



Human Health Risk Assessment of Heavy Metals in Snail (*Archachatina marginata*) from Four Contaminated Regions in Rivers State, Nigeria

S. C. Onuoha¹, P. C. Anelo¹ and K. W. Nkpaa^{1*}

¹Department of Biochemistry, Faculty of Chemical and Biological Sciences, College of Natural and Applied Sciences, University of Port Harcourt, P.M.B 5323, Choba, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author KWN designed the study, wrote the protocol. Author PCA wrote the first draft of the manuscript and managed the literature searches. Authors PCA and SCO performed the statistical analysis and managed the analyses of the study. All authors read and approved the final manuscript

Article Information

DOI: 10.9734/ACSJ/2016/22163

Editor(s):

- (1) Mohammad Luqman, Department of Basic Science, College of Applied Sciences, A'Sharqiyah University, Oman.
(2) Miguel Miranda, National Laboratory of Engineering and Geology, I.P. (LNEG), Portugal.

Reviewers:

- (1) Preeti Parashar, Dr Bhim Rao Ambedkar University, Agra, India.
(2) Anonymous, Jawaharlal Nehru Technological University, Anantapur, India.
Complete Peer review History: <http://sciencedomain.org/review-history/12347>

Original Research Article

Received 21st September 2015
Accepted 6th October 2015
Published 20th November 2015

ABSTRACT

This study assessed the health risks of heavy metals contamination in snail (*Archachatina marginata*) from crude oil producing regions in Rivers State, Nigeria. Cadmium, lead, chromium, nickel, zinc and manganese concentrations in snail muscle tissue taken from the various regions were detected. The potential non-carcinogenic health risks for consumers were investigated by assessing the Estimated Daily Intake and Target Hazard Quotients. Snail caught in the various sites were more contaminated by cadmium and lead (ranged from 0.50 – 0.65 and 2.60 – 5.00 kg/person/day respectively) than Nickel, Manganese, Zinc and Chromium which were below established reference dose. Target Hazard Quotient values indicate that there is no carcinogenic risk for humans except for lead. Carcinogenic Risk for Nickel (4.1 E-3 – 1.0 E-2) indicate that snail from the study sites may not be safe for human consumption and as such consumers of this have the probability of contracting cancer due to Nickel exposure over a lifetime of 70 years or more in future.

*Corresponding author: E-mail: nkwilly@gmail.com;

Keywords: Health risk assessment; *Archachatina marginata*; estimated daily intake; target hazard quotient; carcinogenic risk.

1. INTRODUCTION

Land snail (*A. marginata*) is a major source of protein and consumed by a vast majority of people living around the dense tropical forests and in surrounding cities where snail farming is now a major preoccupation of the people [1]. *A. marginata* meat is highly nutritious [2]. The meat is low in cholesterol and is a source of vital minerals required for normal tissue development and maintenance [3]. Several authors have reported the importance of molluscs as good indicators for monitoring heavy metal pollution [4-6] even though the abnormally high environmental concentrations of heavy metal affects numerous biological processes involved in the development and maintenance of molluscan populations such as feeding, growth, reproduction, general physiological activities and maturity [7,8]. However, there is a growing concern that heavy metals accumulated in human food (*A. marginata*) may represent a health risk, especially for populations with high consumption rates. Metals enter into the food web through direct consumption of water or organisms, or through uptake processes, and potential accumulation in edible food, like snails. Previous reports have shown that consumption of these contaminated food have potential risk to human [9-12].

Health risk assessment in this study seeks to evaluate the consequences of human activities and weighs the adverse effects to public health against the contributions to economic development. It is one of the fastest methods currently used to evaluate the impact of the hazards on human health [13]. Estimation of potential risks to human health of heavy metals in fishes in this study is divided into carcinogenic and non- carcinogenic effects. These methods are typically based on Estimated Daily Intake (EDI), the Target Hazard Quotients (THQ). The contamination of foods by heavy metals has become an inevitable challenge these days [14]. It is therefore necessary to assess daily intake through the consumption of contaminated seafood. On the other hand, The THQ value is a dimensionless index of risk associated with long term exposure to chemicals based upon reference upper safe limits. THQ was developed by the United States Environmental Protection Agency (USEPA) for the estimation of potential health risks associated with long term exposure to chemical pollutants [9]. This method of risk

estimation has recently been used by many researchers [15,16] and has been shown to be valid and useful. Rivers State, South - South, Nigeria has a tragic history of pollution from oil spills and oil well fires. Pollution due to oil exploration activities and spills in this region is very common and have often lead to contamination of farm and forest lands, rivers, creeks and other water bodies [17]. Information on health risk associated with heavy metals is in terrestrial is still limited. This study evaluated the potential health risks associated with heavy metals via consumption of *A. marginata* from contaminated regions in Rivers State, Nigeria using EDI, THQ and CR.

2. MATERIALS AND METHODS

2.1 Study Area

Four (4) crude oil producing communities' namely: Umuajiloke, Umudike, Eleme and Tai in River State, South-South Region of Nigeria were chosen for this study.

2.2 Collection of Test Samples

A. marginata commonly referred to as the giant land snail were collected from farmlands of four (4) different crude oil producing region in Umuajiloke, Umudike, Eleme and Tai, Rivers State, Nigeria. At each site, five individual snails measuring between 6 – 7 inches in length of the same species were collected, cleaned with de-ionized distilled water to remove observable dirt particles and blotted with tissue paper before transported to the laboratory for analysis.

2.3 Quality Assurance and Quality Control

Double distilled deionized water was used throughout the study, i.e. glassware and plastic ware (Merck, Germany) used were thoroughly rinsed with 10% HNO₃ followed by washing with de-ionized distilled water. For quality control data assurance each sample was analyzed in triplicate. Determination of metals was performed with a GBC Avanta AAS (model: PM ver 2.02 Avanta). Analytical blanks were run in the same way as the samples and concentrations were determined using standard solutions prepared in the same acid matrix. Standards for the instrument calibration were prepared on the

basis of monoelement certified reference solution ICP Standard (Merck, Germany).

2.4 Sample Processing

The samples were carefully removed from its shells and oven dried using Memmert drying oven (U27, Germany) for three days for thorough drying at a temperature of 60 – 70°C, after which it was ground to powder form using Silimic mortar (Pyrex). Five grams of the sample was weighed into a crucible container, and then introduced into a furnace to derive the ash for 6 hours. After 6 hours, a crucible thug was used to carry out the crucible from the furnace into a desiccator and allowed to cool. After cooling, 5 mL of 10% hydrochloric acid was used to dissolve ash content to near dryness. After that, it was filtered into a funnel and measuring cylinder and made up to 20 mL with distilled water for the metal analysis using atomic absorption spectrophotometer.

2.5 Atomic Absorption Spectrophotometer

For each of the metals, atomic absorption spectrophotometer (model pm ver 2.02 Avanta) was calibrated using standard of the metals, which are given below as Cr (λ) = 357.90 nm, Cd (λ) = 228.80 nm, Pb (λ) = 283.30 nm, Ni (λ) = 232.0 nm Zn (λ) = 213.9 nm, Mn (λ) = 279.50 nm. Five grams of the samples was digested in 20 ml 10% hydrochloric acid (HCL) on a heating mantle to near dryness. Cr, Zn, Mn and were analyzed using Hollow Cathode Lamp (HCL) in a Flame atomizer AAS. Cd, Ni and Pb were analyzed using Electrode Less Discharge Lamp (EDL) in the Flame atomizer AAS. The extract was aspirated directly into the atomic absorption spectrophotometer machine. The carrier gas was acetylene and air: 70Ψ. In order to analyze a sample for its atomic constituents, the samples were atomized.

2.6 Estimate of Potential Human Exposure to Heavy Metals in Snails

The health risks associated with the consumption of heavy metal contaminated snails were assessed based on the EDI, THQ and CR of heavy metals. The calculations was based on standard assumption for integrate US-EPA risk analysis, FDA and USDOE standard as indicated on Table 1. Estimated Daily Intake (EDI) of heavy metals (Cr, Cd, Ni, Mn, Pb and Zn) dependent on both the metal concentration in the

snail and the amount of consumption of the respective snail.

The Estimated Daily Intake of metals for adults was determined by the following equation:

$$EDI = \frac{\text{Concentration of Metals} \times \text{Daily Snail Intake}}{\text{Average Body Weight}}$$

Where:

EDI is Estimated Daily Intake

The average adult body weights were considered to be 60 kg while average daily snail intake for adults is considered to be 0.10274 kg/person/day.

2.7 Non-carcinogenic Health Effect

2.7.1 Target Hazard Quotient (THQ)

The health risks from consumption of snails by local inhabitants were assessed based on the Target Hazard Quotient (THQ). THQ is the ratio between exposure and reference oral dose (RfD_{ing}), used to express the risk of non-carcinogenic effects. If the ratio is equal to or greater than 1, an exposed population experiences health risks. The method of estimating risk using THQ was provided in USEPA region 3 risk-based concentration Table 1 [15,18,19], is based on the equation below:

$$THQ = \frac{\text{Concentration of metals} \times \text{Daily snail Intake}}{RfD \times \text{Average Body Weight}}$$

Where:

THQ is Target Hazard Quotient

RfD_{ing} is the oral reference dose (mg/kg/day)

Although the THQ-based assessment method does not provide a quantitative estimate for the probability of an exposed population experiencing a reverse health effect, it indeed provides an indication of the risk level due to exposure to pollutants [20]. RfD_{ing} is an estimate of a daily oral exposure for human population, which does not cause deleterious effect during a lifetime, generally used in EPA's non-cancer health assessment.

Values of RfD_{ing} for Cr, Cd, Mn, Pb, Zn and Ni (mg/kg/day) were taken from Integrated Risk Information System [18,19,21]. The THQ

Table 1. Toxicological characteristics of the investigated heavy metals

USDOE, 2011 USEPA, 2011 FAO/WHO, 1993	Ingestion reference dose	Ingestion carcinogenic slope factor
Metals	RfD _{ing} (mg kg ⁻¹ d ⁻¹)	CSF _{ing} (mg kg ⁻¹ d ⁻¹) ⁻¹
Chromium (Cr)	1.5	0.5
Cadmium (Cd)	0.001	0.38
Lead (Pb)	0.0035	0.0085
Nickel (Ni)	0.02	1.7
Zinc (Zn)	0.3	0
Manganese (Mn)	0.8	0

has been recognized as a useful parameter for evaluating risk associated with consumption of metal contaminated snail.

2.8 Carcinogenic Health Effect

2.8.1 Carcinogenic Risk (CR)

Slope factor are used to assess cancer risk. A slope factor and the accompanying weight-of-evidence determination are the toxicity data most commonly used to evaluate potential human carcinogenic risks. Generally, the slope factor is a plausible upper-bound estimate of the probability of a response per unit of a chemical over a lifetime. The slope factor is used in risk assessments to estimate an upper-bound lifetime probability of an individual developing cancer as a result of exposure to a particular level of a potential carcinogen [18].

For carcinogen, which USEPA identifies by a weight-of-evidence classification of the chemical, the estimated daily dose and the cancer slope factor are multiplied together to find the lifetime cancer risk posed by the chemical. Cancer slope factors are estimates of carcinogenic potency and are used to relate estimate daily dose of a substance over a lifetime exposure to the lifetime probability of excess tumors [18].

2.9 Ingestion Cancer Slope Factors

The Ingestion Cancer Slope Factors evaluate the probability of an individual developing cancer from oral exposure to contaminants levels over a lifetime. Ingestion cancer slope factors 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:

$$\text{Lifetime probability of cancer, Carcinogenic Risk} = \text{EDI} \times \text{CSF}_{\text{ing}}$$

Where:

EDI is the estimated Daily Intake of each heavy metal (mg/kg/day)

CSF_{ing} is Ingestion Cancer Slope Factor (mg/kg/day)⁻¹.

The USEPA [22] states that 10⁻⁶ (1 in 1,000,000) to 10⁻⁴ (1 in 10,000) represent a range of permissible predicted lifetime risks for carcinogens. 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 target metal is expressed as the excess probability of contracting cancer over a lifetime of 70 years.

3. RESULTS

The levels of heavy metals (Cr, Cd, Pb, Ni, Mn and Zn) in snails sampled in Umuajiloke, Umudike, Eleme and Tai, Rivers State, Nigeria are presented in Table 2. The concentration of Cd, Cr, Pb, Ni Mn, and Zn in the four samples ranged from 0.50 – 0.63, 0.001, 2.60 – 5.00, 0.78 – 3.54, 5.97 – 12.1 and 11.6 – 27.6 mg kg⁻¹ respectively (Table 2). This study also indicates that Zn among the metals had the highest concentration in the seafood investigated in Eleme, while Cr was the lowest in the three study sites, though. Zn had higher contamination level than Mn and Pb in all the samples from the four sites of study. The level of each metals determined in this study was above the maximum permissible limit by US EPA [18], however, Cr was below the minimum permissible limit of 102 mg kg⁻¹ set by US EPA [18].

The estimated daily intakes (EDI) for individual snails are shown in Table 3. The trend of EDI of heavy metals in all the samples is in the order Pb>Zn>Mn>Ni>Cd>Cr. The EDI of Cr from consumption of *A. marginata* had value of 2.0 E-6 mg kg⁻¹ bw/day; this value was the same for all four snail samples collected from the four

sites. The EDI value is very much below the tolerable daily intake level ($1.5 \text{ mg kg}^{-1} \text{ bw/day}$) established by, USEPA, [18] and USDOE [19] FAO/WHO [21]. In a similar manner, the EDI of Cd was $0.001 \text{ mg kg}^{-1} \text{ bw/day}$. The EDI of Ni from the consumption of these snails was in the range $0.001 - 0.006 \text{ mg kg}^{-1} \text{ bw/day}$ with snail collected from Umudike showing the highest EDI value ($0.006 \text{ mg/kg bw/day}$). Snail collected from Tai had the lowest EDI value ($0.0013 \text{ mg kg}^{-1} \text{ bw/day}$). While the EDI of Mn ranged between $0.011 - 0.020 \text{ mg/kg bw/day}$ with the highest Mn intake from snail collected from Eleme. The EDI values of Pb and Zn from the consumption of snail from the four different sites had ranges of $0.066 - 0.0004 \text{ mg kg}^{-1} \text{ bw/day}$ and $0.047 - 0.02 \text{ mg kg}^{-1} \text{ bw/day}$ respectively, with the highest Pb intake from *A. marginata* collected from Tai, the highest while the Zn EDI in snail from Eleme was the highest. The provisional tolerable intake for Pb and Zn are set at 0.5 and 0.3 mg/kg bw/day respectively by, 1993, USEPA, [18] USDOE, [19]. FAO/WHO [21].

The estimated Target Hazard Quotient (THQ) of heavy metals through the consumption of snails collected from four crude oil contaminated regions in rivers state are shown in Table 4. The THQs for Cd ranged from 0.840 – 1.00, which falls below the standard THQ of value of less than 1. The THQ of Cr from the consumption of snail was 0.0000011 for all four samples from the four regions; it was less than 1. In the same manner, the THQ values of Ni, Mn, and Zn were ranged between 0.070 – 0.300, 0.013 – 0.026, 0.067 – 0.157 respectively, there values were less than 1. Conversely, Pb had a THQ range between 1.257 – 18.86 and it was more than 1.

The averages Carcinogenic Risk (CR) of Cd, Cr, Ni, Mn, Pb and Zn through the consumption of snails from four crude oil contamination regions in rivers state are shown in Table 5. The average carcinogenic risk for Cd, and Ni ranged between $3.4\text{E-}4 - 3.2\text{E-}1$ and $4.1 \text{ E-}3 - 1.0 \text{ E-}2$ respectively, with Umudike showing the highest CR value of $3.2\text{E-}1$ and $1.0\text{E-}2$ respectively.

Table 2. Heavy metals concentration in snail from crude oil contaminated regions (Umuajiloke, Umudike, Eleme and Tai) in Rivers State, Nigeria

Heavy metal	Umuajiloke	Umudike	Eleme	Tai
Cadmium	0.55±0.01	0.50±0.01	0.65±0.11	0.53±0.11
Chromium	0.001±0.02	0.001±0.12	0.001±0.02	0.001±0.15
Nickel	0.82±0.01	3.54±0.02	1.43±0.03	0.78±0.03
Manganese	5.97±0.03	6.23±0.03	12.1±0.14	8.05±0.03
Lead	5.00±0.03	4.40±0.13	2.60±0.02	3.90±0.02
Zinc	21.10±0.02	11.6±0.02	27.6±0.03	16.5±0.01

Table 3. Estimated Daily Intake (EDI) (kg/person/day) of heavy metals through consumption of snail from crude oil contaminated regions (Umuajiloke, Umudike, Eleme and Tai) in Rivers State, Nigeria

Heavy metals	Umuajiloke	Umudike	Eleme	Tai
Cadmium	0.001	0.001	0.001	0.001
Chromium	0.0000017	0.0000017	0.0000017	0.0000017
Nickel	0.001	0.006	0.002	0.001
Manganese	0.010	0.011	0.020	0.014
Lead	0.008	0.007	0.004	0.066
Zinc	0.034	0.020	0.047	0.028

Table 4. Target Hazard Quotients (THQ) of heavy metals through consumption of snail from crude oil contaminated regions (Umuajiloke, Umudike, Eleme and Tai) in Rivers State, Nigeria

Heavy metals	Umuajiloke	Umudike	Eleme	Tai
Cadmium	0.930	0.840	1.000	0.890
Chromium	0.0000011	0.0000011	0.0000011	0.0000011
Nickel	0.070	0.300	0.120	0.065
Manganese	0.013	0.014	0.026	0.018
Lead	2.400	2.114	1.257	18.86
Zinc	0.120	0.067	0.157	0.093

Table 5. Carcinogenic Risks (CR) of heavy metals through consumption of snail from crude oil contaminated regions (Umuajiloke, Umudike, Eleme and Tai) in Rivers State, Nigeria

Heavy metals	Umuajiloke	Umudike	Eleme	Tai
Cadmium	3.5E-4	3.2E-1	3.8E-4	3.4E-4
Chromium	8.5E-7	8.5E-7	8.5E-7	8.5E-7
Nickel	2.4E-3	1.0E-2	4.1E-3	2.2E-3
Lead	7.1E-5	6.3E-5	3.7E-4	5.6E-4

The CR value of Cr for all four snail samples was the same, 8.5 E-7. Also the CR values for Pb ranged between 7.1 E-5 – 5.6 E-4, with Tai showing the highest value.

4. DISCUSSION

Heavy metals are considered the most important constituents of pollution from the terrestrial environment due to toxicity and accumulation by land organisms, such as snails. The entire snail samples collected from the sites contained detectable levels of the elements studied. The accumulation of these heavy metals in snails may represent a health risk, especially for populations with high consumption rates [23,24]. Therefore estimated daily intake or ‘tolerable intake’ is widely used to describe ‘safe’ levels of intake of heavy metals. Chromium has often been described as an essential trace element in humans and some animals [25]. The EDI for Cr in this study was below the established tolerable daily intake of 1.5 mg/kg Bw./day [18,19,21]. The low EDI of Cr through the consumption snails from these study sites could adversely affect balanced glucose metabolism in the population. Cr has been reported to facilitate insulin action [26,27]. The high EDI recorded for Cd in snails in this study is an indication that consumption of snails from these contaminated regions, Umuajiloke, Umudike, Eleme and Tai in Rivers State may lead to severe ingestion of Cd. The mean EDI of Pb in the snails from the study sites far exceeded the RfD values recommended by international regulatory bodies (FAO, WHO, USEPA and USDOE). The introduction of Pb into the food chain may affect human health and thus, studies concerning Pb accumulation in snails are of importance. Ingestion of Pb through the consumption of snails under study may cause mental retardation among children and also hypertension in pregnant women [28]. The estimated daily intake of Mn Ni, and Zn were below established RfD recommended by, USEPA, [18] and USDOE, [19] FAO/WHO, [21]. This may however, not pose a risk to human health.

Target Hazard Quotient (THQ) through the consumption of snails is a measure of chemical

contaminants [29]. It is not a measure of risk but indicates a level of concern. The interpretation of the THQ value is binary; THQ is either > 1 or < 1, where THQ >1 indicates a reason for health concern [30]. The observed THQ of Cr was significantly less than 1 indicating that consumers of snails from these sites are not exposed health risk of chromium. The recorded THQ of Cd was observed to be 1 for Eleme, indicating cause for concern as consumers of snails from this community may experience health effect of chromium. THQ value for Umuajiloke, Umudike, and Tai regions was < 1; hence consumers of snails from these sites are not exposed to the health risk of Cd. Ni, Mn and Zn. This observation is suggestive that consumers of snails from the study sites would not experience significant health risks from intake of individual metals through snail consumption. However, the observed THQ value of Pb which was > 1 may be of concern to consumers of snail from the study sites. Li, et al. [31] reported that highest THQ value poses relatively higher potential health risk to human beings particularly for the people residing in the area with serious metal pollution.

Carcinogenic Risk (CR) is estimated and expressed as a probability of contracting cancer over a lifetime of 70 years. The average value of cancer risk from Cr and Pb accumulation did not show carcinogenicity. However, in comparison with established guideline values, data from this study is suggestive that *A. marginata* collected from Umudike may not be safe for consumers as it shows high CR for Cd, the probability of contracting cancer over a lifetime of 70 years. Similarly, *A. marginata* from Umuajiloke, Umudike, Eleme, and Tai may not be safe for human consumption and as such consumers of this have the probability of contracting cancer due to Ni exposure over a long lifetime of 70 years or more in future.

5. CONCLUSION

This present study showed that precautionary measures need to be taken in order to prevent further heavy metal pollution. It also improves the

base line data and health risk implications of Cr, Cd, Ni, Mn, Pb and Zn in land snail (*A. marginata*) commonly marketed in Umuajiloke, Umudike, Eleme and Tai regions in Rivers State, Nigeria. Such data provide valuable information on safety of snails commonly consumed by the public.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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