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BIOCONCENTRATION OF HEAVY METALS IN THE SNAILS *ARCHACHATINA MARGINATA* AND *LIMICOLARIA SPP* OF THE OUÉMÉ VALLEY IN BENIN

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ABSTRACT

Snails meat of the Achatinidae family is highly appreciated by many African populations and Beninese in particular. But these snails have the ability to absorb and concentrate on their body some toxic chemicals such as heavy metals. This study aims to evaluate the contamination level of heavy metals (Cd and Pb) in snails consumed by the Ouémé Valley populations. Analytical method used to determinate heavy metals levels in samples species *Archachatina marginata* and *Limicolaria spp*sampled was Reverse Voltammetry. Results indicated that for *Archachatina marginata*, the mean cadmium concentrations recorded ranged from 0 to 0.032 mg/kg whereas those from lead ranged from 0.047 to 0.342 mg/ kg. As for *Limicolaria spp*, the average cadmium concentrations recorded range from 0 to 0.187 mg/kg, while those from lead range from 0.012 to 0.878 mg/kg. Heavy metals concentrations are all below the standards accepted by European Union in all communities with the exception of one where lead concentrations exceed the norm. *Limicolaria spp* concentrated lead and cadmium more than *Archachatina marginata*, and heavy metal concentrations are not directly related to the morphological characteristics of snails (length,

diameter and weight). To avoid health issues due to lead, it would be important for the populations of the Ouémé Valley to vary their diet and especially to eat less frequently snails.

Keywords: Snails, bioconcentration, heavy metals, Benin

INTRODUCTION

In Sudanian and Guinean regions of Africa, there is large or small size of snails eaten by populations. These snails belong to the Achatinidae family, whose flesh is highly appreciated by many African populations [1], and Beninese in particular. Snail meat is the most "bush meat" consumed in Côte d'Ivoire, after cane rats, with nearly 17,000 tons per year [2]. In Benin, their consumption was estimated at 300 tons per year [3]. According to Sodjinou et *al*. [4], achatins meat is the most consumed in South Benin in front of grass cutter, chicken, sheep or goat, beef and pork.

In the wild, snails play an important role in the ecological balance of forests, fallows and wetlands. According to Heymans and Evrard [5], they are interesting agents for waste depollution, hence their presence in and around garbage dumps. They are bioaccumulative species, capable of absorbing and concentrating on their bodies toxic chemicals such as heavy metals. In fact, several heavy metals such as copper (Cu), zinc (Zn), manganese (Mn), chromium (Cr), cadmium (Cd), lead (Pb) and others were found in the cephalopod and viscera of different snail species at various concentrations by several authors such as [6], [7] and [8]. Thus, the pollution of the biotope of these snails constitutes a risk of bioaccumulation of chemical pollutants. Since snails occupy a prominent place in Beninese dishes, while heavy metals, almost completely indestructible in the environment [9], are able to penetrate directly into the food chain and concentrate in certain living organisms, exposing humans to slow but certain intoxication; snails contamination would be a real public health problem. Thus, this study aims to assess the degree of contamination by heavy metals (Cd and Pb) of the most consumed snails' species by the populations of the Ouémé Valley in southern Benin.

1. MATERIAL AND METHODS

1.1.Study area

This study was conducted in the Ouémé valley, more precisely in the lower part of the Ouémé valley between 7°12' and 6°23' parallels NORD and 2°21' and 2°33' meridians EAST [10]. Located in the south-eastern region of Benin, it is a flood plain in the shape of an elongated triangle measuring 90 km from north to south (figure 1). Eight stations were selected across the four municipalities of the study area. Localities are: Kessounou, Hozin, Akpadanou, Todé, Affamè, Agonhoui, Bembè and Houinta.

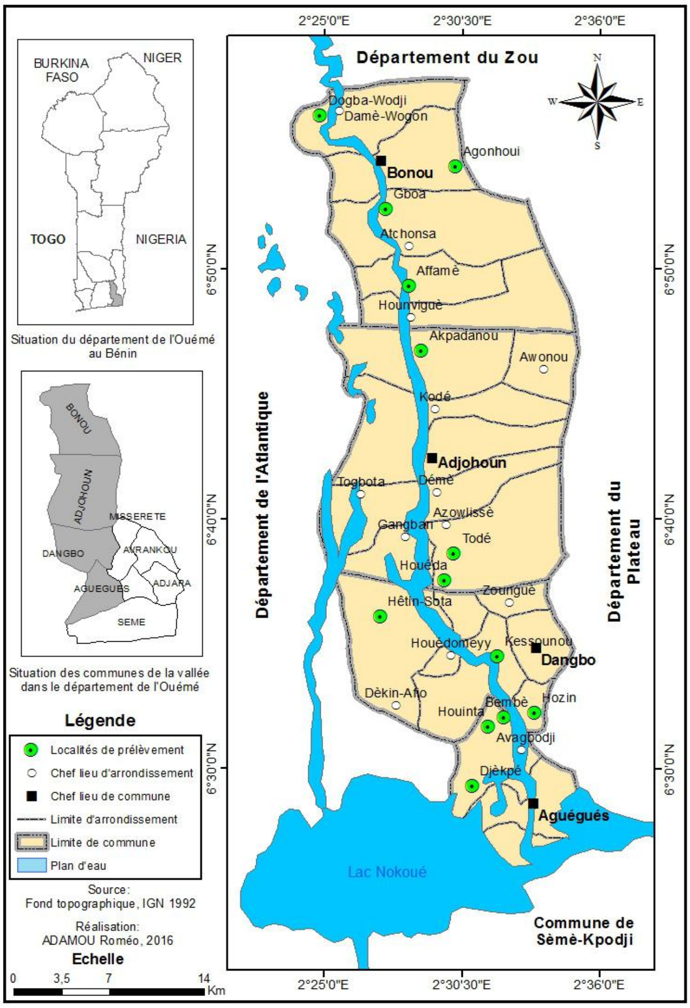

Figure 1: Location of the study area

1.2. Animal material

This study focused on two species of snail belonging to the Achatinidae family. These are *Archachatina marginata* and *Limicolaria spp*. These are highly valued species in southern Benin and particularly in the departments of Ouémé and Plateau.

1.3.Snails sampling

Twenty (20) snails were taken per species directly from collectors in all selected communities. They were immediately put in plastic jars previously washed and sterilized with hydrochloric acid. Samples taken were kept cold until they were sent to the laboratory. A total of 16 samples were collected from the eight sampling area at the rate of two samples per area.

1.4.Samples preparation

Once brought back to laboratory, snails were first fasted for two days (48hours) to remove unabsorbed food and feces from their digestive tract. Then, several measurements were taken on the individuals snails of each sampling area. Weight, length and diameter of its shell were measured and each of 10 snails were pooled, put in sterile pouches and stored at -80 °C until analysis. Determination of heavy metals is based on the cephalopod, i.e. the foot of the snails (the part consumed by the populations).Dissection of each individual snail allowed us to separate foot of the viscera, at the level of the mantle edge, after thawing and shell extraction. Finally, snails were cut into small pieces in each pool mixed together to allow some homogenization of the pollutants.

1.5. Samples mineralization and of heavy metals determination

5g of snails are first weighed per sample in crucibles and then dried in an oven at 105 °C for 24 hours. Mineralization was done in a programmable oven whose temperature was gradually raised to 550 °C until ash was obtained to facilitate the extraction of the metals [11]. The ash was collected in Falcon tubes which also served to digest the ash. The latter was dissolved in 20 mL of HNO_3 (0.5N) and stirred until a clear solution was obtained. The digested sample was filtered through a filter paper WhatmanN°42. Finally, the volume of each sample was increased to 50 ml by adding 30 ml of HNO3 (0.5N) and kept at room temperature for 30 minutes for subsequent analysis.

The analytical method used for the determination of heavy metals is Reverse Voltammetry. The post-mineralized samples are diluted 30% with deionized water and the heavy metals are quantified using Metalyser HM 3000 (Trace2O, Berkshire, UK). The electrodes were placed and packaged according to the desired metal. The metal was quantified by Metalyser in 70 ml of the mineralized material to which reagents of the kit corresponding to the desired metal (buffer and standard) were added. At the end of the analysis, the concentration of the metal is directly displayed on a tablet connected to the machine. The actual metal cation concentrations in the snail cephalopod are determined according to the following equation:

$$\text{C in ppm (mg/kg)} = \frac{Cs^{*}\ Vf^{*}\ Fd^{*}\ 10^{-3}}{P}$$

with:
C: final concentration;
Cs: concentration of the element in the solution;
Vf: final volume of the sample (50 ml);
Fd: dilution factor;
P:portions in grams (fresh weight) taken from the sample

1.6. STATISTICAL ANALYSIS

The data were subjected to the non-parametric comparison test of Kruskall-Wallis and the one of Nruskal Kramer Nemenyi to averaging. For the morphological characteristics of snails, box plots were used to illustrate the extreme values, the first, the second and the third quartile and the median value of each treatment. The link between the morphological parameters and the heavy metals concentration was evaluated through Pearson correlation coefficient. The different diagrams were made using

Microsoft Excel and the map using Arc View. All statistical analyses were performed with the software R 3.2.3.

2. RESULTS AND DISCUSSION

2.1. Results

2.1.1. Morphological characteristics of snails

In order to better physical characteristics of the snails collected on the different selected area, several measurements were taken. The averages of the measurement taken are presented in the following table 1.

Results analysis shown that the measurements of snails collected varied from species and from localities. Average lengths ranged from 6.79 cm to 12.07 cm for *Archachatina marginata* and 3.75 cm to 5.54 cm for *Limicolaria spp*. The average diameters recorded varied between 3.82 cm and 6.52 cm for *Archachatina marginata* and between 1.78 and 2.52 cm for *Limicolaria spp*. As for the average weights recorded, they varied between 35.69 g and 192.68 g for *Archachatina marginata* and between 3.39 g and 10.02 g for *Limicolaria spp*.

For *Archachatina marginata*, maximum and minimum recorded lengths are respectively 15.9 cm (in Agonhoui) and 4.92 cm (in Akpadanou); the maximum and minimum diameters recorded are respectively 8.26 cm (at Agonhoui) and 2.65 cm (in Akpadanou); the maximum and minimum weights are respectively 357.53 g (in Agonhoui) and 14.3 g (in Kessounou). For *Limicolaria spp*, the maximum and minimum recorded lengths are respectively 6.96 cm (in Akpadanou) and 3.2 cm (in Kessounou); the maximum and minimum diameters recorded are respectively 3.09 cm (in Akpadanou) and 1.23 cm (in Kessounou); the maximum and minimum weights are respectively 15.7 g (in Akpadanou) and 2.3 g (in Kessounou and Todé).

Mann-Whitney test carried out based on the characteristics of the two recorded snail species show globally that the median length, median diameter and medium weight of *Archachatina marginata* differs very significantly from those of *Limicolaria spp*(P-value <0.001). Analysis of the average values shown that length and diameter of *Archachatina marginata* is almost twice *Limicolaria spp* (8.26 and 4.54 cm against 4.46 and 2.04 cm respectively) and its average weight (73.71 g) is at least 12 times *Limicolaria spp* (6.07) (Table 1). The size comparison of each snail species between the different communities studied through Kruskall-Wallis test revealed that the development of each species varied very significantly between the different localities for all the characteristics studied (Table 1) P-value <0.001. Nruskal Kramer Nemenyi median structuring test has been used to determine localities for which morphological characteristics of snails are the same. Thus, median length of *Archachatina marginata* individuals is the same for Affamè and Agonhoui and differed from other localities that have similar median lengths. Box plots were established for all characteristics to see the distribution of the individual values of each characteristic in the different communities (figures 2 to 7) and globally for all localities (figure 8).

Table 1: Morphological characteristics of the snails of the Ouémé Valley

	Archachatina Marginata			*Limicolaria Spp*		
Communities	**Average length (cm)**	**Average diameter (cm)**	**Average weight (g)**	**Average length (cm)**	**Average diameter (cm)**	**Average weight (g)**
Kessounou	**6.92±0.94^{b}**	**3.82±0.47^{b}**	**37.66±14.26^{c}**	**3.86±0.37^{c}**	**1.78±0.12^{c}**	**3.76±0.81^{c}**
Hozin	**6.79±0.43^{b}**	**3.84±0.24^{b}**	**35.69±8.46^{c}**	**4.72±0.37^{b}**	**2.09±0.12^{b}**	**6.23±1.38^{b}**
Akpadanou	**7.11±1.71^{b}**	**3.79±0.93^{b}**	**39.74±35.41^{c}**	**5.54±0.39^{a}**	**2.52±0.16^{a}**	**10.02±1.66^{a}**
Todé	**8.50±1.23^{b}**	**4.56±0.64^{b}**	**69.23±29.33^{b}**	**3.75±0.22^{c}**	**1.83±0.09^{c}**	**3.39±0.63^{c}**
Affamè	**10.25±1.45^{a}**	**5.67±0.73^{a}**	**120.51±44.62^{a}**	**4.80±0.48^{b}**	**2.14±0.18^{b}**	**8.52±2.00^{a}**
Agonhoui	**12.07±1.01^{a}**	**6.52±0.50^{a}**	**192.68±45.05^{a}**	**4.01±0.33^{c}**	**1.87±0.13^{c}**	**4.64±1.00^{c}**
Bembè	**7.10±0.4^{b}**	**4.03±0.27^{b}**	**42.26±7.71^{c}**	**4.62±0.41^{b}**	**2.02±0.13^{b}**	**6.16±1.36^{b}**
Houinta	**6.79±0.40^{b}**	**3.88±0.25^{b}**	**37.85±9.09^{c}**	**4.44±0.37^{b}**	**2.02±0.12^{b}**	**5.88±1.20^{b}**
Global	**8.26±0.12**	**4.54±0.07**	**73.71±3.53**	**4.46±0.04**	**2.04±0.02**	**6.07±0.15**
Probability	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**

Averages with the same letters in columns have medians that are not significantly different.

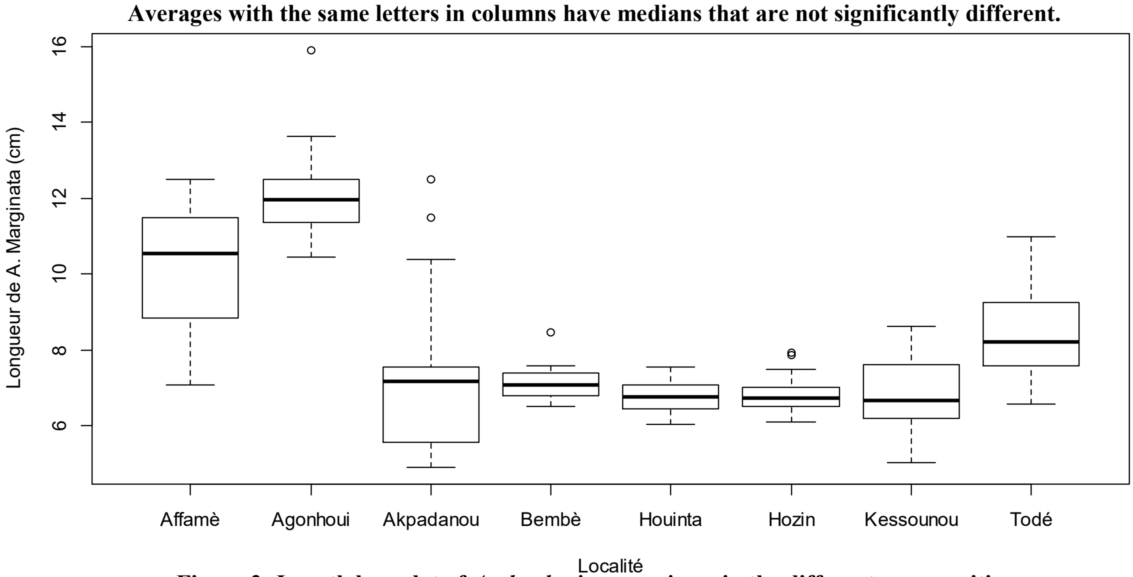

Figure 2: Length box plot of *Archachatina marginata* in the different communities

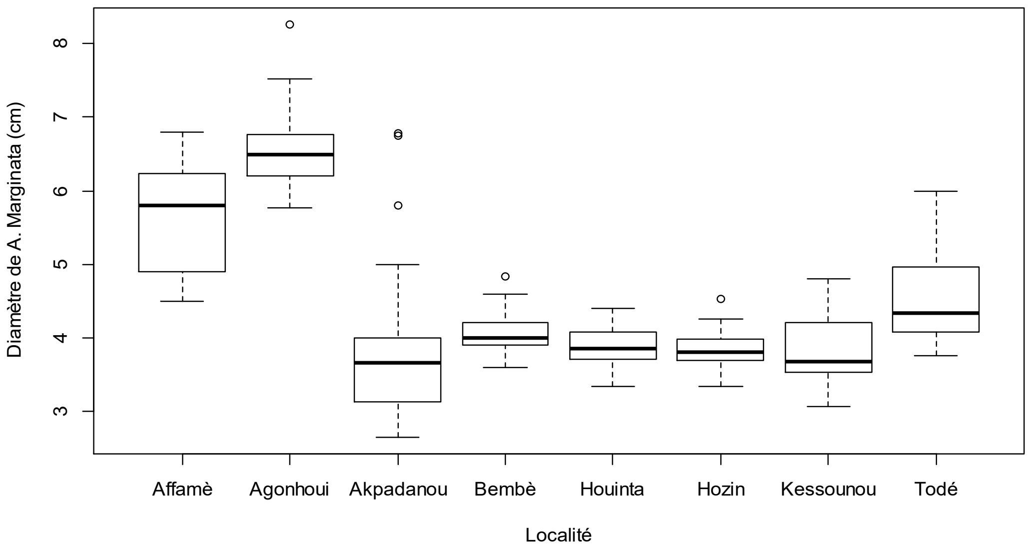

Figure 3: Diameter box plot of *Archachatina marginata*in the different communities

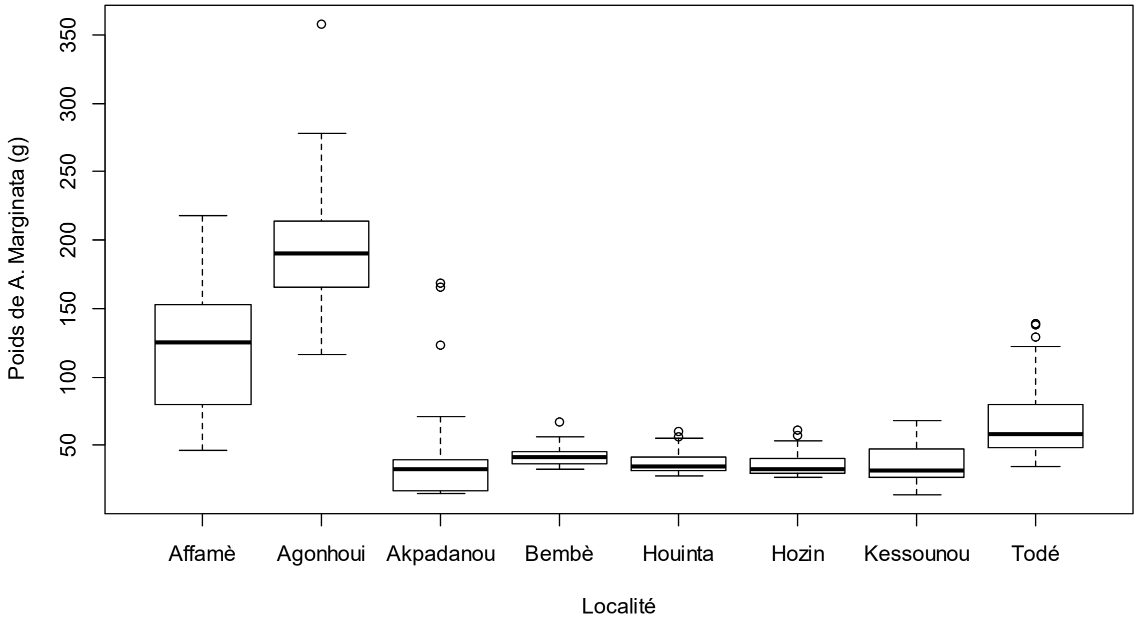

Figure 4: Weight box plot of *Archachatina marginata* in the different communities

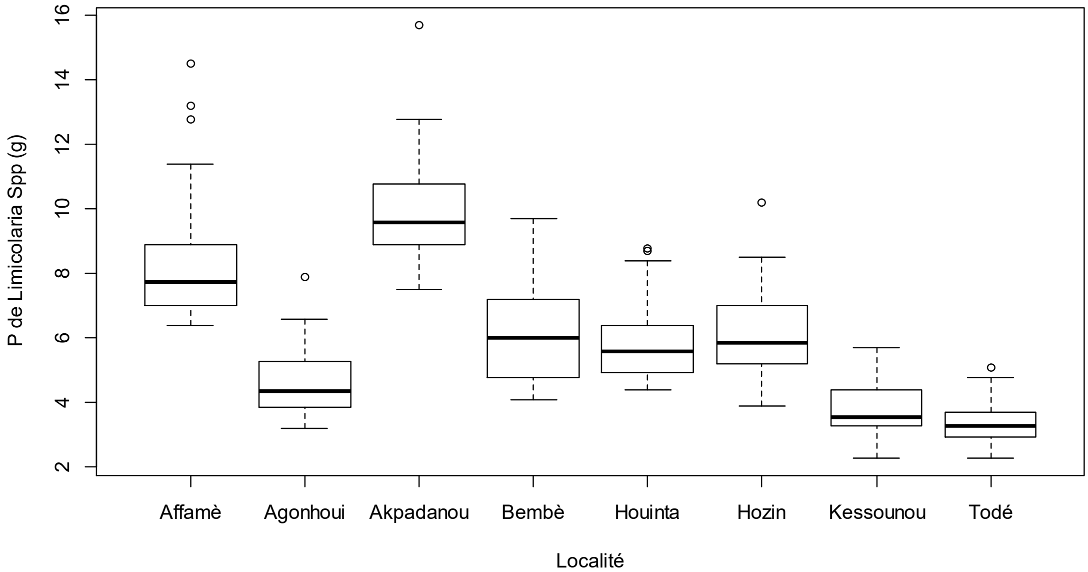

Figure 5: Weight box plot of *Limicolaria spp* in the different communities

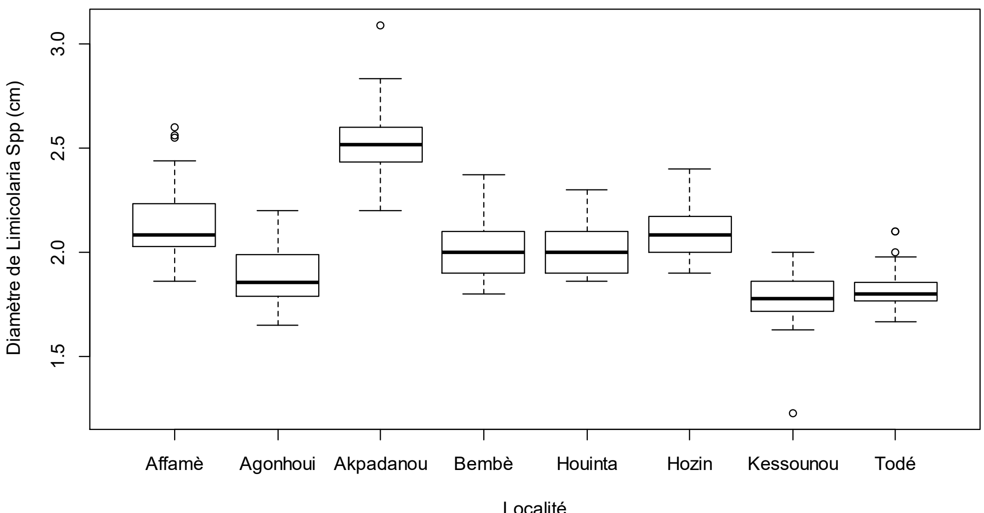

Figure 6: Diameter box plot of *Limicolaria spp* in the different communities

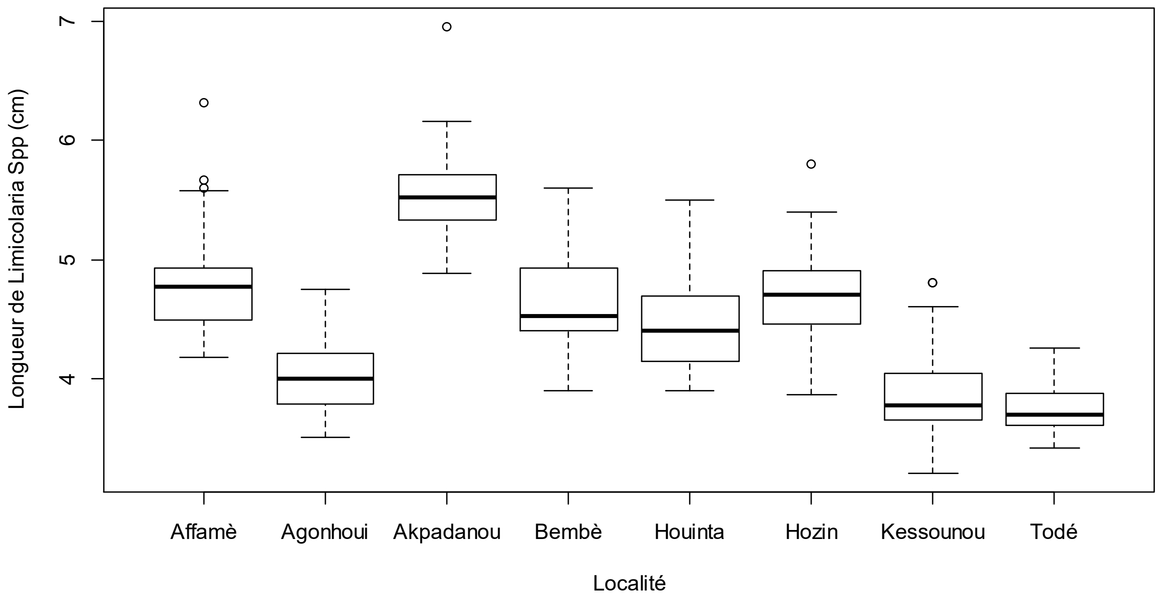

Figure 7: Length box plot of *Limicolaria spp* in the different localities

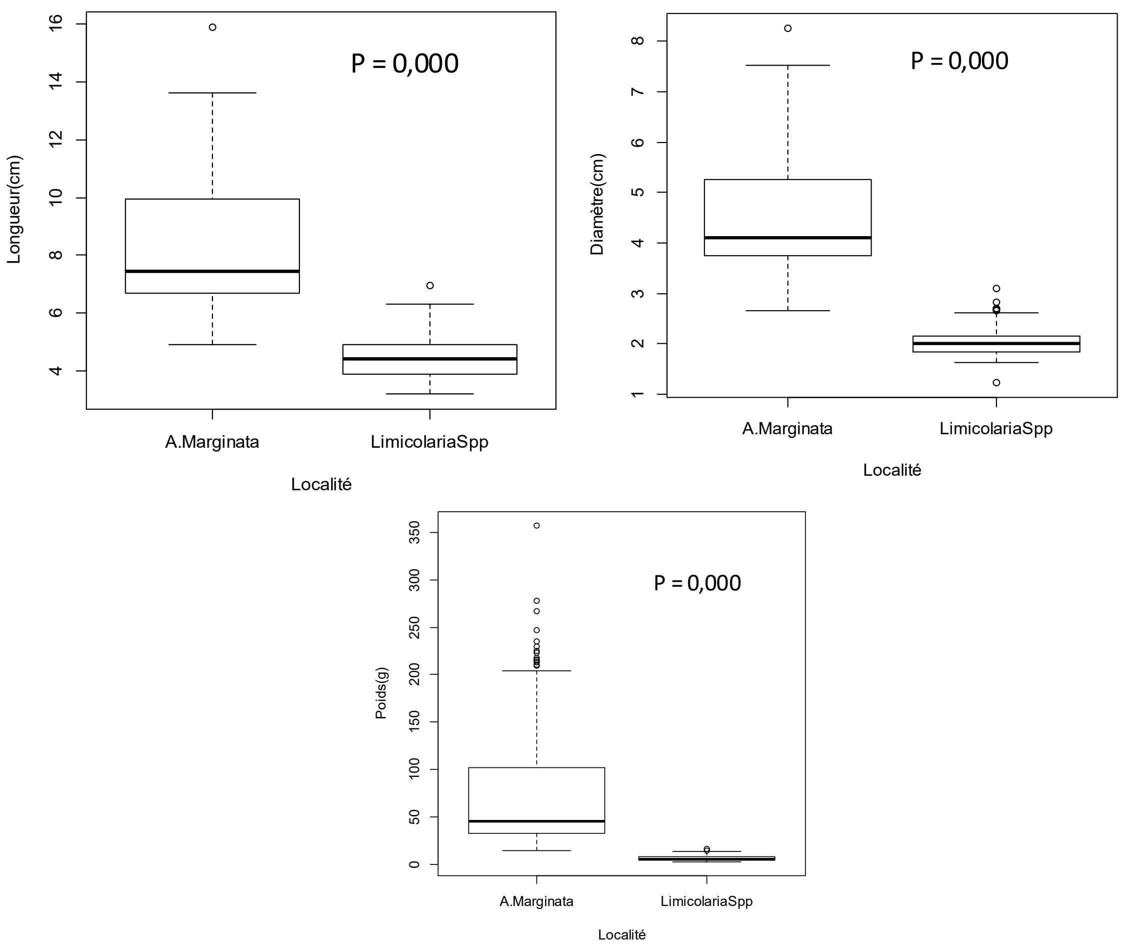

Figure 8: Diameter, length and weight box plot of both species in general

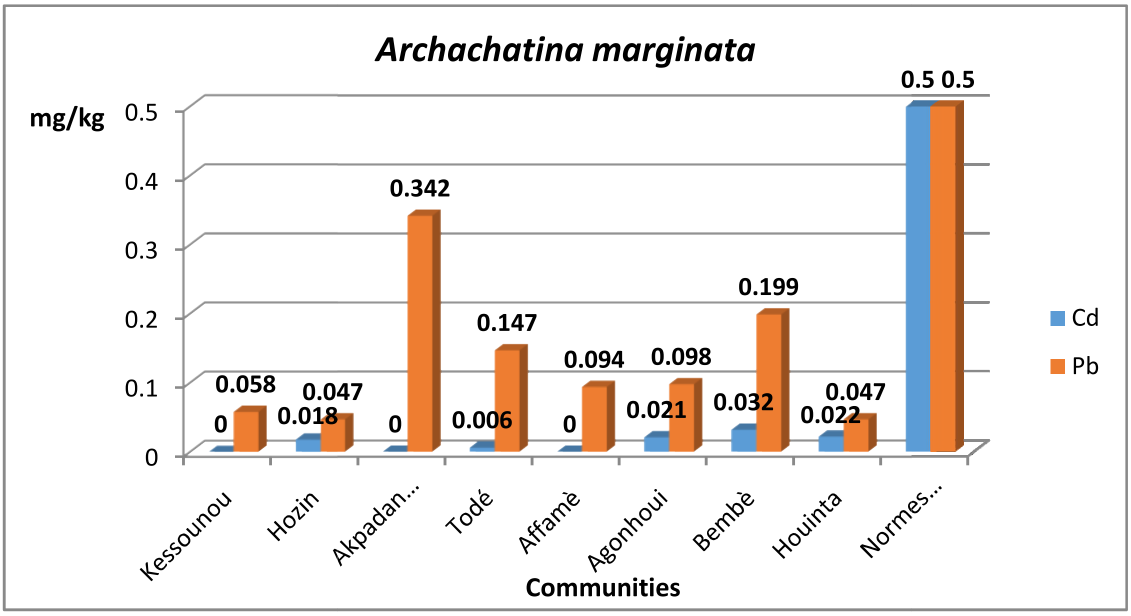

Figure 9: Heavy metal concentrations in the raw species *Archachatina marginata*

2.1.2. Heavy metals concentrations in *Archachatina marginata*

Results obtained from *Archachatina marginata* analysis indicated that cadmium and lead accumulation varied from one locality to another as shown figure 9.

Analysis of the figure 9 shown presence of cadmium and lead in *Archachatina marginata* collected in the different communities. The average cadmium concentrations recorded ranged from 0 to 0.032 mg/kg while those from lead ranged from 0.047 to 0.342 mg/kg. Figure 9 shown that heavy metals levels in the snail samples of each locality could ranked in descending order as followed: Pb> Cd. It could be deduced that *Archachatina marginata* species concentrates more lead than cadmium. Kruskall-Wallis analysis by comparing averages and the one of Nruskal Kramer Nemenyi for averaging shown that there is no significant difference (P> 0.05) between the median concentrations valuesof heavy metals (cadmium and lead) contained in the raw meat of this snail in different localities (table 2).

Table 2: Results of the Kruskall-Wallis and Nruskal Kramer Nemenyi test on the average concentrations of heavy metals in raw *Archachatina Marginata*

Communities	Cd	cv (%)	Probability	Pb	cv (%)	Probability
Affamè	0	*		0.0945	23.196	
Agonhoui	0.0205	65.537		0.098	23.089	
Akpadanou	0	*		0.342	79.394	
Bembè	0.0325	58.744	0.11	0.1995	20.203	0.076
Houinta	0.022	77.139		0.047	87.26	
Hozin	0.018	47.14		0.0475	10.421	
Kessounou	0	*		0.0575	1.23	
Todé	0.0065	141.421		0.1465	72.883	

2.1.3. Heavy metals concentrations in *Limicolaria spp*

Results obtained from the analysis of *Limicolaria spp* samples also revealed that cadmium and lead accumulation varied from one locality to another as shown in figure 10.

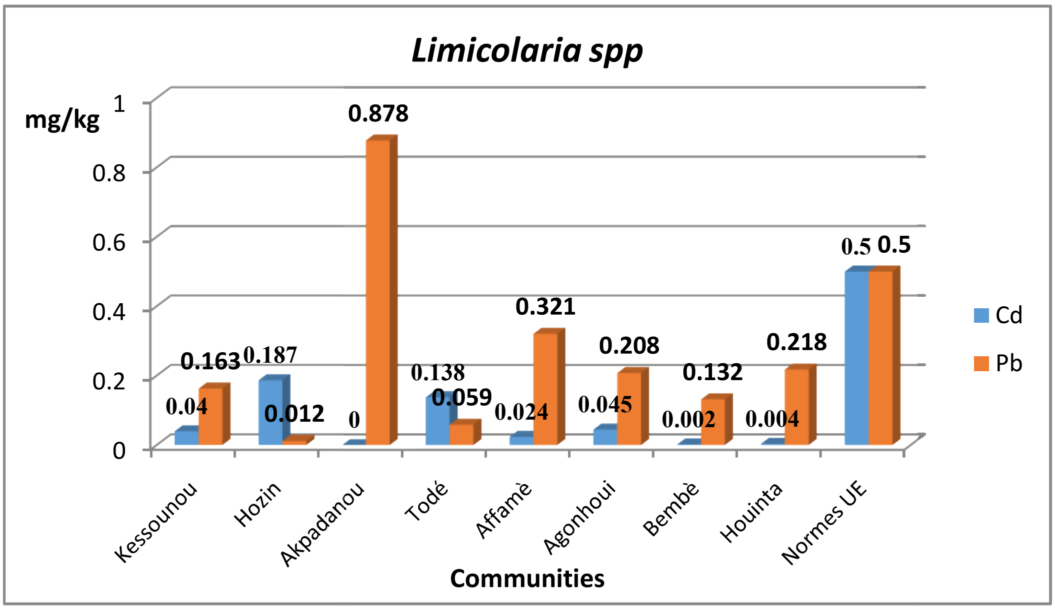

Figure 10: Heavy metals concentrations in raw *Limicolaria spp*

Figure 10 analysis shown presence of cadmium and lead in the snail *Limicolaria spp* collected in the different localities. The average cadmium concentrations recorded ranged from 0 to 0.187 mg/kg while those from lead ranged from 0.012 to 0.878 mg/kg. Concentrations of heavy metals in the snail samples of each locality (with the exception of Hozin and Todé) ranged in descending order as follows: Pb> Cd. We could also deduct that *Limicolaria spp* species concentrates more lead than cadmium. Results analysis after the Kruskall-Wallis test for comparing the averages and Nruskal Kramer Nemenyi for averaging shown that there is no significant difference (P> 0.05) between the median values of concentrations of heavy metals (cadmium and lead) contained in the raw meat of this snail in different localities (table 3).

Table 3: Results of the Kruskall-Wallis and Nruskal Kramer Nemenyi test on the average concentrations of heavy metals in raw *Limicolaria spp*

Communities	Cd	cv (%)	Probability	Pb	cv (%)	Probability
Affamè	0.0245	20.203		0.3205	5.074	
Agonhoui	0.0455	141.421		0.208	73.43	
Akpadanou	0.000	*		0.878	98.415	
Bembè	0.002	141.421	0.151	0.132	36.427	0.101
Houinta	0.0045	141.421		0.2175	49.091	
Hozin	0.1875	82.59		0.0125	141.421	
Kessounou	0.040	70.711		0.1625	36.987	
Todé	0.1375	83.824		0.0595	24.957	

2.1.4. Comparison of heavy metal concentrations between *Archachatina marginata* and *Limicolaria spp*

The comparison of heavy metal content between the two snail species in each community is shown in the following table.

Table 4: Comparison of heavy metal concentrations between *Archachatina marginata* and *Limicolaria spp*

	Cd (mg/kg)		Pb (mg/kg)	
Communities	***Archachatina marginata***	***Limicolaria spp***	***Archachatina marginata***	***Limicolaria spp***
Kessounou	<0.000001	0.040±0.028	0.058±0.001	0.163±0.060
Hozin	0.018±0.008	0.187±0.155	0.047±0.005	0.012±0.018
Akpadanou	<0.000001	<0.000001	0.342±0.271	0.878±0.864
Todé	0.006±0.009	0.138±0.115	0.147±0.107	0.059±0.015
Affamè	<0.000001	0.024±0.005	0.094±0.022	0.321±0.016
Agonhoui	0.021±0.014	0.045±0.064	0.098±0.023	0.208±0.153
Bembè	0.032±0.020	0.002±0.002	0.199±0.041	0.132±0.048
Houinta	0.022±0.017	0.004±0.006	0.047±0.041	0.218±0.106

Analysis of this table shown that in almost all localities, cadmium and lead accumulated levels by *Limicolaria spp* are higher than those accumulated by *Archachatina marginata*, showing that *Limicolaria spp* species would concentrate more heavy metals, namely lead and cadmium, than *Archachatina marginata* species although it is smaller (in length, in diameter and in weight). Each of the two snail species concentrated more lead than cadmium in almost all communities. From this observation, it can be deduced that *Archachatina marginata* and *Limicolaria spp* are bio-indicators designated to control lead pollution.

2.1.5. Relation between morphological characteristics of snails and concentrations of heavy metals

The relationship between morphological characteristics and heavy metal concentration was assessed through the Pearson correlation coefficient. The analysis of the correlation matrices between heavy metal and morphological characteristics of snails shown that there are weak and insignificant links between bioaccumulated heavy metals and morphological characteristics of the two snail species (Table 5 and 6). Thus, heavy metal concentrations in snails depend neither on their length nor their diameter nor their weight. Therefore, large and small snails may have the same heavy metal concentrations. The level of heavy metal would then be rather related to the phenomena of absorption and excretion of these chemical pollutants at the capture time of the snail.

Table 5: Correlation matrices between heavy metal and morphological characteristics of *Archachatina marginata*

	Cd	Pb	Length	Diameter
Pb	-0.163			
	0.547			
Length	-0.325	0.345		
	0.22	0.19		
Diameter	-0.33	0.344	0.972	
	0.212	0.192	0	
Weight	-0.247	0.227	0.977	0.963
	0.357	0.399	0	0

Table 6: Correlation matrices between heavy metal and morphological characteristics of *Limicolaria spp*

	Length	Diameter	Weight	Cd
Diameter	0.876			
	0			
Weight	0.893	0.923		
	0	0		
Cd	-0.112	-0.118	-0.189	
	0.68	0.663	0.483	
Pb	0.445	0.49	0.422	-0.356
	0.084	0.054	0.104	0.176

2.2.DISCUSSION

The two snails species studied are bioaccumulative species. They have a bioaccumulative capacity for chemical pollutants such as heavy metals. Cadmium and lead were identified in the most consumed two species. Their concentrations varied from one species to another and from localities. Differences in metal concentrations between snail species may be due to variation in breeding condition, animal genotype, metabolism, body weight, trophic position, presence or absence of enzymatic system [12]. Cadmium and lead average levels in *Archachatina marginata* do not exceed the N°420/2011 standards of the European Union for cephalopods in all communities. In *Limicolaria spp*, the lead level registered is higher than the EU standards in Akpadanou. Cadmium levels do not exceed standards in all localities. This superiority of the lead content in Akpadanou is due to metal waste thrown into the shallows, bushes and palm groves, where these snails' species were collected. This suggests likely soil pollution by the lead in this locality. These results are much lower than those obtained by Viard et *al*. [13] in the viscera of the snail *Helix aspersa aspersa* transferred to area near the highway A31 (France) where they recorded concentrations up to 21.3 mg Pb.kg^{-1} PS and 5.7 mg Cd.kg^{-1} between 5 and 20 m from the highway. These results are also lower than those obtained by Edorh et *al*.[8]in *Achatina achatina* collected in Okpara Village in Benin where they recorded average levels of 1.24 mg/kg for cadmium and 8.56 mg/kg for the lead. These results differ from those obtained by Waykar and Petare [14] on *Bellamya bengalensis* and *Lymnea accuminata* snails from the Malangaon dam in India where they recorded 83.5 and 1541.3µg/g respectively for cadmium and lead in *Bellamya bengalensis* then 107.63 and 603.3µg/g respectively in *Lymnea accuminata*. These two species of snail each accumulate more lead than cadmium and might therefore be bio-indicators designated for lead pollution. Results also differ from those obtained by Merzouki et *al*. [15] on another gastropod, the mussel *Mytilus galloprovincialis* from coast of El Jadida (Morocco). The recorded concentrations for cadmium go up to 62.629 µg/g in the digestive gland of the mussel. The dissimilarity between the results of the various authors and ours could be due to the difference of the species, the analyzed part (cephalopod or viscera) and the collection area of the species analyzed.

This study revealed that heavy metal concentrations in snails are not directly related to their morphological characteristics (length, diameter and weight). Concentrations of metallic elements in mollusks differ among species because of their ability to regulate or accumulate trace metals ([16, 17]) and could be related to digestive physiology specific to species and to the metal uptake rate across the intestinal epithelium [18]. The morphological characteristics of the snails measured in this study are generally lower than those observed by Edorh et *al*. [8] on *Achatina achatina* species in Okpara who recorded an average length of 12 cm and an average weight of 225 g. The difference in species and the sampling area may justify this inferiority.

CONCLUSION

This study assesses snail's contamination by heavy metals. It appears that the two studied species have the capacity to accumulate heavy metals. *Limicolaria spp.* concentrates lead and cadmium more than *Archachatina marginata*, which is larger than the former. The study shown that heavy metal concentrations are not directly related to the morphological characteristics of snails (length, diameter and weight). Both species shown an affinity to concentrate more lead than cadmium and would therefore be bio-indicators designated to control lead pollution. Thus, to avoid health problems caused by the lead, it would be important for Ouémé Valley populations and surroundings to vary their diet and especially to eat less frequently snails.

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