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Eco-toxicological assessment of heavy metals in the freshwater apple snail (*Pila ovata*) from selected markets in Benin metropolis, Nigeria

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Abstract
Heavy metals have been widely researched around the world as a result of their deleterious and toxic effects on biotic ecosystems. On this premise, the dearth of ecotoxicological data regarding the heavy metal (Pb, Cd, Zn and Cu) content in the African freshwater apple snail (*Pila ovata*) sourced directly from open markets in Benin City, Nigeria warranted this research. Heavy metals in snail specimens of mean weight 76.11±1.07 g were quantified by Atomic Absorption Spectrophotometry technique in order to ascertain their suitability for human consumption and to provide baseline data. The summary statistics for the mean concentrations of heavy metals in *P. ovata* ranged from below detection limit (BDL) for Cd to 0.4192 mg/kg for Zn while market-wise, the mean concentrations of heavy metals ranged from BDL for Cd to 0.4492 mg/kg for Zn at Ekiuwa market. Monthly-wise, the mean concentrations of heavy metals ranged from BDL for Cd to 0.4558 mg/kg for Zn in May. The estimated daily intake (EDI) values (mg/kg/day) for heavy metals in *P. ovata* ranged from zero for Cd to 0.00022 for Zn while the toxic hazard quotient (THQ) for heavy metals in *P. ovata* ranged from zero (Cd) to 0.000002 (Pb). The total heavy metal burden (mg/kg) in *P. ovata* by market ranged from 0.71 at Ekiosa market to 1.09 at Ekiuwa market. Essentially, data from the study revealed that the experimental mollusc should be consumed with caution as a result of potential Pb poisoning over time.

Keywords: Hazard, heavy metals, markets, *Pila ovata*

Introduction

Heavy metals have been widely researched around the world as a result of their deleterious effects on biotic ecosystems (Jones *et al*., 2022) [13]. Furthermore, they are persistent in the environment, contaminate food chains, and produce a myriad of health problems due to their toxicity (Uraku *et al*., 2021) [24]. These metals are especially known for interfering with various physiological, biochemical and cellular activities (Ashish and Lakhyahira, 2021) [3]. Apart from the health impacts that heavy metals have on human health, they are known to disrupt natural ecological systems that exist in aquatic media such as rivers, lakes, estuaries and wetlands (Uwah *et al*., 2021) [25]. It is pertinent to note that heavy metals are naturally present in both aquatic and terrestrial environments; however anthropogenic activities are capable of amplifying natural background concentrations to hazardous levels as a result of urbanization, agricultural and industrial activities (Dubey and Ujjania, 2021) [9]. Living aquatic resources such as shellfish and finfish can be found at the apex of the aquatic food chain and are thus ideal bio indicators for heavy metal accumulation (Kammarchedu and Aluri, 2021) [14]. In Nigeria, a plethora of research has been conducted on heavy metals in a wide variety of ecological compartments including fauna, flora, water, sediment, soil and air. Perhaps the most significant of these are those that dwelt on living aquatic resources particularly shellfish and finfish which serve as food for man. Going further, there is a dearth of ecotoxicological data on the heavy metal levels in freshwater snails sold in open markets in Benin City, Nigeria. Although such snails are cherished as a cheap source of protein, they have been implicated as health hazards being intermediate hosts for helminth parasites (Sanu *et al*., 2020) [21]. The focus of this research was the African freshwater apple snail *Pila ovata* (Olivier, 1804). This gastropod belongs to the Family Ampullariidae and is commonly found in open markets particularly in southern Nigeria.

The snail species feeds on a variety of aquatic plants and is at home in stagnant water, swamps, reservoirs, dams and flood plains. In the West African sub-region, it is found in Nigeria and Chad and can also be found in Egypt and northern Mozambique (Koudenoukpo *et al*., 2020) [15]. A decrease in acidity with a corresponding increase in alkalinity has been observed to contribute to the wide distribution such snails in natural aquatic media (Olomukoro and Azubuike, 2009) [19]. Aquatic snails have been noted to be extremely sensitive to heavy metals in water (Harbar *et al*., 2021) [12] while waters contaminated with heavy metals are known to produce histological damage to the gonads of these species (Wiraatmaja *et al*., 2022) [27]. In addition biochemical, physiological and genotoxic damage by heavy metals have been reported in aquatic molluscan species (Cabral *et al*., 2022) [5]. Externally, the shell of *P. ovata* is typically light brown or olive in colour and shaped like an apple, hence the name "Apple snail". Results from this research are expected to reveal if *P. ovata* is free from hazardous levels of heavy metals (Pb, Cd, Zn and Cu) in order to provide relevant scientific information needed for better informed decision making on the part of potential consumers.

Materials and Details

Description of study area

This study was carried out in Benin City (Latitude 6°20' 00''N and Longitude of 5 37' 20'' E) in Edo state, Nigeria. The population of the City is estimated to be about 1, 782,000.The amount of rainfall in the City ranges between 1750 mm and 2000 mm annually with an average temperature of 34^0C. The "Bini" people have four distinct market days *viz*: *Agbado, Eken, Ekioba* and *Ekenaka*. The research focused on four purposely selected major markets *viz*: Ekiuwa, Ekiosa, Oliha and Oba markets after a preliminary survey which took into consideration the number of markets that sell *P.ovata* in the City. The specific GPS points for the selected markets are; Oba Market (L atitude 6.3348N and Longitude 5.6201E), Ekiuwa market (Latitude 6.3361N and Longitude 5.6879E), Ekiosa market (Latitude 6.1649 N and Longitude 5.6879 E) and Oliha Market (Latitude 6.2298N and Longitude 5.5409E).

Collection of samples

Samples (n=128) of *P. ovata* (Plates 1 and 2) were purchased from the aforesaid markets between February and May, 2022. February and March represented the dry months while April and May represented the wet months. Purchased samples were placed in labeled DANA PLAST® PVC receptacles with lids and conveyed to the laboratory within 24 hours for further studies.

Plate 1: *Pila ovata* (Dorso-vertical view)

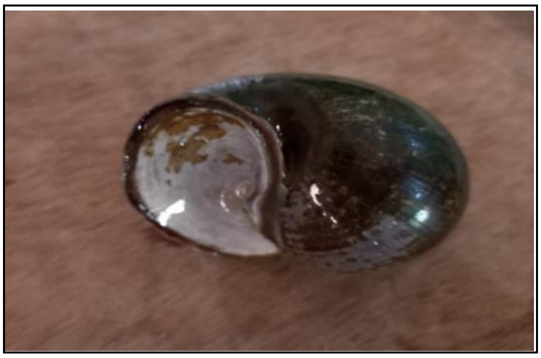

Plate 2: *Pila ovata* (Apperture view)

Laboratory protocol and procedures

In the laboratory, the snails were thoroughly washed with running tap water in order to remove all adhering debris and any other visible external foreign matter. The identities of the snails were verified using using a key in which their unique morphological characteristics were applied (Brown, 1994) [4] while the MolluscaBase website (www.molluscabase.org) was used for cross referencing. Snail samples were weighed (76.11±1.07 g) using an ATOM A-110C® electronic compact scale while widths (5.62±1.06 cm) and lengths (6.71±1.98 cm) were measured with a Vernier caliper and translucent ruler. The shells were cracked open by applying pressure with a metal rod while the fleshy components were extracted using stainless steel scalpel and forceps. The extracted parts were thereafter dried to constant weight within the enclosure of a Surgifield-Uniscope® SM 9023 model laboratory oven at a temperature of 75 °C. Dried samples were milled individually using a porcelain mortar and pestle while triple-acid digestion as described by Wangboje and Oghenesode (2017) [26], was carried out thereafter. All digests were analyzed for Pb, Cd, Zn and Cu by means of an Atomic Absorption Spectrophotometer (Unicam® 696 series) equipped with solar software using air acetylene flame as an oxidant. Concentrations of metals in snails were expressed in mg/kg. Blanks, spiked samples and duplicate analyses were performed for all analytes as part of the quality assurance procedures. All reagents used were of analytical grade and were manufactured by BDH, Poole, England.

Total toxicity of mixtures (TTM) index for heavy metals

Whether or not a mixture of heavy metals in a particular medium exceeds the quality guideline value for that medium, can be determined by applying the TTM index (ANZECC/ARMCANZ, 2000) [2].

$$TTM = \Sigma \, {}^{CI}/_{GV1}$$

Where: C1 = Concentration of the 'ith' component of mixture; GV1= Guideline value for the 'ith' component; TTM >1= The mixture has exceeded the Guideline value.

Estimation of Annual intake (EAI) and Estimation of Daily intake (EDI) of heavy metals

$$\text{EAI (Mg/person/year)} = \frac{\text{Concentration of heavy metal in snail} * \text{Per capita figure}}{\text{Adult body weight (Assumed to be 70kg)}}$$

Where: Per capita figure is 13.3 kg/person/year for Nigeria (Word Fish Center, 2021)

$$\text{EDI (Mg/person/day)} = \frac{EAI}{365\ Days}$$

Target hazard quotient (THQ) for non-carcinogenic risk
The target harzard quotient (THQ) for non-carcinogenic risk was calculated using the following equation (USEPA, 2016).

$$\text{THQ}= {Ed * Ef * EDI * Ct}/{At * Rfd} \times 10^{-3}$$

Where Ed is the exposure duration of 30 years, Ef is exposure frequency of 350 days/ year, Ct is a conversion factor of 0.208 to convert fresh wet weight to dry weight, "A"t is the average time for the non-carcinogenic element i.e. 365 days/ year for 65 years or 23,725 days, Rfd is the reference dose in mg $kg^{-1}day^{-1}$ for heavy metals (Pb=0.01, Cd=0.005, Zn=0.25 and Cu=0.25) (USEPA 2011).

Total target hazard quotient (TTHQ) for non-carcinogenic risk
This is the arithmetic total of the individual metal THQ values taking into consideration that a synergistic effect of heavy metals may be in operation in nature (Paul *et al*., 2021) [20].
$TTHQ = THQ^1 + THQ^2 + THQ^3 + THQ^{ith}$

Statistical procedure
The research was a factorial experiment within a Completely Randomized Design taking into cognizance 4 heavy metals, 4 markets, 4 months and 1 source (*P. ovata*) replicated twice. Statistical software (GENSTAT® version 13.3 for Windows) was used for analyzing generated data. One-way analysis of variance (ANOVA) was used to test for significant differences ($p<0.05$) between mean values of heavy metals while the New Duncan Multiple Range Test (Post-hoc test) was used to separate significant means. Microsoft Excel (for Windows 2010), was used for all graphical presentations

Results
As presented in Table 1, the summary statistics for the concentrations of heavy metals in *Pila ovata* ranged from below detection limit (BDL) for Cd to 0.4192 mg/kg for Zn while market-wise, the mean concentrations of heavy metals ranged from BDL for Cd to 0.4492 mg/kg for Zn at Ekiuwa market as shown in Table 2. Monthly-wise, the mean concentrations of heavy metals ranged from BDL for Cd to 0.4558 mg/kg for Zn in May (Table 3) while the total toxicity of mixtures (TTM) value for heavy metals in *P. ovata* was 1.18 as shown in Figure 1. The estimated annual intake (EAI) values (mg/kg/year) for heavy metals in *P. ovata* ranged from zero for Cd to 0.079 for Zn (Fig.2) while the estimated daily intake (EDI) values (mg/kg/day) for heavy metals in *P. ovate* ranged from zero for Cd to 0.00022 for Zn (Fig. 3). The toxic hazard quotient (THQ) for heavy metals in *P. ovata* ranged from zero (Cd) to 0.000002 (Pb) while the total toxic hazard quotient (TTHQ) was 0.00000123 as shown in Fig.4. The percentage quota of heavy metals in *P. ovata* ranged from 0% for Cd to 41.41% for Zn (Fig. 5) while the total heavy metal burden (mg/kg) in *P. ovata* by market ranged from 0.71 at Ekiosa market to 1.09 at Ekiuwa market (Fig. 6).

Table 1: Summary statistics for concentrations (mg/kg) of heavy metals in *Pila ovata*

Heavy metal	Mean concentration	Standard Deviation (SD)	Maximum limit(mg/kg)
Cd	BDL	Nil	0.05*
Pb	0.3496	0.001	0.3**
Cu	0.2435	0.005	30***
Zn	0.4192	0.06	40***

BDL= Below Detection Limit *Commission Regulation (2010) [7], **CODEX Alimentarius (2015) [6], ***FAO (1983) [10]

Table 2: Mean concentrations (mg/kg) of heavy metals in *Pila ovata* by market

Market	Cd	Cu	Pb	Zn
Oba	BDL	$0.2017±0.0266^a$	$0.3192±0.0412^a$	$0.3875±0.0186^a$
Ekiuwa	BDL	$0.2508±0.03^a$	$0.3917±0.0217^a$	$0.4492±0.0168^a$
Ekiosa	BDL	$0.2533±0.03^a$	$0.0392±0.0287^b$	$0.4167±0.0192^a$
Oliha	BDL	$0.2683±0.0461^a$	$0.3383±0.0134^a$	$0.4233±0.0046^a$

BDL= Below Detection Limit; Means with the same letters on the same column are not significantly different ($p>0.05$)

Table 3: Mean concentrations (mg/kg) of heavy metals in *Pila ovata* by month

Month	Cd	Cu	Pb	Zn
February	BDL	$0.2325±0.0314^a$	$0.3367±0.0431^a$	$0.3983±0.0393^b$
March	BDL	$0.2306±0.0311^a$	$0.3362±0.0431^a$	$0.4010±0.0452^b$
April	BDL	$0.2333±0.032^a$	$0.3342±0.0454^a$	$0.4225±0.0383^a$
May	BDL	$0.2758±0.058^a$	$0.3858±0.0227^a$	$0.4558±0.0480^a$

BDL= Below Detection Limit; Means with the same letters on the same column are not significantly different ($p>0.05$)

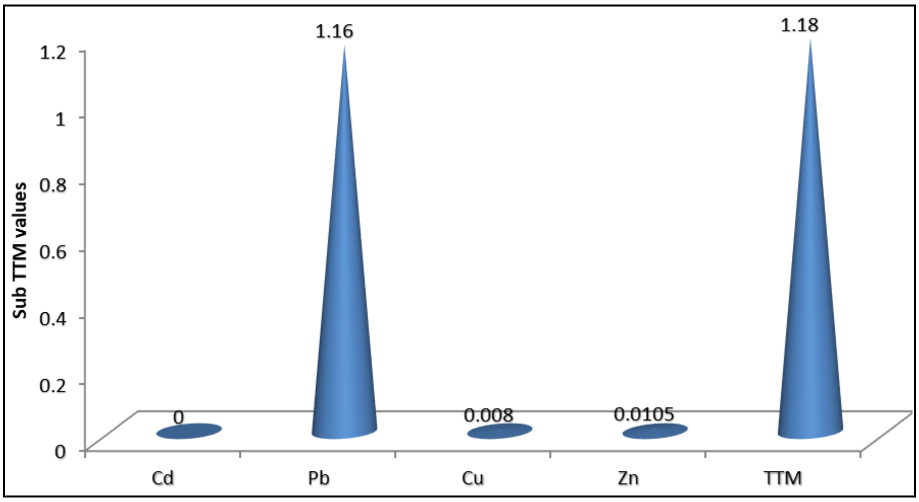

Fig 1: Total toxicity of mixtures (TTM) value for heavy metals in *Pila ovata*

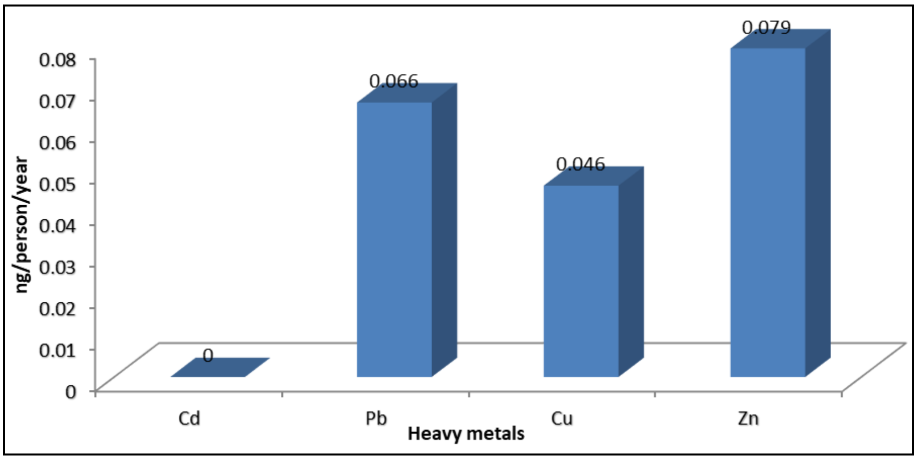

Fig 2: Estimated annual intake (EAI) values for heavy metals in *Pila ovata*

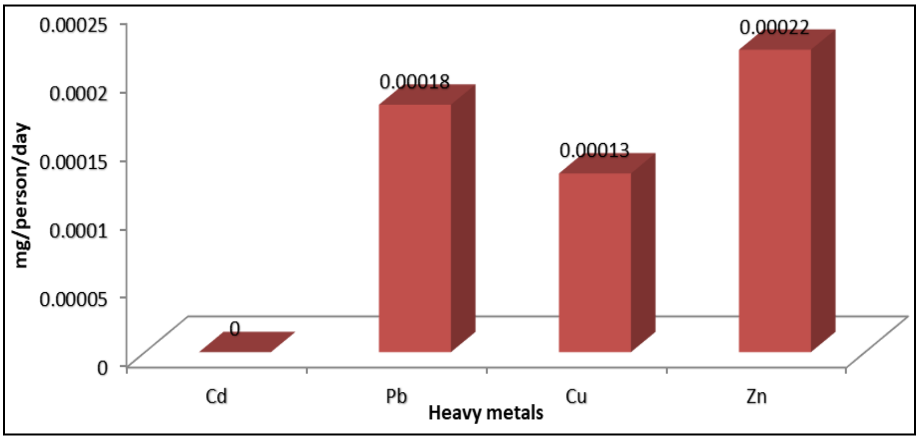

Fig 3: Estimated daily intake (EDI) values for heavy metals in *Pila ovata*

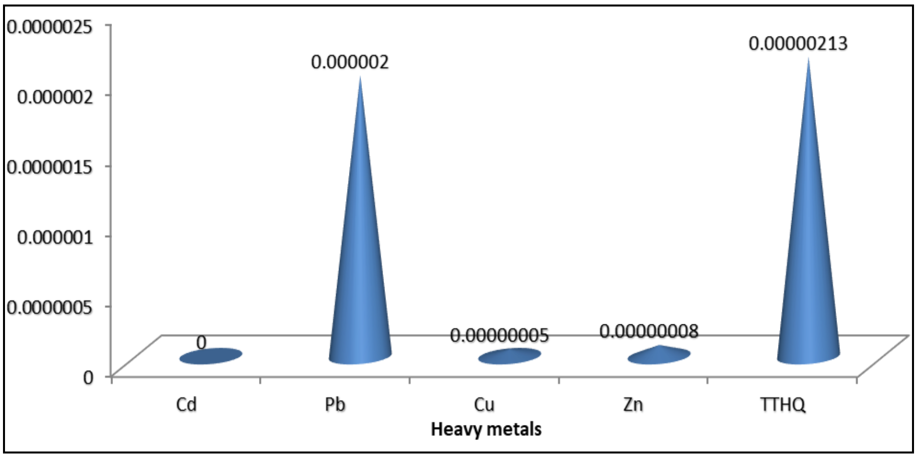

Fig 4: Toxic hazard quotient (THQ) and Total toxic hazard quotient (TTHQ) for heavy metals in *Pila ovata*

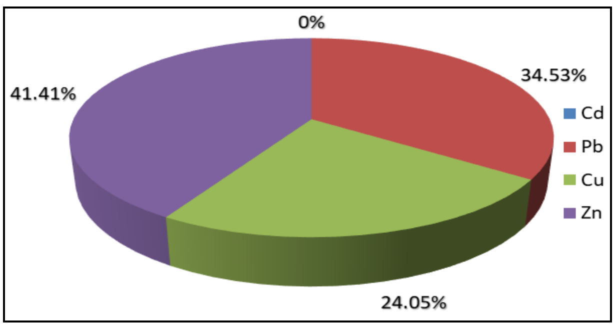

Fig 5: Percentage (%) quota of heavy metals in *P. ovata*

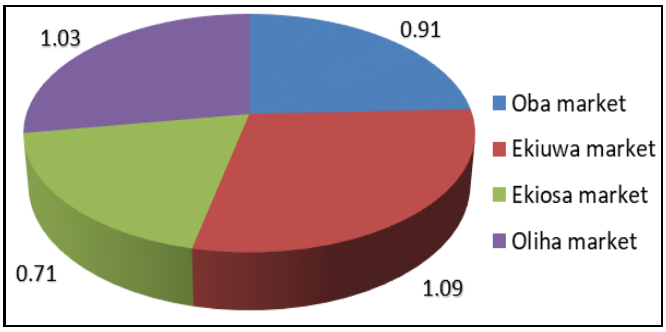

Fig 6: Total heavy metal burden (mg/kg) in *P. ovata* by market

Discussion

Aquatic snails are good bio indicators of elemental contamination and may give an estimation of these substances in their ambient media (Culha *et al.*, 2022.) [8] In this research, Cd was the only metal that was below detection level (BDL) in *P. ovata* while Pb, Cu and Zn were detected at varied levels, with an observed heavy metal profile of Zn>Pb>Cu>Cd. The essential elements were Zn and Cu while the non-essential elements were Cd and Pb. The presence and indeed the absence of heavy metals in aquatic species and hydrobionts is linked to the ability of such species to take up elemental species from their surrounding and the ability to metabolize same. Furthermore, when the rate of uptake of elements exceeds the rate of loss or depuration, elemental bioaccumulation is bound to occur. Alnashiri (2022) [1] investigated the heavy metal concentrations in selected gastropods (*Barbatus barbatus, Lambis truncata* and *Strombus tricornis*) from the Red Sea and attributed the presence of these metals to bioaccumulation from the Red Sea environment. In the aforesaid study, the mean concentrations of metals peaked at 13.4 mg/kg for Cu, 3.56 mg/kg for Mn, 0.129 mg/kg for Zn, 3.22 mg/kg for Pb and 300.5 mg/kg for Fe. The mean concentrations of Pb, Cu and Zn observed in *P. ovata* in this research were all below the mean values observed for the same metals in the Red Sea investigation indicating far higher bioaccumulation of metals in the latter research. Similarly, Culha *et al.*, (2022) observed higher mean concentrations of Cd (41.03 mg/kg), Pb (0.73 mg/kg), Cu (18.15 mg/kg) and Zn (59.83 mg/kg) in molluscs (i.e. *Rissoa splendida, Bittium reticulatum, Cystoseira barbata* and *Tritia neritea*) from the Black Sea than what was obtainable in this research. In another research, Paul *et al.*, (2021) [20] also attributed the presence of elemental species in fish and shellfish in the Rupsa River, Bangladesh, to bioaccumulation and bio magnification. Sanu *et al.*, (2020) [21] observed that the presence of heavy metals in natural aquatic media apart from impacting negatively on aquatic species could lead to loss in diversity of these species. Menon *et al.*, (2023) [16] examined the bioaccumulation of selected heavy metals in a sister species *Pila globosa* in the Kole wetlands of India and attributed the spiked presence of heavy metals in the wetland to the unabated influx of agro-chemicals such as pesticides and fertilizers. Market-wise, there was no significant (p>0.05) difference in the mean concentrations of Cu and Zn in *P. ovata* between markets, the only exception being Pb. This observation may be linked to the fact that the gastropod may have been sourced from similar distibutors or obtained from the same aquatic body. With regard to the total heavy metal burden in *P. ovata* by market, the highest and lowest burdens were observed at Ekiuwa and Ekiosa markets respectively giving an indication that potential consumers should obtain *P. ovata* from the latter due to a lower heavy metal load. Monthly-wise, there was no significant (p>0.05) difference in the mean concentrations of Cu and Pb in *P. ovata* between months, the only exception being Zn. This observation gives a clear pointer into the relative stability of the metal content in *P. ovata* over the study period. The TTM value of 1.18 clearly exceeded unity because the guideline value for Pb was surpassed. However, the guideline values for the other elements were not surpassed. The EAI and EDI values were dominated by Zn owing to the fact that this element had the highest mean concentration in *P. ovata*. These figures also indicate that potential consumers of *P. ovata* would be consuming more of Zn compared to the other elements as further corroborated by the dominant quota (41.41%) of the element in *P. ovata*. Since Cd was BDL, potential consumers of this gastropod are thus free from any perceived health threat associated with Cd. The THQ values observed in this research point to the potential threat of Pb. Similarly, the TTHQ value was dominated by Pb again pointing to the potential health risk of the metal even in tandem with the other metals. At this point, it is pertinent to note that the mean concentration of Pb in *P. ovata* exceeded the guideline value/limit for Pb in aquatic produce thus positing the metal as a potential threat to consumers. Lead has been described to be cumulatively toxic (Ashish and Lakhyahira, 2021) [3] with sources of the metal coming from mining, smelting, batteries, pipes, ammunition and paint. In children, the metal is known to cause damage to the brain and nervous system while in adults it increases the risk of high blood pressure, kidney damage and stillbirths (World Health Orgainization, 2022) [29].

Conclusion

The research successfully presented the heavy metal profile of selected metals in *P. ovata* particularly sourced from open markets in Benin Metropolis, Nigeria, with a view of protecting the health of potential consumers and to provide baseline data on the heavy metal content of this species. Indeed, this was a pilot investigation which revealed that the heavy metal of potential threat to human health was Pb via the prolonged consumption of *P. ovata*. It is suggested that future research should embrace or include other heavy metals not covered in the present research and that relevant regulatory bodies should undertake periodic monitoring exercises in order to ascertain the safety of this aquatic mollusc for human consumption.

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