

Assessment of the Content of Metallic Trace Elements (Cu, Zn, Pb, Cd, Hg) in the Tissue of *Achatina Fulica* Snails Boiled and Consumed by the Population of the Town of Daloa (Côte d'Ivoire)

Kacou Alain Paterne Dalogo^{1,4*}, Ano Guy Serge Ehouman^{2,4}, Adé Hortense Hampoh³,
Djamatchè Paul valery Akesse¹, Djédoux Maxime Angaman², Karim Sory Traore³

¹Laboratory of Environmental Sciences and Technologies, Jean Lorougnon Guede University, BP 150 Daloa, Côte d'Ivoire

²Agrovalorisation Laboratory, Jean Lorougnon Guede University, BP 150 Daloa, Côte d'Ivoire

³Nutrition and Food Security Laboratory, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire

⁴Laboratory of Environmental Sciences, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire

Abstract

The giant *achatina fulica* snails that have recently been introduced to West Africa are increasingly consumed by people in Côte d'Ivoire because they are readily available in urban areas, particularly in shallows, marshy areas and rubbish dumps. The presence of snails in these potentially polluted areas exposes them to metallic contamination, especially as these molluscs have a great capacity to accumulate these toxic compounds. The aim of this study was to evaluate the level of contamination by metallic trace elements (TMEs) (Cu, Zn, Pb, Cd, Hg) in boiled *achatina fulica* snails consumed by the population of the town of Daloa in west-central Côte d'Ivoire. The eighteen (18) snail samples taken from six (6) sampling sites were boiled before analysis using an inductively coupled plasma optical emission spectrometer (ICP-OES). The results show that the snails sampled were heavily loaded with the oligo-elements Cu and Zn, with average concentrations reaching 343 ± 39.38 mg/Kg and 177 ± 3.218 mg/Kg respectively. Pb and Cu were detected in levels that even exceeded the maximum acceptable reference concentrations for molluscs at some sites. Hg was very weakly present in the samples, with the maximum average concentration not exceeding 0.007 ± 0.0008 mg/Kg.

Keywords: *achatina fulica*, metallic trace elements, contamination, maximum reference concentrations

*Correspondence

Author: Kacou Alain Paterne Dalogo
Email: pak_dalogo@yahoo.fr

Introduction

The snail is a terrestrial, shell-covered mollusc of the gastropod class that is eaten in its natural state by humans and animals [1]. In Africa, the giant snail species belonging to the Achatinidae family is highly prized by many populations [2, 3], who mainly eat its flesh, which has proven nutritional properties. A source of protein, snail meat also contains minerals (Mg, Fe, Ca, K), vitamins (B, D, E) and omega-3 fatty acids, all of which contribute to the proper functioning of the nervous system [4-7]. According to a study by Mbétid-Bessane [8], snail flesh is the most prized and consumed "bush meat" in Côte d'Ivoire, after the aulacode, with almost 17,000 tonnes consumed each year. In Daloa, in the centre-west of the country, *achatina fulica* snails, which are invasive and ubiquitous species originating in East Africa, are becoming increasingly present in consumers' culinary practices. Indeed, compared to the species *achatina achatina* (Linné, 1758) or *archachatina marginata*, which were once prized by the Ivorian population [5], the *achatina fulica* species are much more accessible and widely available. These snails, commonly known as "all-near snails", are mostly collected in urban and peri-urban areas, near cultivated land, low-lying areas, marshes and household and industrial waste dumps, which are generally sites with high concentrations of chemical pollutants. These pollutants include metallic trace elements. TMEs are compounds that are present in all environmental compartments, either naturally or as a result of human activity. They pose numerous public health problems because of their persistence, mobility and toxicity [9-11]. They can therefore affect organisms directly by accumulating in their bodies or indirectly by transfer via the food chain [12]. Because of their place in the terrestrial ecosystem, snails are capable of integrating multiple sources of contamination (soil, atmosphere, plants) by various routes (digestive, respiratory and/or cutaneous). They

are bioaccumulative species, capable of absorbing and concentrating in their bodies certain toxic chemical substances such as heavy metals. Several authors, including Swaileh et al [13], have found several heavy metals such as copper (Cu), zinc (Zn), cadmium (Cd) and lead (Pb) in the feet and viscera of different snail species in varying concentrations. Viard [14], and Etorh et al [15]. The consumption of snails therefore represents a risk of slow but definite toxicity for the population. Although studies have investigated the health risks associated with the consumption of *Archachatina marginata* and *Limicolaria* spp snails contaminated with heavy metals, particularly in Benin [16], very little attention has been paid to the level of TMEs contamination in *achatina fulica* species, particularly in Côte d'Ivoire where they have been introduced in the recent past.

The aim of this study is therefore to assess the level of contamination in boiled *achatina fulica* snails consumed by the population of the town of Daloa.

Material and Methods

Study area

The study was carried out in the town of Daloa, in central-western Côte d'Ivoire, 383 km from Abidjan, the economic capital. It covers an area of 5,305 km² and will have a population of 4,279 in 2021. It comprises around 40 districts, six (6) of which have been selected for this study. These are: Tazibouo Université, quartier Suisse, Abattoir 1 and 2 and Orly 1 and 2. The figure below (**Figure 1**) shows the geographical map of the study area and the various sampling sites.

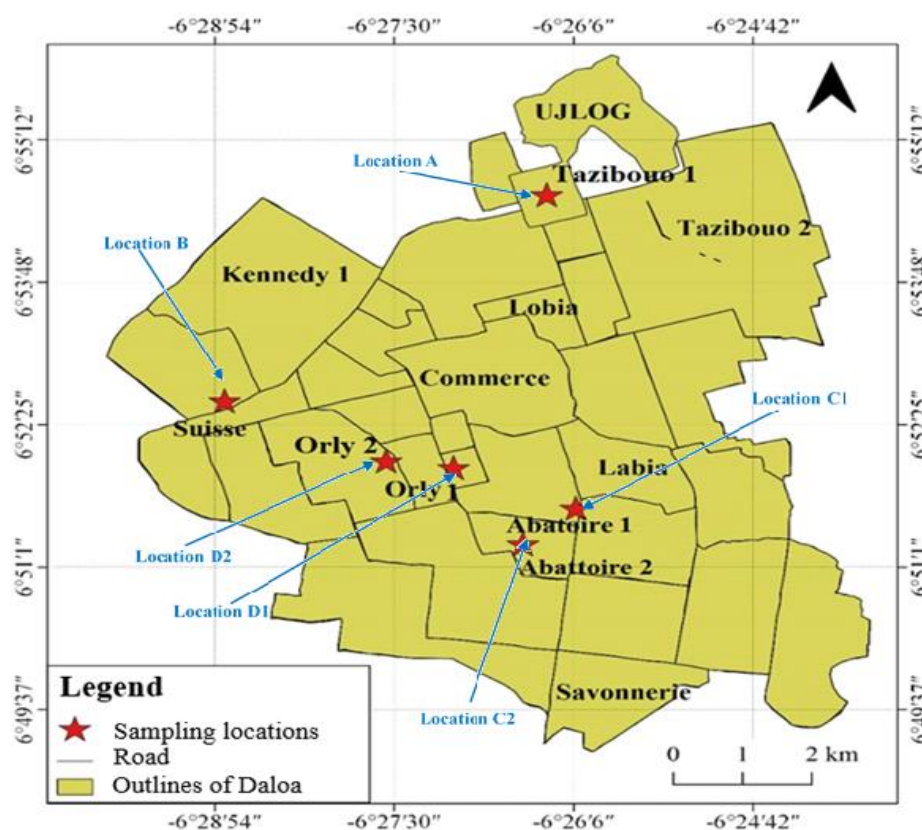


Figure 1 Location of the study area and sampling sites

Biological material

This study concerns a species of snail belonging to the Achatinidae family. This is the *Achatina fulica* or *Lissachatina fulica* (**Figure 2**).

Methodology

Food survey

The survey provided essential information on people's eating habits, and on when and where the snails studied (*achatina fulica*) are procured or collected. It emerged that these snails are collected in urban areas, particularly in low-lying areas and other wetlands. Consumer interest in this species is justified by the fact that supply is less restrictive than for other

species, which are found in remote fields or bought at high prices. The survey also revealed that, in terms of cooking habits, the boiled form was more common than the grilled form for the majority of consumers.



Figure 2 Photography of *Achatina fulica* in its living environment

Sampling

The information collected during the survey enabled us to choose the various neighbourhoods and, more specifically, the snail sampling sites. Low-lying areas where local residents are accustomed to collecting snails were therefore preferred. A total of eighteen (18) samples were taken from the six (6) sites selected following the survey (Figure 1), with three (3) samples per site. The snails were then placed in plastic bags before being transported to the laboratory, where they were fasted for 48 hours to rid them of unabsorbed food and faeces from their digestive tract before cooking.

Cooking snails and pre-treatment

Before cooking, the snails are sacrificed by freezing them at -80° for 48 hours and then dissected; the soft bodies are removed from the shells using forceps. They are then washed several times in distilled water and heated until boiling. After cooking, the soft tissues were dried to constant mass at 85°C and stored for further analysis.

Mineralisation of samples

Dried snail soft tissue samples placed in silica crucibles were calcined at 550°C using a muffle furnace until ash was obtained to facilitate metal extraction [17]. The 0.5 g of ash obtained per sample was digested in Falcon tubes by adding 2 mL of 50% concentrated nitric acid (HNO_3). The solution was stirred on a hot plate to obtain a homogeneous, transparent solution. After cooling in ambient air, the mineralisate was filtered through $0.45\ \mu\text{m}$ Whatman filter paper into a 50 mL volumetric flask. The filtrate was then made up to the mark with distilled water and kept at room temperature for subsequent analysis.

Determination of the TMEs of interest

The molecules of interest are determined using the Avio 100 inductively coupled plasma optical emission spectroscopy (ICP-OES) technique. The computer system is controlled by Syngitix ICP Continuous operating software.

Statistical analysis of the data

The statistical data were processed using STATISTICA 7.1 and Sphinx Me software. The descriptive statistics were used to determine the mean concentrations and standard deviations of the trace metals found in the samples analysed.

Results

Contamination of snails by metallic trace elements

The choice of sites was based on the responses of the populations at the various collection sites of *Achatina fulica* snails whose level of contamination by TMEs is being studied. These sites are Tazibouo Université (site A), Quartier Suisse (site B), Abattoir 1 (site C1), Abattoir 2 (site C2), Orly 1 (site D1), Orly 2 (site D2).

The results obtained after analysing the samples showed that snails from the various sites were contaminated with trace metals. **Table 1** shows the average levels of copper, zinc, cadmium, lead and mercury in the boiled snails analysed.

Table 1 TMEs content of various snails sampled (mg/kg)

ETM	Site A	Site B	Site C1	Site C2	Site D1	Site D2
Cu	0,35±0,050	126±27,22	50,6±21,68	185±25,30	343±39,38	41±2,271
Zn	0,156±0,036	111±11,40	98,9±3,218	177±3,218	139±25,95	163±26,60
Cd	0,11±0,013	1,13±0,180	0,167±0,041	0,763±0,130	1,22±0,15	1,61±0,45
Pb	0,12±0,044	1,9±0,455	5,05±0,314	0,237±0,033	0,18±0,028	1,18±0,052
Hg	0,003±0,000	0,054±0,013	0,007±0,0008	0,003±0,000	0,005±0,000	0,004±0,001

We note that all the ETMs we researched were found in varying concentrations in the samples taken from all six sites: Tazibouo University (site A), Swiss (site B), Abattoir 1 (site C1), Abattoir 2 (site C2), Orly1 (siteD1), Orly2 (siteD2). We note that copper and zinc are the most abundant elements found in samples from sites B, C1, C2, D1 and D2, in comparison with site A, where concentrations of these TMEs are almost non-existent (**Figure 3**).

The classification in ascending order of recorded levels is as follows:

- Site A: Cu > Zn > Pb > Cd > Hg
- Site B: Cu > Zn > Pb > Cd > Hg
- Site C1: Zn > Cu > Pb > Cd > Hg
- Site C2: Cu > Zn > Cd > Pb > Hg
- Site D1: Cu > Zn > Cd > Pb > Hg
- Site D2: Zn > Cu > Cd > Pb > Hg

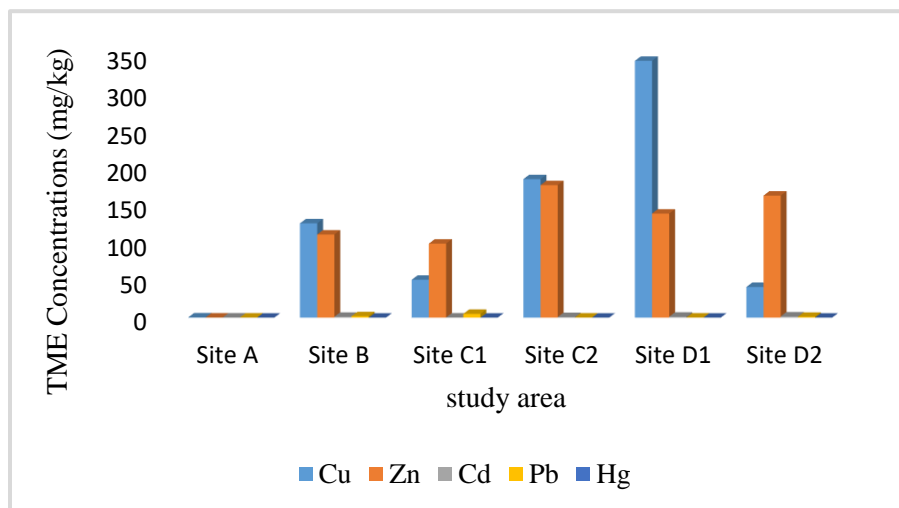


Figure 3 TMEs levels in *Achatina fulica* snails per sampling site

Differential study of contamination according to sampling sites

The table below (**Table 2**) shows the results of the tests carried out to examine, from a statistical point of view, the significance of the difference between the concentrations of the trace elements measured in the samples from the different sites 2.

Table 2 Results of tests on the statistical significance of data

TME	A	B	C1	C2	D1	D2	F	P
Cu	0,35±0,050 ^a	126±27,22 ^b	50,6±21,68 ^a	185±25,30 ^b	343±39,38 ^c	41±2,271 ^a	181,64	0,0000
Zn	0,156±0,036 ^a	111±11,40 ^b	98,9±3,218 ^b	177±3,218 ^c	139±25,95 ^{bc}	163±26,60 ^c	1572,9	0,0000
Cd	0,11±0,013 ^a	1,13±0,180 ^b	0,167±0,041 ^a	0,763±0,130 ^b	1,22±0,15 ^{bc}	1,61±0,45 ^c	45,392	0,0000
Pb	0,12±0,044 ^a	1,9±0,455 ^c	5,05±0,314 ^d	0,237±0,033 ^a	0,18±0,028 ^a	1,18±0,052 ^b	222,2	0,0000
Hg	0,003±0,000 ^a	0,054±0,013 ^b	0,0075±0,0008 ^a	0,0037±0,000 ^a	0,0059±0,000 ^a	0,0045±0,001 ^a	39,33	0,0000

The probability P determined was below the risk threshold of 5% ($P < 0.05$). There is therefore a significant difference between the concentrations obtained per site for each molecule.

In addition, a comparison of the overall concentrations of all the contaminants studied per site was carried out using the TUKEY test. The results shown in **Table 3** indicate that there is a certain homogeneity between sites B, C1, C2 and D2, unlike site A and site D1, where there is no similarity with the other sites.

Table 3 Comparison of overall TMEs concentrations per sampling site

Sites	A	B	C1	C2	D1	D2
[ETM]	0,777 ^a ±1,422	48,01 ^{ab} ±60,80	30,94 ^{ab} ±41,14	63,41 ^{ab} ±90,08	96,43 ^b ±140,3	41,04 ^{ab} ±65,96

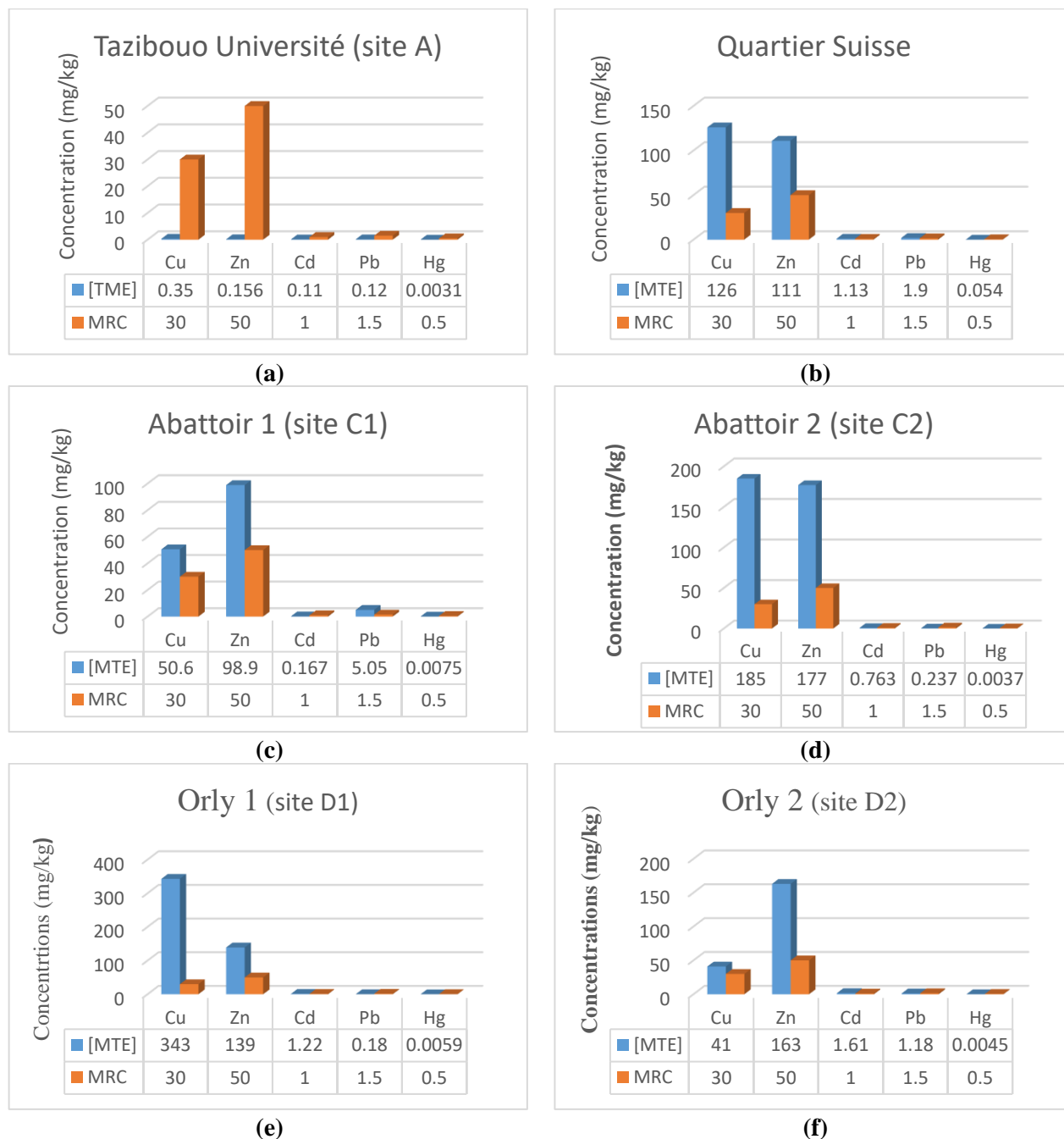


Figure 4 Comparison of ETM levels observed in snails from different sites with maximum reference concentrations [18]

Comparison of MTE levels observed in snails analysed with maximum reference concentrations (MRC)

The graphs below (**Figures 4a-f**) compare the levels observed in the snail samples collected at the various sites with the maximum reference concentrations set by European Union Commission Regulation (EC) No. 2021/1317 of 9 August 2021 [18].

The various tables in Figure 4 above show:

- the mercury (Hg) concentrations of all the samples are well below the reference values in force ;
- Zinc (Zn) and copper (Cu) levels are above MRC levels, with the exception of samples from site A (Figure 4a)
- lead levels exceed MRC levels in samples from sites B and C1, while cadmium levels exceed limit values in samples from sites B, D1 and D2

Discussion

The various levels of trace metal elements detected in the samples of *achatina fulica* taken show the level of contamination of these molluscs. This contamination differs significantly depending on the sampling site, with statistical tests giving a probability below the 5% threshold. The cumulative concentrations of all the TMEs per site show that boiled snails from site A are the least contaminated. This could be explained by the fact that site A is located in the Tazibouo Université area, one of the most sanitized residential neighbourhoods in the city of Daloa. Copper and zinc alternate in the top two places in descending order of concentration of the molecules studied and found in the samples from all six (6) sampling sites. Moreover, the concentrations of these two trace elements are above the limit values, with the exception of those obtained in the samples from site A. The high levels of zinc in snail meat could be linked to the plant cover in the sampling areas, as shown in a previous study by [19]. Snails are plant eaters living at the soil-plant-air interface, ingesting the soil on which they move. They are therefore exposed by the cutaneous, digestive or respiratory route to numerous chemical contaminants, including trace elements, which are drained into the various shallows that were the main sampling areas. This also explains the high concentration of copper recorded, since this element is a constituent of many chemical fertilisers and household appliances, the residues of which are found on the soil, leaves, flowers and other waste ingested by the gastropods studied. This study also showed that lead and cadmium concentrations exceeded standards in boiled snails from sites (B and C1) and (B, D1 and D2) respectively, while mercury levels were lowest in all the samples analysed. These results call into question the high number of sites where samples with Pb and Cd concentrations exceeding standards were detected, especially when compared with the results obtained by Adamou and colleagues [16]. When analysing boiled snails of the species *Limicolaria* spp and *Archachatina marginata* in the Ouémé valley, these Beninese scientists obtained maximum concentrations of 1.024 mg/Kg for lead and 0.422 mg/Kg for Cd for the two species mentioned above, values that are below the accepted average reference concentrations [18]. The high levels of Pb and Cd observed in our study could be justified by the bioavailability and high accumulation capacity that characterise lead, cadmium, zinc and copper mentioned above, and indeed all the TMEs in the environment. In fact, studies by Grara et al [20] and Viard et al [21] have demonstrated a high capacity for accumulation of TMEs, particularly Cd, Pb, Cu and Zn, in the snail *Helix aspersa* and gastropod molluscs respectively. Coeurdassier [22] showed that the accumulation of these same TMEs (Cd, Pb, Cu and Zn) by gastropod molluscs is linked to their environmental concentrations and their bioavailability within the organism. The presence of lead, cadmium and mercury in food, even at low concentrations, would constitute a health hazard for consumers [23, 24]) compared with Zn and Cu, which are described as essential metals [25].

Conclusion

This study, the aim of which was to assess the level of contamination by trace metals (Cu, Zn, Pb, Cd, Hg) in boiled *achatina fulica* snail meat, did indeed show the presence of the sought-after TMEs in varying concentrations.

Cu and Zn were the compounds most frequently detected, with average levels even exceeding the maximum reference concentrations for samples from all the sampling sites except Tazibouo Université (site A). The main cause for concern was the presence of Pb and Cd in the flesh of boiled snails at levels above the standards, particularly at sites B and C1 for lead and B, D1 and D2 for cadmium, due to the toxic nature of these compounds. Hg was detected in very low concentrations in all eighteen (18) samples in the study. This study showed that there was no statistical link between the contamination of snails at the different sites, since the average concentrations calculated were significantly different.

This work needs to be taken further by assessing the health risks associated with eating these giant snails. This will involve assessing the dose-effect relationship in humans of the metal compounds studied. In other words, the concentrations obtained in the snail samples analysed will be compared with the maximum reference concentrations

required for human consumption. This will ultimately enable us to conclude whether there are any potential effects on humans.

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Publication History

Received	26.10.2024
Revised	14.11.2024
Accepted	14.11.2024
Online	30.01.2025