavy metal concentration in *Lymnaea auricularia* (mg kg^{-1}); C_{HMW} = Heavy metal concentration in water (mg kg^{-1}).

Heavy Metal Index (MI) and Heavy Metals Evaluation Index (HEI)

The MI is a water quality indicator that evaluates the contamination level of water by heavy metals by comparing it with their maximum allowable concentrations. The HEI, on the other hand, sums up the ratios of the measured concentration to the maximum allowable concentration for each parameter (Badeenezhed *et al*., 2023).

MI is calculated as:

$$MI = \Sigma \frac{Metal\ concentration}{maximum\ allowable\ concentration}$$

When MI is less than 1 = low/no pollution; between 1 – 3 = moderate pollution; greater than 3 = high pollution/ severe contamination.

The HEI is calculated as:

$$HEI = \sum_{i=1}^{n} \frac{metal\ concentration}{maximum\ allowable\ concentration\ of\ metal}$$

HEI value below 10 indicates a low level of metal pollution, while HEI value between 10 and 20 suggests a medium or moderate level of contamination. HEI values above 20 indicate a high level of heavy metal pollution, which poses a significant risk to water quality and potentially to human health (Edet and Offiong, 2002).

Statistical Analysis: The values obtained from the analysis were expressed as Mean ± SD for triplicate determinations. Simple standard formulae were employed, and results compared with standard permissible values. Preliminary risk calculation using mathematical formulas was used to determine the potential risk of water snail (*Lymnaea auricularis*) exposure to contaminants.

Results and Discussion: The concentration of heavy metals in water and the water snail is shown in Table 1. The concentration of Fe in water samples varied widely, with site 3 having the highest concentration of 8760 µg/L, while site 2 had the lowest concentration of 2929.7 µg/L. Water 1 also had relatively high levels of Fe, at a concentration of 6217.7 µg/L. Iron levels in water exceeding 1,000 µg/L often indicate contamination from industrial effluents or natural geological leaching (Chapman, 1996). Elevated levels of Fe in water can cause bioaccumulation in aquatic organisms, as observed in water snails harvested from site 1. However, the extent of bioaccumulation in snails is not proportional, which suggests variability in uptake of Fe or other environmental factors such as pH, oxygen levels and other competing ions affecting the solubility of Fe.

Copper (Cu) was detected in water samples for site 3 at a concentration of 312 µg/L, but was below the detection limit in sites 2 and 3, respectively. Similarly, Cu was detected in only the Snail from site 1 at a concentration of 172 µg/kg. Cu levels in water below 20 µg/L are generally considered safe (WHO, 2011).

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The higher concentration of Cu in site 1 may indicate localised contamination, possibly from oil exploration activities. The bioaccumulation of Cu in snails from site 1 suggests exposure to the contaminated site.

Table 1: Concentration of heavy metals in water and water snail (*Lymnaea auricularis*) from experimental sites.

Samples	Metals concentration				
Water	Iron (Fe)	Copper (Cu)	Manganese (Mn)	Lead (Pb)	Zinc (Zn)
Site 1 (µg/L)	6217.7±59.7	312.0±9.50	1083±6.20	<0.001	<0.001
Site 2 (µg/L)	2929.7±111.4	<0.001	1144.7 ±6.10	<0.001	<0.001
Site 3 (µg/L)	8760.0±52.4	<0.001	2559.7±61.4	<0.001	148.3
Water Snail Site 1 (µg/kg)	2559.3 ±51.8	172.0 ±3.50	885.7±3.50	<0.001	243.3±6.60
Site 2 (µg/kg)	265.7±5.00	<0.001	2460.0±10.0	<0.001	180.7±1.50
Site 3 (µg/kg)	2016.7 ±2.00	<0.001	1238.3±11.5	<0.001	58.0 ±2.00

Site 1 = Imiringi 1, Site 2 = Imiringi 2, Site 3 = Emeyal.
Values are presented as mean ± SD for water (n=3) and water snails (*L. auricularis*) (n=5)

The level of Mn in water samples was consistently high, with water samples from site 3 showing the highest concentration (2559.7 µg/L) and site 1 showing the lowest concentration (1083 µg/L). Elevated levels of Mn in water above 50 µg/L are indicative of contamination through natural weathering of rocks, mining or industrial discharges (USEPA, 2004). Similarly, the water snail from site 2 shows the highest concentration of manganese (2460.0 ± 10.0µg/L).

For Pb, the determined concentration in both water and tissue samples was below the detection limit of the measuring instrument. The absence of Pb in both matrices suggests minimal contamination from anthropogenic sources. The bioavailability of Pb in aquatic systems is highly dependent on pH and organic matter. Zinc was below the detection limit in water samples from sites 1 and 2, but was detectable at 148.3 µg/L in site 3. Zinc levels below 300 µg/L in water are generally considered acceptable (WHO, 2011). The elevated level of Zn in site 3 might reflect localised contamination sources. In *L. auricularis* samples, Zn was detected in sites and 3

Bioaccumulation Factor (BAF): The computed BAF for metals is shown in Table 2. The BAF reflects the ability of an organism to concentrate metals from its environment. A higher BAF indicates greater bioaccumulation potential and bioavailability of the metal.

Generally, Fe and Mn show significant bioaccumulation in water snails, suggesting they are highly bioavailable in the studied ecosystems. For Cu and Zn, they are observed to exhibit site-specific bioaccumulation, while Pb remains undetected in both systems. High concentrations of Fe, Mn and Cu can exhibit toxic effects on aquatic life, affecting growth, reproduction and metabolic processes (Nriagu, 1996). Bioaccumulation of metals in water snails thus highlights their role as bioindicators of metal pollution in aquatic ecosystems (Oehlmann and Schulte–Oehlmann, 2003).

Table 2: Bioaccumulation factor of metals

Locations	Bioaccumulation Factor (BAF)				
	Fe	Cu	Mn	Pb	Zn
1	0.41	0.55	0.82	0	0
2	0.091	0	2.15	0	0
3	0.23	0	0.484	0	0.40
Min.	0.091	0	0.48	0	0

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Max.	0.41	0.55	2.15	0	0.40

Site 1 = Imiringi 1, Site 2 = Imiringi 2, Site 3 = Emeyal.

The BAF for Fe is below 1 at all locations, suggesting that Fe has a low bioaccumulation potential in water snails. The bioavailability of Fe is often influenced by water chemistry, such as pH and organic matter. The low BAF values may result from Fe being bound to particulate matter or existing in less bioavailable forms, such as ferric hydroxide (WHO, 2011). Site 2 showed the lowest BAF for Fe, with a value of 0.091.
Bioaccumulation of Cu was only observed in site 1, with a BAF value of 0.55. This indicates moderate bioaccumulation, possibly due to higher Cu concentration in water at this location (312 ug/L). Mn had the highest BAF values compared to the other studied metals, with site 2 showing significant bioaccumulation. This suggests that Mn is highly bioavailable in this environment, and this could be a result of its solubility in water under reducing conditions or weak complexation with organic matter (Ali *et al*., 2019). BAF values greater than 1 at this location indicate potential biomagnification concerns for Mn in the food chain.
The BAF for Pb is 0 at all locations, and this is consistent with its non-detectable concentrations in both water and snails. Pb is known to have low solubility in water and often binds to particulates or sediments, thereby limiting its bioavailability (Nriagu, 1996). This suggests either minimal pollution by Pb or that Pb exists in forms inaccessible to the snails. For Zn, its bioaccumulation was observed only at site 3. The bioaccumulation of Zn was observed only at site 3, with a BAF value of 0.4. The higher Zn concentration in water at this location likely contributed to its detectability in snails. Zn is an essential trace element, and its bioaccumulation in snails may reflect physiological uptake mechanisms. However, the BAF value below 1 suggests that Zn is not significantly biomagnified in this ecosystem.

Heavy Metals Index (Mi) and Heavy Metals Evaluation Index (Hei)

The computed MI and HEI values are presented in Table 3. The MI is a water quality indicator that evaluates the contamination level of water by heavy metals by comparing it with their maximum allowable concentrations. The HEI, on the other hand, sums up the ratios of the measured concentration to the maximum allowable concentration for each parameter (Badeenezhed *et al*., 2023).
The MI for Fe and Mn were greater than 3 in all water samples, indicating high pollution/severe contamination. The computed heavy metals evaluation index (HEI), as shown in Table 3, suggests that sites 1 and 3 are contaminated with Fe, while site 2 is moderately polluted. The three sites (1, 2 and 3) were observed to be moderately polluted with Mn.

Table 3: Ecological risk assessment of heavy metals

Sampling location	Metals	Average metal concentration, *n=3,* (µg/L)	Standard permissible limit in water (µg/L)	Metals index (MI)	Heavy metals evaluation index (HEI)
1	Fe	6217	300	20.7	23.6
	Cu	312	2,000	0.16	
	Mn	1083	400	2.71	
	Pb	0	10	0	
	Zn	0	5,000	0	
2	Fe	2929.7	300	9.76	12.6
	Cu	0	2,000	0	
	Mn	1144.7	400	2.86	
	Pb	0	10	0	
	Zn	0	5,000	0	
3	Fe	8760	300	29.2	35.6
	Cu	0	2,000	0	
	Mn	2559.7	400	6.40	
	Pb	0	10	0	
	Zn	148.3	5,000	0.03	

Site 1 = Imiringi 1, Site 2 = Imiringi 2, Site 3 = Emeyal.

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Conclusion

The study reveals significant heavy metal contamination in both water and freshwater snails (*Lymnaea auricularis*), with pronounced levels of Iron (Fe) and moderate levels of Manganese (Mn) in both matrices. However, *L. auricularis* tends to accumulate Mn more in site 1 than all other metals investigated. Fe and Cu were moderately accumulated. These values demonstrate the potential for heavy metals to enter the food chain and may pose a serious ecological risk to higher trophic levels. Thus, it is recommended that regular assessment of heavy metal concentrations in water bodies around Kolo Creek and in *L. auricularis* be carried out to ascertain the environmental health

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