

## Health Risk of Lead Poisoning in Four Edible Snail Samples Obtained from Bayelsa State, Nigeria

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### Abstract

Over the years, lead has been a major environmental nuisance, and lead poisoning is a significant epidemic in many countries in the world including Nigeria. Most often, lead poisoning has been identified as a chronic environmental disease which later develops long-term adverse health effects. However, this study investigated the concentration, fractionation, and potential health risk of lead in four edible snails (*A.achatina*, *L.flammea*, *P.aurita*, and *T.fuscatus*) obtained from Bayelsa State, Nigeria using Flame Atomic Absorption Spectrometer (FAAS). The mean concentrations of lead (mg/kg dry wet basis, mean±SD) were; *A.achatina* (29.5±5.41), *L.flammea* (8.00±1.00), *P.aurita* (37.7 ± 2.47), and *T.fuscatus* (27.8 ± 2.89). These values were higher than the permissible limits of FAO/WHO and FEPA. Speciation analysis showed that the water soluble fractions were below the limits of WHO and FEPA. Polar and non-polar fraction were below detection limits (BDL), indicating non-availabilities of polar and non-polar lead species in the snails. While the residual fractions were higher than the acceptable limits of WHO and FEPA. Health risk assessments results revealed that the chronic daily intake (CDI) of lead in the snails were in the decreasing order of *P.aurita* > *A.achatina* > *T.fuscatus* > *L.flammea* with values of 15.52, 12.14, 11.14, and 3.29 respectively. These values are higher than the provisional daily intakes of lead set by WHO and FEPA. The non-carcinogenic health risks of lead in the snails were generally low (THQ = HI < 1), indicating non-cancer adverse health risk at the moment. However, the carcinogenic risk indices of lead in the snails were within the threshold values of  $1.0 \times 10^{-6} - 1.0 \times 10^{-4}$  set by USEPA. Therefore, considering the bioaccumulative nature of lead, these snails should be consumed moderately

**Keywords:** Lead poisoning; Lead levels; Snails, Risk assessment; Bayelsa State; Nigeria.

### Introduction

Lead poisoning is an old environmental epidemic which is presented everywhere. It is a type of metal poisoning caused by lead in a biological organism. In human, the brain is the most sensitive [1]. Symptoms include abdominal pain, nausea, vomiting, diarrhea, constipation, headache, irritability, weight loss, memory problems, infertility, damage of the nervous system and kidneys, weakness in fingers, wrists, or ankles, increase in blood pressure and in severe cases, anemia, seizures, coma, or death may occur [2].

Lead poisoning and its adverse effects were known to the ancients, since BC due to high lead levels in the environment. In the 2<sup>nd</sup> century BC a Greek botanist Nicander described the colic and paralysis seen in lead-poisoned people [3]. Lead was also used extensively in Roman aqueducts from 500 BC to 300 AD [4]. According to Julius Caesar's engineer, Vitruvius, "water is much more wholesome from earth ware pipes than from lead pipes, since it is made injurious by lead because white lead is produced by it which is harmful to the human body [5].

Recent studies have also revealed the adverse effects of lead poisoning due to high Lead levels in the ecosystem. In March 2010, Team members came from the CDC-Nigeria office in Abuja, the Nigerian Federal Ministry of Health, the Nigerian

Field Epidemiology and Laboratory Training program, the World Health Organization, and Medicines Sans Frontieres (MSF), and discovered an epidemic of lead poisoning in Zamfara State, North Western Nigeria which affected at least 10,000 people and led to the death of hundreds of people [6]. The source of the outbreak was associated with artisanal gold ore processing that occurs in that region [7]. The exposure pathways were drinking water, food, inhalation of contaminated dust, oral ingestion of particles particularly by children and breast feeding. In 2013, the World Health Organization estimated that lead poisoning resulted in 143,000 deaths, and contributed to 6,000,000 new cases of children with intellectual disabilities” each year [8]. Since 2014, lead contamination in drinking water has been an issue in the US City of Flint, Michigan. The source of the contamination has been attributed to corrosion in the lead and iron pipes that distributed water to the citizens [9]. Also, lead levels in North-Eastern Tasmania, Australia, were reported to reach over 50 time’s national drinking water guidelines in 2015. The contamination source was attributed to a combination of dilapidated drinking water infrastructure, including lead jointed pipelines, end-of-life polyvinyl chloride and household plumbing [10].

Humans can be exposed to lead and lead compounds via contaminated air, water, dust, food or consumer products due to human activities such as fossil fuel burning, mining, smelting, battery, wire or pipe manufacturing and so on [11,12]. Children are more vulnerable to lead, because most often they put objects in their mouth such as those that contain lead paint and absorb a greater proportion of lead [2]. While in adults, the most common exposure to lead poisoning is via occupational factors at a particular risk [13]. Diagnosis is typically by measurement of the blood level [2], elevated lead levels may also be detected through changes in red blood cells or dense lines in the bones of children as seen on X-rays [14].

Land Snails (*A. achatina* and *L. flammea*) and Marine Snails (*P. aurita* and *T. fuscatus*) are the most common gastropods found in terrestrial and aquatic media used as important sources of animal protein for many coastal communities in Nigeria, Bayelsa State in particular. Many consumers in Nigeria obtain their snails from the conventional wild life source [15]. These organisms are easily harvested by local residents, and are typically processed for consumption and commercial purposes. Since the snails are filter feeders, they are likely to accumulate lead and other biotoxins in their tissue which are very dangerous to human health [16]. Thus, lead accumulation due to regular intake of these snails could pose a serious health risk to Bayelsans and communities in Nigeria. Therefore, it is imperative that continuous investigation be carried out to assess the lead levels in snails and health risk associated with lead exposure in these snails.

The United States Environmental Protection Agency (USEPA) health risk assessment method [17-20] has been employed by many researchers [21-26] to determine the quantitative risks of heavy metals exposure to humans through the consumption of contaminated food, water, air and soil, which are proven valid and useful.

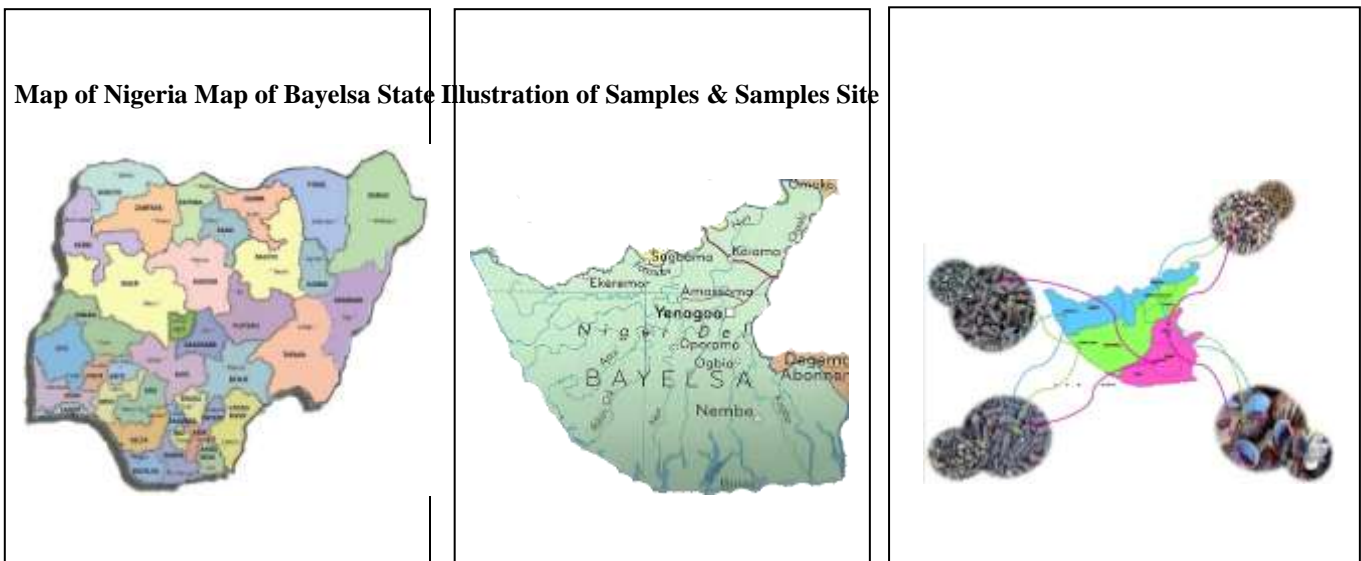
Therefore, this study is aimed at investigating the lead levels and its fractionation in four edible snail samples and assesses the health risks associated with the consumption of this organism by humans in Bayelsa State, Nigeria.

## **Materials and Methods**

### **Study area**

Bayelsa State is located in the Southern part of Nigeria with an area of about 21110 square kilometers. Geographically the state is located within latitude 04° 15” North, 05° 25” South and longitude 05° 22” West and 06° 45” East. The state lies in the tropical rain forest belt and has the heaviest rainfall in Nigeria with short dry season (from November to March). The state shares boundary with Delta State in the North, Rivers State in the East and the Atlantic Ocean in the West and South. More than three quarters of the state is covered by water with moderately low land stretching from Ekeremor to Nembe. The major occupations in the state are fishing, farming, palm wine tapping and local gin, trading, carving and weaving. However, Bayelsa is a major state in Nigeria in which there is massive oil exploration and exploitation accounting for over 30% of the Nigerian oil production. There are hundreds of oil wells and flow station across the state. These and many more other activities no doubt, have release lead and it compounds into the environment of Bayelsa State.

**FIG.1. Map of Nigeria, Bayelsa State and illustration of samples and samples site.**



**Sampling**

In this study, sample collections were based on the three senatorial districts in Bayelsa State. The land snails (*A. achatina* and *L. flammea*) were collected from Yenagoa, Ogbia and Sagbama local government areas of Bayelsa State, Nigeria. While the marine snails were collected from Nembe, Southern Ijaw and Ekeremor Local Government Areas of Bayelsa State, Nigeria. The spatial distribution of the sampling sites is shown in **FIG. 1**.

**Sample preparation and analysis**

Upon collection, the snail samples were wrapped in a cellophane bag labelled accordingly and transported to the department of Animal and Environmental Biology, University of Port Harcourt, Nigeria where they were identified properly. The edible parts (soft tissues) were obtained by cracking the shells that house the soft tissue. Thereafter, samples were thoroughly washed several times and rinsed with distilled water, dried in the oven at 105° C to a constant weight, and then ground into

fine powder. The snail samples were digested using 10ml of 3:1 (v/v) of con HCl/NHO<sub>3</sub> (aqua regia) and 1 ml of HClO<sub>4</sub> for total lead concentration [27].

Fractionation analysis of lead in the samples were carried out using three stage sequential extraction procedures with water, (1 g of samples in 15 ml of distilled water), hexane (10 ml in the residues of fraction one, F<sub>1</sub>), and methanol (10ml CH<sub>3</sub>OH/DCM,8:2) in to the extraction bottles containing the dried residues of fraction two, F<sub>2</sub>. All mixtures for each stage were kept on an electrical shaker for 24 hours and centrifuged 3500 rpm for 15minutes to obtain:

- Water soluble fractions (F<sub>1</sub>)
- Hexane (non-polar) fractions (F<sub>2</sub>)
- Methanol (polar/fractions (F<sub>3</sub>).

Then, the residual fractions were obtained by digesting the dried residues obtained from fraction three (F<sub>3</sub>) with aqua regia cone HCl/NHO<sub>3</sub> and 1ml HClO<sub>4</sub> [28,29].

Lead concentrations were analyzed using flame atomic absorption spectrometer, (FAAS) GBC, Avanta ver 2.02 models. The fuel used was acetylene gas, while the oxidant is compressed air. For quality assurance/quality control program, lead concentrations were calculated with reference to a standard curve and calibration of the instrument was done by preparing standard solutions of lead and analyzing the certified reference material (CRM) with samples. All samples were analyzed in triplicate and the results obtained from this analysis were the average of triplicate determination.

#### **Health risk assessment for snail consumption**

The exposure assessments from consuming lead contaminated snail were evaluated using the Chronic Daily Intake (CDI), Target Hazard Quotient (THQ) and Target Cancer Risk (TR) as per USEPA Region III Risk-Based concentration TABLE 1 [19].

**Chronic Daily Intake (CDI):** The chronic daily intakes (EDI) of lead via consumption of the snails were evaluated using the formula;

$$CDI (mg/kg bw /day) = \frac{MI_s \times MC}{BW_a} \quad (1)$$

Where MI<sub>s</sub> is the mass of the snail ingested per day (the per capital consumption of fish and shell fish in Nigeria for human food is averaged 9.0 kg which is equivalent to (24.7 g per day) 30, MC is the concentration of lead in snail, BW<sub>a</sub> is the body weight of the consumers (60 kg for adults).

**Target Hazard Quotient (THQ):** To assess the health risk consumption of these snail species, the target hazard quotient (THQ) values were calculated by using the measured concentrations of lead in the snails. The THQ is an estimation of risk level for non-carcinogens due to pollutant exposure, and is the ratio between measured concentration and the oral reference dose (R<sub>FD</sub>), weighed by the length and frequency of exposure, amount ingested and body weight. The THQ was calculated using the formula established by the USEPA given as follows;

$$\frac{EF \times ED \times MI \times MC}{Rfd \times BW_a \times AT_n} \times 10^{-3} \quad (2)$$

Where EF is the exposure frequency (365 days/year), ED is the exposure duration (51.86years), which corresponds to an average life expectancy of a Nigerian [30]. AT<sub>n</sub> is the averaging exposure time for non-carcinogens (365 days/year × ED) .

Note MI, MC and  $BW_a$  have been previously defined in equation (1).  $R_{FD}$  is the oral reference dose ( $Lead = 3.5 \times 10^{-3} \text{ mg/kg per day}$ ), [17,24] which is an estimate of daily exposure to human population that is likely to be without an appreciable risk of deleterious effect during life time. Thus, THQ index value  $< 1$  is assumed to be safe over a lifetime [17-19].

**Target Cancer Risk (TR):** The TR index value was used to assess carcinogenic risks. The acceptable level of TR according to USEPA and WHO is respectively less than  $1 \times 10^{-6}$  (one cancer in 1,000,000 people) and less than  $1 \times 10^{-4}$  (one cancer in 10,000 people) in some circumstance [19,31]. The TR of lead due to the ingestion of snails in Bayelsa State was calculated as follows;

$$TR = \frac{EF \times ED \times MI_s \times CPS_o \times MC}{BW_a \times AT_c} \times 10^{-3} \quad (3)$$

Where TR is the target cancer risk,  $CSP_o$  is the carcinogenic potency slope, oral ( $Pb = 8.5 \times 10^{-3} \text{ mg/kg bw/day}$ )[32]. ED for carcinogens is 70 years (that is an incremental probability of individual developing cancer over a life time of 70 years)[19].  $AT_c$  is the averaging time, carcinogens ( $365 \text{ days/year} \times ED$ ). Recall, EF,  $MI_s$ , and MC have been given in equation I and II. **TABLE I** shows the summary statistics of input parameters in the health risk assessment of lead via four edible snails intake from Bayelsa State, Nigeria.

## Result and Discussion

**TABLE 2** shows the concentrations, CDI, THQ and TR values of lead in four edible snail samples from Bayelsa State. Lead concentrations in the four snails samples ranged from 23.5 – 34.0  $\text{mg/kg}$  with mean value of  $29.5 + 5.41 \text{ mg/kg}$  for *A. achatina*, *L. Flammea* values ranged from 7.00 – 9.00  $\text{mg/kg}$  with mean value of  $8.00 + 1.00 \text{ mg/kg}$ , *P. aurita* values ranged from 36.0-40.5  $\text{mg/kg}$  with mean value of  $37.7 + 2.47 \text{ mg/kg}$  and *T. fuscatus* ranged from 24.5 – 29.5  $\text{mg/kg}$  with mean value of  $27.8 + 2.89 \text{ mg/kg}$ . Among the four edible snails studied, *P. aurita* has the highest mean value of lead while *L. flammea* recorded the lowest lead concentration. Thus, the concentrations of lead in the four snail samples are in the decreasing order of  $P. aurita > A. achatina > T. fuscatus > L. flammea$ , respectively.

The concentrations of lead in these snails are all higher than the standards of WHO, FEPA, UNEP, EC and international criteria which are 0.01, 2.0, 0.3, 0.2 and 0.1 ( $\text{mg/kg}$ ) [33-37] as shown in **TABLE 2**. The concentrations of Pb in this study are also higher than the values reported by Alinor and Obiji [39], Ubalua [40] in fish and shell fish from Nworie River and Aba River, Nigeria. The high Pb concentration in these snails is a source of concern considering the fact that Pb has no biological or beneficial role in the human system rather, research showed that high levels of Pb causes permanent damage to the central nervous system, brain, liver, kidney and higher levels result in death [2,3].

TABLE 1. Summary statistics of input parameter of lead assessment via Snails Intake.

Abbreviation	Description	Unit	Values
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MIS	Mass of fish ingested	g/day	
MC	Metal (Pb) concentrations	Mg/kg	Presented in table 2
BW <sub>a</sub>	Body weight adult	Kg	60
EF	Exposure frequency	Days/year	365
ED	Exposure duration	Year	51.86
R <sub>f</sub> D	Oral reference dose	Mg/kg/day	Pb = $3.5 \times 10^{-3}$
AT <sub>n</sub>	Averaging time non-carcinogens	Days	$365 \times 51.86 = 18928.9$
AT <sub>c</sub>	Averaging time carcinogens	Days	$365 \times 70 = 25550$
CPS <sub>o</sub>	Carcinogenic potency slop, oral	Mg/kg/day	Pb = $8.5 \times 10^{-3}$

TABLE 2. Concentrations (*mg/kg*) EDI, THQ and TR of Pb in four edible snails samples from Bayelsa state, Nigeria and standard guide line values.

Snail sample	Range	Mean $\pm$ SD ( <i>mg/kg</i> )	CDI	THQ	TR
<i>A. achatina</i>	23.5-34.0	$29.5 \pm 5.41$	12.14	$3.5 \times 10^{-3}$	$1.0 \times 10^{-4}$
<i>L. flammea</i>	7.00-9.00	$8.00 \pm 1.00$	3.29	$9.41 \times 10^{-4}$	$2.8 \times 10^{-5}$
<i>P. aurita</i>	36.0-40.5	$37.7 \pm 2.47$		$4.4 \times 10^{-3}$	$1.3 \times 10^{-4}$
<i>T. fuscatus</i>	24.5-29.5	$27.8 \pm 2.89$		$3.3 \times 10^{-3}$	$9.7 \times 10^{-5}$
Standard Guideline Values					
WHO[33]		0.01			
FEPA[34]		2.0			
UNPA[35]		0.3			
EC[36]		0.2			
International Criteria[37]		0.1			

WHO[38]			0.0035		
USEPA[17-19]				$\leq 1$	$1 \times 10^{-6} - 1 \times 10^{-4}$
WHO[31]					$1 \times 10^{-6} - 1 \times 10^{-4}$

The assessment of health risk in human through the consumption of contaminated food is dependent on the contaminant concentration in the food and the amount consumed. The chronic daily intake is the maximum amount of contaminant to which a person can be exposed per day over a life time without appreciable risk of health effects. The Target Hazard Quotient (THQ) is the ratio of determined dose of a pollutant or contaminant to a reference dose level. That is a daily oral intake rate that is estimated to pose no appreciable risk of adverse health effects, even to sensitive populations over 51.86 years (non-carcinogens) for life time. THQ is either less than (<)1 or equal to(=)1, where THQ is > 1 indicates reasons for concerns.

As indicated in **TABLE 2**, the CDI of Pb in the snail samples are the decreasing order of *P. aurita* > *A. achatina* > *T. fuscatus* > *L. flammea* with values of 15.52,12.14,11.44 and 3.29 respectively. These values are higher than the provisional tolerable daily intake of Pb set by WHO [38] which is 0.0035 mg/kg. THQ values of Pb in the four edible snails are in the order of *P. aurita* > *A. achatina* > *T. fuscatus* > *L. flammea* with values of  $4.4 \times 10^{-3}$ ,  $3.5 \times 10^{-4}$ ,  $3.3 \times 10^{-3}$  and  $9.41 \times 10^{-4}$ . These values are all lower than the THQ values of 1 set by USEPA for non-carcinogens, indicating no potential adverse health risk to local consumers at the moment.

The Target Cancer Risk (TR) for the four edible snails were also calculated and presented in **TABLE 1**. The TR values are also in the order of *P. aurita* > *A. achatina* > *T. fuscatus* > *L. flammea* with values of  $1.3 \times 10^{-4}$ ,  $1.0 \times 10^{-4}$ ,  $9.7 \times 10^{-5}$  and  $2.8 \times 10^{-5}$ . However, these values are within the acceptable values of USEPA and WHO which ranged from  $10^{-6} - 10^{-4}$  respectively. Therefore, excessive consumption of these snails should be avoided in order to prevent health risk in future.

#### Lead speciation

**TABLE 3** showed the speciation analysis of lead concentrations in the four edible snail samples and CDI, THQ, TR values. The concentration of Pb in the water soluble fraction are as follows; *A. achatina* values ranged from 1.5 – 1.44 mg/kg ( $1.23 \pm 0.16$ ), *L. flammea* values ranged from 0.75-2.55 mg/kg ( $1.43 \pm 0.79$ ), *P. aurita* values ranged from 0.30 – 4.50 mg/kg ( $1.95 \pm 1.83$ ) and *T. fuscatus* values ranged from 1.05-2.10 mg/kg ( $1.5 \pm 0.44$ ). The concentration of Pb in the hexane (non-polar) and methanol (polar) fractions were below detection limits (BDL), indicating non-bioavailability of polar and non-polar species of Pb in these snails. However, in the residual fraction, the concentration of lead in the snails ranged from 22.20-30.0 mg/kg ( $27.3 \pm 3.57$ ) in *A. achatina*, from 5.75-6.75 mg/kg ( $6.40 \pm 0.46$ ) in *L. flammea*, from 27.55-38.00 mg/kg ( $34.35 \pm 4.85$ ) in *P. aurita* and from 25.80-27.00 mg/kg ( $26.72 \pm 0.06$ ) in *T. fuscatus*. *P. aurita* also ranks the highest with a mean value of 34.35 mg/kg while *L. flammea* ranks the lowest with a mean value of 6.40 mg/kg. Generally, the mean concentration of Pb in the water soluble fraction were below the standards of WHO [33] and FEPA [34], but the values

obtain from the residual fraction were all higher than the standards of WHO and FEPA. These values were also higher than the values of Pb reported by Ibe [41] in catfish (*Claria gariepinusi*).

The health risk assessment of the four edible snail samples pose potential health adverse effect with respect to the chronic daily intakes of Pb which is 0.0035 mg/kg set by WHO [38]. The residual fraction pose the highest risk with *P. aurita* ranking the highest with value of 14.14 and *L. flammea* ranks the lowest with value of 2.635. However, THQ values for non-carcinogenic effects of Pb in the snail samples were all below the standard of USEPA ( $THQ > 1$ ), while the TR values for all the fractions were within the acceptable limits of  $1 \times 10^{-6} - 10^{-4}$  set by USEPA, and WHO with the residual fraction posing the highest health risk. The THQ and TR values of Pb in this study are similar to the values reported by Yadolah [32] in bottle water from Bandar Abbas Ciry, Iran.

TABLE 3. Speciation of Pb (mg/kg) and CDI, THQ and TR of Pb in four edible snail samples from Bayelsa state, Nigeria.

Fraction	Snails	Range	Mean $\pm$ SD (mg/kg)	CDI	THQ	TR
Water soluble water	WA	1.50-1.44	1.23 $\pm$ 0.16	0.502	1.4 $\times$ 10 <sup>-4</sup>	4.3 $\times$ 10 <sup>-6</sup>
	WL	0.75-2.55	1.43 $\pm$ 0.79	0.579	1.7 $\times$ 10 <sup>-4</sup>	5 $\times$ 10 <sup>-6</sup>
	WP	0.30-4.50	1.95 $\pm$ 1.83	0.803	2.3 $\times$ 10 <sup>-4</sup>	6.8 $\times$ 10 <sup>-6</sup>
	WT	1.05-2.10	1.5 $\pm$ 0.44	0.618	1.8 $\times$ 10 <sup>-4</sup>	5.2 $\times$ 10 <sup>-6</sup>
Hexane (no-polar fraction)	HA	BDL	BDL	-	-	-
Hexane (no-polar fraction)	HL	BDL	BDL	-	-	-
	HP	BDL	BDL	-	-	-
	HT	BDL	BDL	-	-	-
Methanol (polar) fraction	MA	BDL	BDL	-	-	-
Methanol (polar) fraction	ML	BDL	BDL	-	-	-
	MP	BDL	BDL	-	-	-
	MT	BDL	BDL	-	-	-
Residual fraction	RA	22.20-30.0	27.3 $\pm$ 3.57	11.241	3.2 $\times$ 10 <sup>-3</sup>	9.6 $\times$ 10 <sup>-5</sup>
Residual fraction • WA, WL, WP & WT = water	RL		6.40 $\pm$ 0.46	2.635	7.5 $\times$ 10 <sup>-4</sup>	2.2 $\times$ 10 <sup>-5</sup>
	RP		34.35 $\pm$ 4.85	14.14	4.0 $\times$ 10 <sup>-3</sup>	1.2 $\times$ 10 <sup>-4</sup>
	RT		26.72 $\pm$ 0.06	10.99	3.1 $\times$ 10 <sup>-3</sup>	9.35 $\times$ 10 <sup>-4</sup>



<p>soluble fractions of <i>A. achatina</i>, <i>L. flammea</i>, <i>P. aurita</i>, and <i>T. fuscatus</i>.</p> <ul style="list-style-type: none"> <li>• HA, HL, HP &amp; HT = the hexane (non-polar) fractions of <i>A. achatina</i>, <i>L. flammea</i>, <i>P. aurita</i>, and <i>T. fuscatus</i>.</li> <li>• MA, ML, MP &amp; MT = methanol (polar) fractions of <i>A. achatina</i>, <i>L. flammea</i>, <i>P. aurita</i>, and <i>T. fuscatus</i>.</li> <li>• RA, RL, RP &amp; RT = the residual fractions of <i>A. achatina</i>, <i>L. flammea</i>, <i>P. aurita</i>, and <i>T. fuscatus</i>.</li> </ul> <p>BDL = Below Detection Limits.</p>						
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**Conclusion**

This study has investigated the concentration, speciation and health risk of Pb in four edible snail samples. The total Pb content in the snails was all higher than the standards set by regulatory bodies. The speciation analysis revealed that the water soluble fraction were below the limits of WHO and FEPA, the polar and non-polar fraction of Pb in the snails were not detected, while the residual fraction values were all higher than the WHO and FEPA standards. The CDI values of Pb in the snails were all higher than the provisional tolerable daily intake of Pb set by WHO. The THQ values of all the snails were < 1, indicating non-carcinogenic health risk at the moment. However the TR values of Pb in these snails were within the threshold value set by USEPA, indicating a potential threat to consumers in future. Therefore, continuous monitoring of Pb and other heavy metals in these snails should be carried out regularly to prevent adverse health risk in future via snail consumption.

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