



## Research Article

**DETERMINATION OF HEAVY METALS AND GLYPHOSATE IN ACHATINA ACHATINA(SNAILS) COLLECTED IN NAWA REGION (SOUTH WESTERN CÔTE D'IVOIRE) AND EVALUATION OF THE HEALTH RISKS WITH THEIR CONSUMPTION****\*N'Guessan N. O<sup>1</sup>, Kouassi K. D<sup>2</sup>, Kouamé K. V<sup>3</sup>, Yapi D. A. C<sup>4</sup> and Kouadio K. N<sup>5</sup>**<sup>\*1,2,3,4</sup>Laboratoire Biodiversité et Ecologie Tropicale (BioEcoTrop)<sup>\*</sup>Doctorant à Université Jean Lorougnon Guédé, Daloa, Côte d'Ivoire, Ivoirien<sup>5</sup>Laboratoire d'Environnement et de Biologie Aquatique, Université de Man. Ivoirien**ARTICLE INFO****Article History:**Received 12<sup>th</sup> October, 2022Received in revised form 23<sup>rd</sup>

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Snails, heavy metals, exposure, Nawa.

**ABSTRACT**

Due to their herbivorous and scavenger diet, snails are contaminated by various pollutants present in the soil and flora. However, the edible part of *Achatina* is one of the most consumed meats in some regions of Africa, particularly in Côte d'Ivoire. This study aims to determine the concentrations of mercury, cadmium, lead, copper, zinc and glyphosate in *Achatina achatina* and evaluate risks of exposure associated to their consumption. Analytic methods used for the determination of heavy metals and glyphosate are respectively atomic absorption spectrophotometry and HPLC. The results revealed the presence of heavy metals and glyphosate in the cephalopod *Achatina Achatina*. The hazard quotient assessment of exposure to chemical pollutants revealed that consumers are most exposed to cadmium because hazard quotient is greater than 1 in all locations for both children and adults. The remaining chemical pollutants have hazard quotients below 1. However, consumers are not exempt from the remaining chemical pollutants. So, people should consume *Achatina* meat in moderation.

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**INTRODUCTION**

Trace metals and active substances are pollutants generated in general by human activity (Naert *et al.*, 2017). They have everyday a toxicological impact on plants, consumer products, and humans (Ben Ghnaya *et al.*, 2013). These pollutants are considered as serious environmental pollutants because of their persistence and tendency to bioaccumulate in living organisms (Schuurmann and Markert, 1998). However, naturally available food resources play an important role in the diet of populations (Sodjinou *et al.*, 2002). Among these resources, we have African land snails or achatines which belong to the Achatinidae family. Snails have been consumed throughout the world since prehistoric times and remain one of the most popular meats in Europe, Africa and the Americas (Dafem *et al.*, 2008). Unfortunately, they are bio-accumulative animals of trace metals. Metal concentration in invertebrate tissues varies considerably not only between taxa, but also between individuals of the same species (Tyler *et al.* 1989). The form in which the pollutant appears is probably related to the physiological properties of the species and not the trophic level (Spurgeon & Hopkin 1999). Metal content in invertebrates depends on ingestion conditions, the physiological characteristics of the species, and especially the type of food (Lindqvist & Block 1997, Purchart & Kula 2007). The giant African snail feeds on detritus that may be contaminated with heavy metals and organic pollutants. Thus, this

animal could accumulate these pollutants in its organs or tissues at varying levels (Viard *et al.*, 2004). Also, bioaccumulation involves a food web (Scheifler, 2002). Thus, it goes up to the consumer of higher level such as the man, more exposed than any other link of the chain (Miquel, 2001). In Côte d'Ivoire several studies have shown the contamination of fish by heavy metals (Coulibaly *et al.*, 2018; Coulibaly *et al.*, 2013; Coulibaly *et al.*, 2012; Kouamé, 2020). On the other hand, very little work has been done to assess the risks of heavy metal and glyphosate contamination by snails. Moreover, very little research has been conducted on the bioaccumulation of metals and glyphosate in the giant African snail consumed in Côte d'Ivoire. The purpose of this study is to determine the concentrations of mercury, cadmium, lead, copper, zinc and glyphosate in the flesh of the *Achatina achatina* snail and to assess the health risks associated with its consumption.

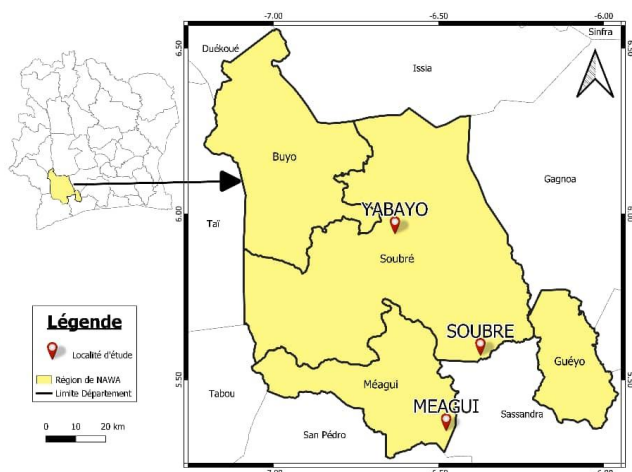
**MATERIALS AND METHODS****Study area**

The present work was carried out in the Nawa region of southwestern Ivory Coast (Figure 1). This region is located between 5°60' and 9°50' North latitude and 6°0' and 8°20' West longitude and covers an area of 9193 km<sup>2</sup> with a population of 314,192 inhabitants (INS, 2014). Three localities (Yabayo, Soubré, Meagui) were selected for this

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study. The Yabayo site is located between 5°35'17.052 N and 6°22'25.14 W. The Soubré site is located between 5°57'19.93 N and longitude 6°37'56.57 W. As for the Meagui site, it is located between 5°21'38.75 N and longitude 6°28'75 W. The choice of these sites is justified by the fact that these three localities are supply areas for the Ivorian markets in snails.



**Figure 1** location of the study area

### Biological material

This study focused on a species of land snail belonging to the family Achatinidae. It is *Achatina achatina* (Fig.2). It is the most prized species in Ivory Coast in general and by the populations of the study area in particular.



**Figure 2** *Achatina achatina*

Eight (8) snails were collected from under the cocoa orchards after a rainfall in each of the three locations. The collected samples were placed in a stomacher bag and stored in a cooler before being transported to the laboratory the next morning.

### Preparation of snail samples

Once in the laboratory, snails were fasted for two days (48 hours) to remove unabsorbed food and feces from their digestive tract. The live weight, shell length and shell diameter of each snail were determined before dissection. This was done according to the method described by Chukwujindu *et al.*, (2009).

### Mineralization and determination of heavy metals

Mineralization for the determination of lead, copper, zinc and cadmium was performed according to AOAC method 999.10 (2003). For mercury, the official AOAC method No. 977.13 was adopted (AOAC, 1978). For the digestion of each sample, a 0.5 g test sample of shredded material is placed in a Teflon tube under a fume hood. Then 5 mL of 65% concentrated nitric acid (HNO<sub>3</sub>) and 15 mL of 37% hydrochloric acid (HCL) are successively added to the shredded material in the

ratio (HNO<sub>3</sub>/HCL1/3). Another Teflon tube containing bidistilled water is used as a blank (neutral control of the elements to be investigated or treated). To this blank tube, the same reagents that were used for the matrix digestion are added in the same order. The Teflon tubes are put in a water bath for 1 hour (1H) at a temperature of 90°C for the digestion of the content. The Teflon tubes are removed and placed on the bench for cooling. Thirty minutes (30) later, a decompression of 5 minutes is done under the hood by opening the Teflon tubes to let the gas escape. After cooling, the mixture is filtered into a 100 mL volumetric flask and supplemented with bidistilled water up to the mark and then homogenized by manual stirring. Then, the solutions obtained after the mineralization are transferred into a clean tube bearing the references of the samples and kept at 4°C until the analysis.

### Quantification of metallic trace elements

The detection of the metallic trace elements was done with the help of the Atomic Absorption Spectrophotometer with graphite furnace, controlled by the LC solution software equipped with a non-specific background corrector (Deuterium lamp), an automatic sample changer. A calibration was done before the preparation of the standard solutions for each metal with increasing concentrations of 10ug/l, 800ug/l, and 1500ug/l. The coefficient of determination of each element chosen is between 0.995 and 1. The metal contents in mg/kg (ppm) were obtained from the concentrations read directly on the spectrometer.

### Determination of glyphosate content

Twenty-five (25) g of snail meat samples are taken and weighed into a 50ml conical tube. Then, demineralized water is added up to the 50ml gauge, the whole is vortexed for 3 min. The resulting solution V<sub>2</sub> is filtered into another 50ml conical tube. At the end, a corresponding volume of 10% TCA (trichloroacetic acid) is added to V<sub>2</sub> up to the 50 ml gauge and then vortexed for 3 min. The whole is filtered into another 50 ml graduated conical tube to obtain the volume V<sub>3</sub>.

### Preparation of samples for quantification

To 1 ml of the diluted V<sub>3</sub> solution, 1 ml of the tetraborate solution (5%) and 1 ml of the FMOC solution are added in order. The resulting solution is stirred for 1 hour in the dark at room temperature, then centrifuged at 4500 rpm for 5 min. The supernatant is collected in a vial and ready for HPLC detection and quantification.

### Method for assessing the health risks associated with the consumption of snails contaminated with heavy metals

The assessment of health risks related to the consumption of snails contaminated by trace metals followed three main steps: the identification of the hazard, the choice of toxicological reference values (TRV) and the assessment of population exposure (Bisson *et al.*, 2009). This leads to the calculation of the daily exposure dose. By assumption, it was considered that the average daily quantity of snail ingested by a child is the same as that ingested by an adult. This quantity is determined by a field survey of households in the study area. This interview revealed that people consume an average of 6 *Achatina achatina* snails per meal every two weeks.

### Metal concentrations in cephalopods

$$C = \frac{Cs \cdot Vf \cdot Fd \cdot 0,001}{P}$$

With

C= final concentration in mg/kg,

Cs=concentration of the element in the solution

Vf= final volume of the sample,

Fd= dilution factor

P= sample weight in g

### Daily Exposure Dose (DEL)

$$DEL=C \cdot Q \cdot F/P$$

With

TDI=daily exposure to trace elements (mg/kg/d)

C=trace element concentration in snails

F=frequency of exposure (F=1) without unit

P=body weight of the target

Q= amount of snails ingested per day (kg/d)

The quantity Q of snails ingested per day was calculated by considering that the cephalopod (the consumed part of the snail) represents, according to a study carried out in Ivory Coast by Otchoumou (2005), 30% of the live weight of the snail and by taking into account the 6 *Achatina achatina* snails consumed per meal then the measurement of the weight of the snails in the laboratory. Table I presents the toxicological reference values of acceptable daily intake (ADI) of the heavy metals and glyphosate analyzed.

**Table 1** Toxicological reference values of acceptable daily doses (ADI) of the heavy metals and glyphosate analyzed.

Trace elements Metallic	Sources	Toxicological Reference Values
Cadmium	Bisson (2009)	0,0002 mg/kg/day
Copper		0,140 mg/kg/day
Zinc		0,3 mg/kg/day
Lead		0,0036 mg/kg/day
Glyphosate	ANSES (2019)	0,5 mg/kg/day
Mercury	Bisson (2018)	0.00066 mg/kg/day

**Table 3** Health risks of eating lead contaminated *Achatina achatina* snails in the sampled localities

Lead	Average heavy metal concentration C (mg/kg)	Average weight of snails (Kg)	Quantity of snails eaten per day Q (kg/day)	Target weight (kg)		Daily exposure dose TDI (mg/kg/day)		Acceptable Daily Intake ADI (mg/kg/day)	Healthrisks (Hazard Quotient)	
				Adulte	Child	Adulte	Child		Adulte	Child
Soubre	0,27	0,096875	0,174375			0,00068	0,002		0,19	0,57
Meagui	0,25	0,16275	0,29295	70	28	0,001	0,003	0,0035	0,29	0,85
Yabayo	0,24	0,1745	0,3141			0,001	0,003		0,29	0,85

**Table 4** Health risks of eating cadmium contaminated *Achatina achatina* snails in the sampled localities

Cadmium	Average heavy metal concentration C (mg/kg)	Average weight of snails (Kg)	Quantity of snails eaten per day Q (kg/day)	Target weight (kg)		Daily exposure dose TDI (mg/kg/day)		Acceptable Daily Intake ADI (mg/kg/day)	Healthrisks (Hazard Quotient)	
				Adulte	Child	Adulte	Child		Adulte	Child
Soubre	0,127	0,096875	0,174375			0,0003	0,0008		1,5	4
Meagui	0,12	0,16275	0,29295	70	28	0,0005	0,0012	0,0002	2,5	6
Yabayo	0,13	0,1745	0,3141			0,0006	0,0014		3	7

### Hazard Quotient (HQ)

$$HQ= DEL/TDI$$

If

HQ >1 the appearance of a toxic effect cannot be excluded

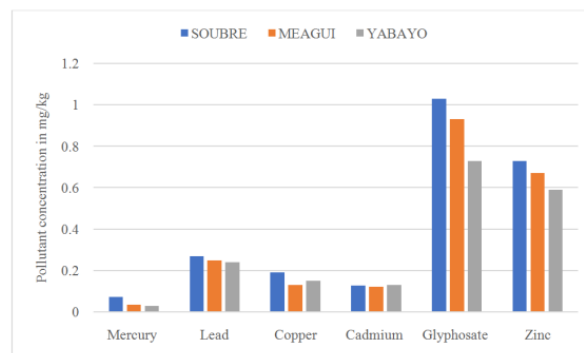
HQ <1 the appearance of a toxic effect is very unlikely

### Statistical analysis

## RESULTS

### Concentrations of TMEs and glyphosate in the flesh of the snail *A. achatina*

Figure 3 below shows the content of chemical pollutants in the flesh of *Achatina achatina*.



**Figure 3** Heavy metal and glyphosate levels in the snail *A. achatina*

Results obtained after analysis of snail samples collected in the Nawa region reveal the presence of heavy metals and glyphosate in the cephalopod (Fig. 3). The average values of these toxic substances in snail meat vary from one locality to another and from one element to another. The lowest values are recorded in the order mercury, cadmium, copper and lead. Zinc and glyphosate levels are relatively higher in snail meat. Mercury levels ranged from 0.029 mg/kg (Yabayo) to 0.072 mg/kg (Soubre), while lead levels ranged from 0.27 mg/kg (Yabayo) to 0.24 mg/kg (Soubre). Copper levels ranged from 0.13 mg/kg (Meagui) to 0.19 mg/kg (Soubre), while cadmium levels ranged from 0.12 mg/kg (Meagui) to 0.3 mg/kg (Yabayo). Glyphosate concentrations range from 0.73 mg/kg

(Yabayo) to 1.03 mg/kg (Soubre). Zinc levels ranged from 0.59 mg/kg (Yabayo) to 0.73 (Soubre).

**Population exposure assessment**

The results of the exposure assessment for heavy metals and glyphosate related to snail ingestion and the corresponding hazard quotient (HQ) for adults and children are presented in Tables 3 to 8.

The hazard quotients of lead and mercury are less than 1. However, since they have no beneficial activity in living organisms, a harmful effect cannot be excluded even at very low concentrations because there is no threshold below which

**Table 5** Health risks of eating copper contaminated *Achatina achatina* snails in the sampled localities

Copper	Average heavy metal concentration C (mg/kg)	Average weight of snails (Kg)	Quantity of snails eaten per day Q (kg/day)	Target weight (kg)		Daily exposure dose TDI (mg/kg/day)		Acceptable Daily Intake ADI (mg/kg/day)	Healthrisks (Hazard Quotient)	
				Adulte	Child	Adulte	Child		Adulte	Child
Soubré	0,19	0,096875	0,174375			0,00048	0,0011		0,0032	0,008
Meagui	0,13	0,16275	0,29295	70	28	0,0005	0,0014	0,15	0,004	0,009
Yabayo	0,15	0,1745	0,3141			0,0007	0,0017		0,0045	0,011

**Table 6** Health risks of eating zinc contaminated *Achatina achatina* snails in the sampled localities

Zinc	Average heavy metal concentration C (mg/kg)	Average weight of snails (Kg)	Quantity of snails eaten per day Q (kg/day)	Target weight (kg)		Daily exposure dose TDI (mg/kg/day)		Acceptable Daily Intake ADI (mg/kg/day)	Healthrisks (Hazard Quotient)	
				Adulte	Child	Adulte	Child		Adulte	Child
Soubré	0,73	0,096875	0,174375			0,002	0,004		0,007	0,013
Meagui	0,67	0,16275	0,29295	70	28	0,003	0,007	0,3	0,01	0,023
Yabayo	0,59	0,1745	0,3141			0,003	0,007		0,01	0,023

**Table 7** Health risks of eating glyphosate contaminated *Achatina achatina* snails in the sampled localities

Glyphosate	Average heavy metal concentration C (mg/kg)	Average weight of snails (Kg)	Quantity of snails eaten per day Q (kg/day)	Target weight (kg)		Daily exposure dose TDI (mg/kg/day)		Acceptable Daily Intake ADI (mg/kg/day)	Healthrisks (Hazard Quotient)	
				Adulte	Child	Adulte	Child		Adulte	Child
Soubré	1,03	0,096875	0,174375			0,002	0,006		0,004	0,012
Meagui	0,93	0,16275	0,29295	70	28	0,004	0,010	0,5	0,008	0,020
Yabayo	0,73	0,1745	0,3141			0,003	0,008		0,006	0,016

**Table 8** Health risks of eating mercury contaminated *Achatina achatina* snails in the sampled localities

Mercure	Average heavy metal concentration C (mg/kg)	Average weight of snails (Kg)	Quantity of snails eaten per day Q (kg/day)	Target weight (kg)		Daily exposure dose TDI (mg/kg/day)		Acceptable Daily Intake ADI (mg/kg/day)	Healthrisks (Hazard Quotient)	
				Adulte	Child	Adulte	Child		Adulte	Child
Soubré	0,072	0,096875	0,174375			0,00018	0,00045		0,28	0,68
Meagui	0,035	0,16275	0,29295	70	28	0,00015	0,00037	0,00066	0,28	0,56
Yabayo	0,029	0,1745	0,3141			0,00013	0,00033		0,2	0,5

Analysis of Tables 3, 4, 5, 6, 7 and 8 reveals that the hazard quotient (HQ) for cadmium in adults and children in all localities is greater than 1. In fact, in all localities, the hazard quotients for children are four, six and seven times higher respectively in Soubré (HQ =4), Meagui (HQ =6), and Yabayo (HQ =7). We also note that the hazard quotient is greater than 1 for adults in Soubré (DQ=1.5), Meagui (HQ =2.5) and Yabayo (HQ =3). The hazard quotients for lead and mercury are less than 1 for all three sites sampled, for both children and adults. Copper, zinc, and glyphosate all have hazard quotients strictly below 1 at all sites for both adults and children.

**DISCUSSION**

Heavy metals and glyphosate were observed in *Achatina achatina* samples after analysis. The mean concentrations of lead, mercury and cadmium in the snail cephalopod from the three sampled localities were all above the toxicological reference values set by WHO, INERIS and ATSDR (daily standards) for oral exposure to these metals, respectively.

these three metals (Hg, Pb, Cd) do not produce undesirable effects (WHO 2005).

The average lead concentrations in snails sampled at the three sites exceeded sixty-six (66) times the WHO standard. However, the positive effect of boiling on the flesh of achatine could reduce the risk of lead contamination. In their study on the effect of two cooking methods (frying and boiling) on the concentration of lead and cadmium, Adamou *et al* (2019) showed that the concentration of lead decreased by 2 to 78% after boiling, depending on the location. Lead (Pb) is also a powerful poison to the nervous system and can cause irreversible damage to a child's development (Roeckx, 1986).

As for cadmium, its average concentrations in the snails sampled in the three localities exceed six hundred (600) times the toxicological reference value set by ATSDR (daily standards), i.e. 0.0002 mg/kg/day for exposure to this metal by the oral route. The health risk related to the consumption of this species of snail is very high because of its cumulative toxicity whose biological half-life is about 20 to 30 years

(Loubna, 2009). Furthermore, this result is very alarming because of the negative effect of cooking temperature on cadmium. Adamou *et al* (2018) highlighted the negative effect of cooking on cadmium. According to these authors, the concentration of cadmium in the flesh of *Archachatina marginata* species increases from 0% to 119% after boiling and from 144 to 219% after frying. Children are the most exposed. These results are similar to those obtained by Adamou *et al.* (2018). This is confirmed by Hounkpatin *et al.* (2012) who justify it to their low body weight and their physiological fragility since contaminants are easily absorbed in their body. Indeed, children's bodies potentially absorb more contaminants and remain unable to eliminate them as easily as adults since their elimination systems are less developed (Hounkpatin *et al.*, 2012). Mariam *et al.* (2004) reported average levels of 0.33 ppm, 0.37 ppm and 0.31 ppm Cd in beef, sheep and poultry, respectively. Saviperumal *et al.* (2007) found concentrations ranging from below the detectable level to 0.98 mg/kg Cd in some mollusc species in India. Wegwu and Wigwe (2006) further observed a cadmium concentration of 0.60 to 0.84 mg/kg. The cadmium levels found in this study are lower than those reported by the above authors, but lower than the 5.7 mg kg<sup>-1</sup> of cadmium reported by Viard *et al.* (2004) on land snails collected near a highway in France.

Average mercury concentrations differ from one locality to another. In fact, the mercury content of snails in Soubré is one hundred and nine (109) times higher than the norm. The level in Méagui is fifty-three (53) times higher than the standard. The locality of Yabayo also records a high concentration, forty-three (43) times higher than the toxicological reference value. It is considered by the WHO as one of the ten chemicals of extreme concern for public health and especially a threat to the development of children at an early age. Its slight liposolubility allows it to easily cross the placental barrier (Palkovicova *et al.*, 2008) as well as the pulmonary alveoli and to pass into the bloodstream through which it is distributed in different organs (Cercy and Wankmuller, 2008) where it is able to induce oxidative stress by affecting intracellular levels of reduced GSH (Sener G *et al.*, 2007).

The average concentrations of glyphosate in Méagui, Soubré and Yabayo are all above the ANSES (2019) guideline. The high concentration of glyphosate recorded in Soubré could be explained by the fact that the samples were taken under cocoa trees that are only treated with the herbicide GLYPHADER, of which glyphosate is the active substance. The occurrence of a toxic effect from glyphosate is very unlikely because its hazard quotient is less than 1. However, it still remains a potential threat to public health. Indeed, the gardener Dewayne Johnson had the firm Monsanto (group producer of glyphosate) condemned to pay 78.5 million American dollars responsible for his cancer.

With respect to copper, only the average concentration in the Méagui locality is below the standard. The values of the other two localities are higher than the standard. As for zinc, its average concentration in the snails of the three sites was found to be two (2) times higher than the standards established by INERIS (2009). Having a role in the metabolism of living beings, and a hazard quotient lower than 1, the occurrence of a toxic effect related to copper and zinc is very unlikely.

## CONCLUSION

This study assesses population exposure to heavy metals and glyphosate in the flesh of *Achatina achatina* edible by people in the Nawa region. *Achatina achatina* snail is one of the heavy metal and glyphosate accumulating species. The snails studied all concentrate cadmium above the accepted standards. Consumption of snails from the Nawa region presents a probable hazard to human health. All these results require that additional measures have to be taken to preserve human health, biodiversity and integrate sustainable development to ensure the nutritional quality of snails, an important source of protein to be developed in this century where food security is on the agenda. We only recommend the consumption of snails from controlled breeding conditions.

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