Human Health Risk Assessment of Heavy Metals in Three Species of Mollusks (*Egeria radiata, Limicolaria flammea* and *Viviparus contectus*) from Yenagoa, Bayelsa State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Authors IF, MOM and BAA designed the study. Author IF wrote the first draft of the manuscript and managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

Heavy metals pose a threat to human health and their presence in specific matrices is anthropogenic. The work focuses on the penetration of the food chain through the ingestion of mollusk proteins. This study evaluated the composition of heavy metals (Cd, Cr, Mn, Pb and Zn) in three species of mollusks (*Limicolaria flammea, Viviparus contectus, Egeria radiata*) from Yenagoa, Bayelsa State, Nigeria and the health risk associated with their consumption. Heavy metals concentration was determined by Atomic Absorption Spectrophotometer (AAS). Health risk associated with consumption of the samples were assessed by Estimated Daily Intake (EDI), target Hazard Quotient (THQ) and Carcinogenic Risk (CR). EDI, THQ and CR were done by calculation following standards. Heavy metals concentration range in the samples were: Cd (0.289±0.00 mg/kg - 0.667±0.00 mg/kg), Mn (0.816±0.00 mg/kg - 0.934±0.00 mg/kg), Pb (0.082±0.00 mg/kg - 0.092±0.00 mg/kg) and Zn (4.114±0.00 mg/kg - 8.534±0.00 mg/kg); Cr was not detected in neither of the samples. EDI of heavy metals through consumption of these samples were within acceptable limits for all heavy metals detected. THQ ranges were as follows: Cd (0.4949-1.1420), Mn

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(0.000017-0.000019), Pb (0.0401 - 0.0450) and Zn (0.0016 - 0.0066).   THQ values indicate that there is no human risk concern of risk for humans except Cd (in *E. radiata*). CR values for Cd were as follows: *L. flammea* (3.8×10-3), *E. radiata* (4.34×10-4) and *V. contectus* (1.88×10-4). CR values were within acceptable limits with the exception of Cd in *L. flammea*. The present study shows that the samples contained detectable levels of heavy metals however their consumption do not pose any form of health risk with the except for Cd in *L. flammea*, hence the carcinogenic role of *L. flammea* stands out for its high content of Cd.

1. INTRODUCTION

There are various species of mollusks (aquatic and terrestrial) which are very palatable and serve as major sources of cheap and affordable protein to individuals residing in the oil-rich Niger Delta region of Nigeria. These molluscan species (*Limicolaria flammea, Egeria radiata* and *Viviparus contectus*) are actively harvested and sold at the markets in areas where they abound and have been found to be very nutritious with great positive impact on human health [1]. Mollusks have been widely usedas good indicators of environmental heavy metals pollution by several authors [2-4]. The occurrence of high concentrations of heavy metals in the tissues of edible organisms have been shown to cause several harmful effects including interference with reproduction, maturity and metabolic activities [5,6]. As consumption rates of mollusks increase, an inherent concern on their safety with respect to heavy metals has arisen. Heavy metals enter the food web through various channels such as direct consumption of water or organisms, or through uptake processes, and potential accumulation in edible food, like snails which is mainly due to their feeding mechanisms [4]. Consumption of heavy metals via food negatively impacts on the body's metabolism of animals and humans [7]. Health risk assessment of heavy metals evaluates the impact of crude oil exploration activities and its adverse effects on the locals' health who consume animals or plants products from polluted environments. Health risk assessment of heavy metals is quick method of assessing the effects of pollutants (heavy metals) on human health especially through diet [4]. There are two components in this assessment: non-carcinogenic and carcinogenic effects. The assessment of daily intake via consumption of contaminated animal products (especially seafood) is very necessary for populations which depend on these seafood as their protein sources. The methods employed include Estimated Daily Intake (EDI), Target Hazard Quotients (THQ) and carcinogenic risk (CR). These methods are usually classified as carcinogenic and non-carcinogenic risk. This study evaluated the potential health risks associated with heavy metals (Cd, Cr, Pb) through consumption of *L. flammea, E. radiata* and *V. contectus* from Bayelsa State, Nigeria.

2. METHODOLOGY

2.1 Collection and Preparation of Samples

The three test samples (*L. flammea, E. radiata* and *V. contectus*) were harvested and purchased from Yenagoa in Bayelsa State. A total of 4 kg of each sample was obtained for analyses. Sample collection was done between May and September during the peak periods of their availability., cleaned with deionized distilled water to remove particles of dirt and blotted with tissue paper before transportation to the laboratory for analysis. After collection, the samples were washed properly with tap water and the soft tissues removed from the shells. The samples were thoroughly washed several times and rinsed with distilled water. The samples were dried to constant weight in a laboratory oven (TT-9023A Techmel & Techmel, USA) at 80°C for 72 h. The samples were then ground to fine powder using an electric mill (Crownstar, China).

2.2 Determination of Heavy Metal Concentration

Mineral concentration in the samples was determined using an Atomic Absorption Spectrophotometer (FS240AA, Agilent, USA) according to the American Public Health Association [8].

2.2.1 Sample digestion

Five grams (5 g) of the dried sample was weighed and transferred into a digestion flask and 20 mL of the acid mixture (650 mL conc. HNO_3; 80 mL perchloric acid; 20 mL conc.

H_2SO_4) was added. The mixture was heated in the digestion flask until a clear digest was obtained. It was then transferred to a 50 mL volumetric flask and diluted to 50 mL with distilled water.

2.2.2 Procedure

The AAS machine was calibrated for each metal based on different wavelengths (λ) as follows: Cd (228.80 nm), Cr (357.90 nm), Mn (279.5 nm), Pb (283.30 nm) and Zn (213.9 nm). The extracts were aspirated directly into the AAS machine. The carrier gas consisted of air-acetylene mixture (70Ψ). Samples were atomized prior to determination of their atomic constituents. The sample was thoroughly mixed by shaking and 100 mL of it was transferred into a glass beaker of 250 mL volume, to which 5mL of conc. HNO_3 was added and heated to boil until the volume was reduced to about 18 mL, by adding conc. HNO_3 in increments of 5 mL until all the residue were completely dissolved. The mixture was then cooled, transferred into a volumetric flask, filtered into a measuring cylinder and made up to 100 mL using metal free distilled water, ready for mineral determination. The sample solution was at this point aspirated into the burner system and the concentration of the mineral present was displayed on the recording system against the corresponding absorbance and recorded from the AAS readout.

2.3 Health Risk Assessment of Heavy Metals

The health risks associated with the consumption of the samples analyzed in this study were assessed based on the EDI, THQ and CR of the heavy metals. The standards shown in Table 1 were used for these calculations.

2.3.1 Estimated Daily Intake (EDI)

EDI of heavy metals depends both on the concentration of the metal in the sample and average consumption of the sample. EDI is calculated using the equation below:

$$EDI = \frac{\text{concentration of metals } \times \text{Daily sample intake}}{\text{Average Body weight}}$$

Where average body weight is 60 kg and average daily sample intake is 0.10274 kg/person/day.

2.3.2 Target Hazard Quotient (THQ)

THQ is the ratio between exposure and ingestion reference dose (RfD_{ing}) and it is normally used to express the risk of non-carcinogenic effects. According to this ratio, exposed populations are prone to experience health risks if the value ≥ 1 [4]. Values ≤ 1 consequently indicate no health risk for those metals for the exposed population. THQ was calculated based on the equation below:

$$THQ = \frac{\text{concentration of metals } \times \text{daily sample intake}}{RfDing \times \text{Average Body weight}}$$

Where RfD_{ing} is the ingestion reference dose and average body weight is 60 kg, average daily intake is 0.10274 kg/person/day[4].

2.3.3 Carcinogenic Risk (CR)

CR factor generally assesses cancer risk associated with consumption of toxic substances. The carcinogenic factor is a function of the EDI and ingestion cancer slope factor (CSF_{ing}). The CSF_{ing} evaluate the probability of an individual developing cancer from oral exposure to contaminants levels over a lifetime. CSF_{ing} are expressed in units of $(mg/kg/day)^{-1}$. Lifetime probability of contracting cancer due to exposure to site-related chemicals is calculated as follows:

$$CR = EDI \times CSFing$$

According to toUnited States Environmental Protection Agency (USEPA) standards, 10^{-6} (1 in 1,000,000) to 10^{-4} (1 in 10,000) represent a

Table 1. Toxicological characteristics of heavy metals [4,9]

Metals	RfD_{ing}(mg/kg/day)	CSF_{ing} (mg/kg/day) $^{-1}$
Cd	0.001	0.38
Cr	1.5	0.5
Mn	0.8	0
Pb	0.0035	0.0085
Zn	0.3	0

RfD_{ing} is ingestion reference dose, ingestion cancer slope factor (CSF_{ing})

range of permissible predicted lifetime risks for carcinogens [9]. The chemical for which the risk factor falls below 10^{-6} may be eliminated from further consideration as a chemical of concern. The risk associated with the carcinogenic effect of the target metal is expressed as the excess probability of contracting cancer over a lifetime of 70 years [4].

3. RESULTS AND DISCUSSION

The concentration of heavy metals (Cd, Cr and Pb) in *L. flammea*, *V. contectus* and *E. radiata* from Bayelsa State are shown in Table 2. The ranges of heavy metals were as follows: 0.475±0.00 - 0.667±0.00 mg/kg, 0.816±0.00 - 0.934±0.00 mg/kg, 0.082±0.00 - 0.092±0.00 mg/kg and 4.114±0.00 - 8.534±0.00 mg/kg for Cd, Mn, Pb and Zn respectively. It is evident that the samples had the highest concentration of Zn while Cr was not detected in all samples and Pb was not detected in *E. radiata*. The EDI for the samples are shown in Table 3. The EDI for Cd were 0.001 kg/person/day, 0.000494 kg/person/day and 0.001142 kg/person/day for *L. flammea*, *V. contectus* and *E. radiata* respectively. EDI for Mn was the same for all samples (0.001 kg/person/day) while Pb had 0.00014 kg/person/day and 0.00157 kg/person/

day respectively. EDI values for Zn ranged from 0.007 kg/person/day – 0.014 kg/person/day in the samples analyzed. The results for the THQ of heavy metals through consumption of the samples are presented in Table 4. The heavy metal with the highest THQ for *L. flammea* was Cd (1.0086) while the lowest was Pb (0.0401). The highest THQ value in *V. contectus* was Cd (0.4949) while the lowest was Pb (0.0450). Cd being the only heavy metal detected in *E. radiata* had a THQ value of 1.142. The results of CR are presented in Table 5. CR values for Cd were as follows: *L. flammea* (3.8×10^{-3}), *E. radiata* (4.34×10^{-4}) and *V. contectus* (1.88×10^{-4}). THQ value for Pb was 3.83×10^{-4} in *V. contectus.*

The heavy metals concentration of *L. flammea* (except Cd) in the present study was much lower than those reported by Kadanga [10]. Previous work [11] reported lower levels of Zn and Mn in *Limicolaria spp* compared to the findings of this study while Pb was undetected. Heavy metals concentration in *Archachatina marginata* was much higher than the reported value for *V. contectus* in this study [10]. This may be due to species differences, feeding pattern and the environmental conditions from which the samples were sourced. Literature is scarce on heavy metals concentration in *V. contectus* however

Table 2. Heavy metals concentration (mg/kg) of samples

Metals (mg/Kg)	*L. flammea*	*V. contectus*	*E. radiata*
Cadmium (Cd)	0.475±0.00[b]	0.289±0.00[a]	0.667±0.00[b]
Chromium (Cr)	Nd	nd	nd
Manganese (Mn)	0.816±0.00[a]	0.846±0.00[a]	0.934±0.00[a]
Lead (Pb)	0.082±0.00[a]	0.092±0.00[a]	nd
Zinc (Zn)	4.114±0.00[a]	4.294±0.00[a]	8.534±0.00[b]

Values are presented as mean ± standard deviation (n=3); Values with different superscripts differ significantly (p=.05); nd-not detected

Table 3. Estimated daily intake (EDI) (kg/person/day) of heavy metals through consumption of samples

Metals (mg/Kg)	*L. flammea*	*V. contectus*	*E. radiata*
Cadmium (Cd)	0.001	0.000494	0.001142
Manganese (Mn)	0.001	0.001	0.001
Lead (Pb)	0.00014	0.00157	nd
Zinc (Zn)	0.007	0.007	0.014

nd-not detected

Table 4. Target hazard quotient (THQ) of heavy metals through consumption of samples

Metals (mg/Kg)	*L. flammea*	*V. contectus*	*E. radiata*
Cadmium (Cd)	1.0086	0.4949	1.1420
Manganese (Mn)	0.000017	0.000018	0.000019
Lead (Pb)	0.0401	0.0450	nd
Zinc (Zn)	0.0016	0.0017	0.0066

nd-not detected

Table 5. Carcinogenic risk (CR) of heavy metals through consumption of samples

Metals (mg/Kg)	*L. flammea*	*V. contectus*	*E. radiata*
Cadmium (Cd)	3.8×10^{-3}	1.88×10^{-4}	4.34×10^{-4}
Lead (Pb)	3.41×10^{-4}	3.83×10^{-4}	nd

nd-not detected

the following minerals have been detected in *V. Contectus*: Ca, Mg, Fe, Zn and Mn [12]; Mn and Zn values corroborates with the findings of this study. A previous research work Akpang and Oscar [13] reported composition of Mn values in *E. radiata* which were agreeable with the findings of the present study. According to a previous research Ekpo et al. [14] the composition of Cd, Pb, Zn and Cr in *E. radiata* as follows: 0.006±0.0007 mg/100 g, 0.003±0.0007 mg/100 g, 0.050±0.0009 mg/100 g and 0.027±0.0006 mg/100 g respectively. These findings are similar to the results of this study. A previous report Nwabueze [15] showed that the mean concentrations of heavy metals in *E. radiata* tissues followed an increasing sequence (Cd<Cr<Mn <Pb) which was different from the trend observed in this study (Mn<Cd; Cr and Pb were not detected). The findings of other researchers [16] on heavy metals concentration in *E. radiata* from McIver market in Warri Nigeria were significantly higher than those obtained in this study. Health risk assessment of heavy metals evaluates the impact of crude oil exploration activities and its adverse effects on the locals' health who consume animals or plants products from polluted environments. Consumption of contaminants has become a daily occurrence by locals especially those living in the oil-rich Niger Delta in the South-South region of Nigeria. The health risk assessment of heavy metals is a quick method of assessing the effects of pollutants (heavy metals) on human health especially through diet [4]. EDI for all heavy metals in the samples studied were within safe limits [4]. THQ values are interpreted as either ≥1 (health concerns with respect to that pollutant) [17]. THQ values for Cd were > 1 in *L. flammea* and *E. radiata*. Thus, the consumption of these protein sources is not advised as they pose health threats. However, *V. contectus* THQ was < 1 indicating that its consumption is safe. THQ for Cr and Pb were < 1 for all samples analyzed. Thus, Cr poses no risk through consumption of the samples. The findings of this study indicate that CR values for all heavy metals (Cd, Cr and Pb) in the samples were within the permissible limits (1.88×10^{-4} to 5.99×10^{-4}) except for Cd in *L. flammea* (3.8×10^{-3}). Thus, the consumption of *L. flammea* with respect to carcinogenic risk of Cd must be carefully considered.

4. CONCLUSION

Heavy metals are known to pose threats to the health of humans in many ways. Mollusks due to their habitats and feeding habits tend to bioaccumulate harmful levels of heavy metals in their tissues. The present study showed that *L. flammea, E. radiata* and *V. contectus* from Yenagoa, Bayelsa State contain detectable levels of heavy metals (Cd, Cr, Mn, Pb and Zn). However, their consumption may not pose any health risk to consumers except for Cd in *L. flammea*. This study also improves the baseline data and health risk implications of Cd, Cr, Mn, Pb and Zn marketed in Yenagoa, Bayelsa State, Nigeria and such data provide very vital information on safety of these mollusks commonly consumed by the locals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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