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Impact of Metallic Trace Elements (MTE) Content of the Sheds of the Lagoon Ebrie (Ivory Coast) on the Biometric Parameters of *Achatina Fulica*

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Abstract: The biometric parameters of *Achatina fulica* living on five bays of the Ebrié lagoon of Abidjan were the subject of this study. The five bays chosen for this study are: the berries of Azito, Banco, Cocody, M'badon and Anan. Another area blessed with the same climatic conditions was chosen as a control site. A sample of 50 snails was collected per site. The living masses, the masses of shells, consumable flesh, and viscera of these snails have been noted and compared. Soil samples from the different snail collection sites were collected and assayed in metallic trace elements (ETM). The results showed that the biometric characteristics of snails decrease with the increase of soil ETM content in the living environment. Thus, the living mass, empty shell, consumable flesh and visceral mass are strongly influenced by the concentration of soil ETM.

Keyword: *Achatina fulica*, Bio-indicator, Bay, Trace elements (ETM).

INTRODUCTION

The city of Abidjan has developed around the lagoon Ébrié. Abidjan is located on the coast of the Ivorian continental shelf with a growing population estimated at 4.5 million inhabitants¹. It corresponds

to a space of intense economic activities and on the other hand the engine of the natural economy for our country as it concentrates more than half of the industries of the country. All this makes the Ebrié lagoon, the receptacle for large quantities of substances of natural or anthropogenic origin, many of which possess toxic properties. This lagoon communicates with the many bays which constitute the points of transport of many wastes. However, the banks of these different bays are a key link in the supply of the city of Abidjan in market garden products such as lettuce, cabbage and cucumbers². Increasing awareness and concern about the impact of chemical pollution on human life and health is generating interest among researchers in characterizing and monitoring the quality of the environment³. This monitoring of the environment requires the help of gastropod molluscs, which are known for their great capacity for the accumulation of metallic trace elements, as has been proven in several studies⁴⁻⁷

In this study, it will be necessary to make a biometric study of the giant snail *Achatina fulica* in relation with the concentrations of the ETM of the medium.

MATERIAL AND METHOD

Sampling sites: This study was carried out on the banks of five bays of the Ébrié lagoon extended over 125 km along the coast of Côte d'Ivoire between the longitudes 3 ° 40' and 4 ° 50' West, and the latitude 5 ° 50' North. The five bays whose shores formed the sites of these works are: Azito Bay, Anan Bay, Banco Bay, Cocody Bay and M'badon Bay, all located in the metropolis of Abidjan. A site located far away (Belleville ALEPE road) from urban areas benefiting from the same climatic conditions was chosen as a control site (**Figure 1**).

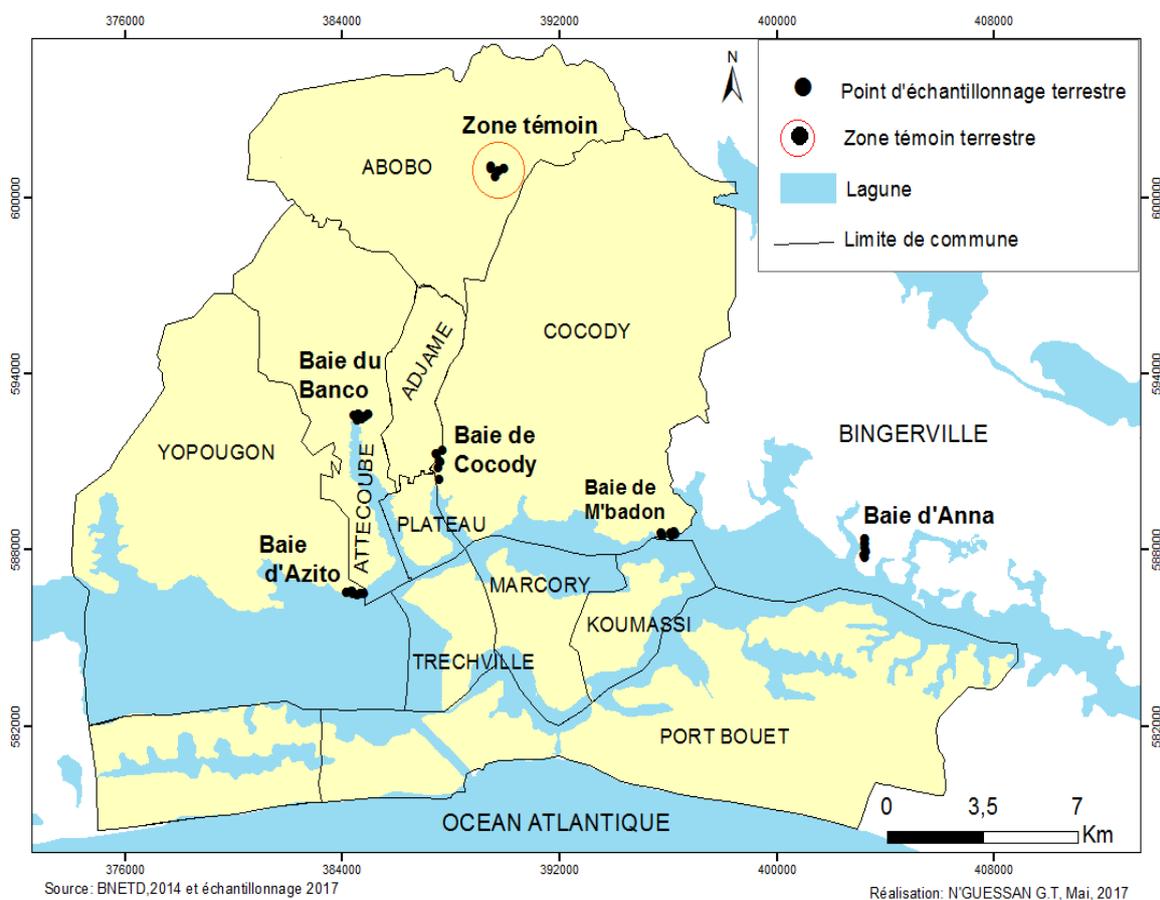


Figure 1: Map showing the different sampling areas

Biological material: The biological material that was the focus of this study is essentially *Buyina fulica* snail (Bowditch, 1720). It was chosen for its sedentary lifestyle and for its abundance in the study area⁸.

Sampling of biological material: Sampling was done on the various banks during the month of July (2016) corresponding to a period of the great rainy seasons in Côte d'Ivoire. At each site, 50 live snails were collected at random by simple collection on the ground, on / under shrub leaves and in the grass at cool hours of the day (at dusk in the morning and evening).

Determination of the shell and weight characteristics of snails: The snails collected at each bay were weighed individually using an ISO 9001 sartorius electronic scale, sensitive to 1/100 mg. Also, their lengths and shell heights were measured using calipers brand VERNIER CALIPER precision 1 mm of Chinese origin.

Determination of the masses of the different body parts: The snails of each bay after weighing and measuring saw their flesh eradicated from their shells. The pedal mass was then separated from the visceral twist. The different body parts (empty shell, visceral twist and pedal mass) of each snail thus obtained were weighed using the over collared scale.

Collection of soil samples at the banks: An area of 100m by 100m or one hectare (10000m²) has been defined at each bay. In this space, samples were manually taken randomly, using a rubber shovel to a depth⁹ of about 1-10 cm . Twelve (12) samples were collected per bay taking into account the different compartments in which *Achatina fulica* live. These samples were stored in polythene bags and transported to the laboratory in a cooler to be dried at room temperature. After drying, the samples were sieved using a sieve with a mesh diameter of 63 µm. Three composite samples were obtained per site for the assay.

Mineralization of soil samples (ISO 11885 method: June 2007): A quantity of 1 g of soil sample is taken and placed in a capped vial to which 20.0 ml of nitric acid prepared at 7 mol / L are added from the commercial solution. The whole is stirred and then autoclaved at 120 ° C for 3 hours finally to allow digestion. After 3 hours, the solution is cooled in the open air and then filtered with a filter paper to collect the filtrate in a volumetric flask. The digestion vessel is rinsed with distilled water and the rinsing water is passed through the filter paper. The filtrate is collected in a vial and the volume is completed to the mark. The different concentrations were read using an Atomic Absorption Spectrometer (AAS) of the VARIAN AA-20 type.

Calculated parameters: The average contamination index (ICM) at each bay was calculated on Excel assuming that all micropollutants have the same polluting power. This index is represented by an arithmetic mean for the bay considered and thus makes it possible to characterize each bay globally.

Statistical test: The Newman-Keuls Test from the XLSTAT software was used to compare the morphometric and weight characteristics of the snails of the different study sites. This test also made it possible to compare the concentrations of ETM of the different study sites. The mean contamination indices of these different snail collection sites were compared to the nonparametric Kolmogorov test at a confidence level of 5%. The significance of the correlation between the ETMs, the ICMs and the biometric parameters was tested using the STATISTICA 7.1 software.

RESULTS

Morphometric parameters: The biometric parameters and the masses of the different body parts of the snails collected at the study sites are compared in **Table 1**. The mean values of living masses, lengths and shell height of all the snails collected vary respectively from 35.91% . at 49.86g; 6.33 to 7.92 cm and 3.02 to 3.81 cm. As for the masses of the body parts, they have extreme values of 5,41 and 9,47g

for the empty shells, 25,53 and 32,14g for the visceral twists then 7,85 and 10,44 for the flesh. The statistical analysis of the results shows a variation of the physical characteristics and body masses of the animals according to their collection site. Control animals have a mean mass, length, and shell height that is statistically higher than those collected from urban bays (**Figure 2**).

Table I: Averages of *Buyina Fulica* Biometric Parameters Collected at the Level study sites (June to July 2016) (n = 50).

Site	Cocody	M'badon	Azito	Banco	Anan	Témoïn
Longueur	7,01±0,74 ^c	6,33±0,91 ^d	6,49±0,85 ^d	7,49±0,82 ^b	7,36±1,16 ^{bc}	7,92±1,18 ^a
Hauteur	3,02±0,45 ^d	3,58±0,5 ^b	3,29±0,45 ^c	3,27±0,32 ^c	3,27±0,43 ^c	3,81±0,81 ^a
Masse vive	36,6±9,91 ^c	42,44±9,62 ^{bc}	35,91±12,56 ^c	41,56±10,23 ^{bc}	44,69±17,87 ^b	49,86±15,76 ^a
Masse coq.	6,36±1,98 ^{bc}	7,34±2,68 ^b	5,41±3,63 ^c	6,64±2,16 ^{bc}	8,87±5,79 ^a	9,47±3,68 ^a
Masse tortillon	23,33±7,1 ^c	28,95±5,6 ^{ab}	25,53±7,16 ^{bc}	28±7,07 ^b	29,22±9,93 ^{ab}	32,14±9,94 ^a
Masse Chair	8,45±2,05 ^{bc}	9,58±1,75 ^{ab}	7,85±1,54 ^c	8,92±2,35 ^{ab}	9,48±3,34 ^{bc}	10,44±3,3 ^a

For the same metal, the indexed average values of the same letters are not significantly different from the Newman-Keuls test ($p > 0.05$)

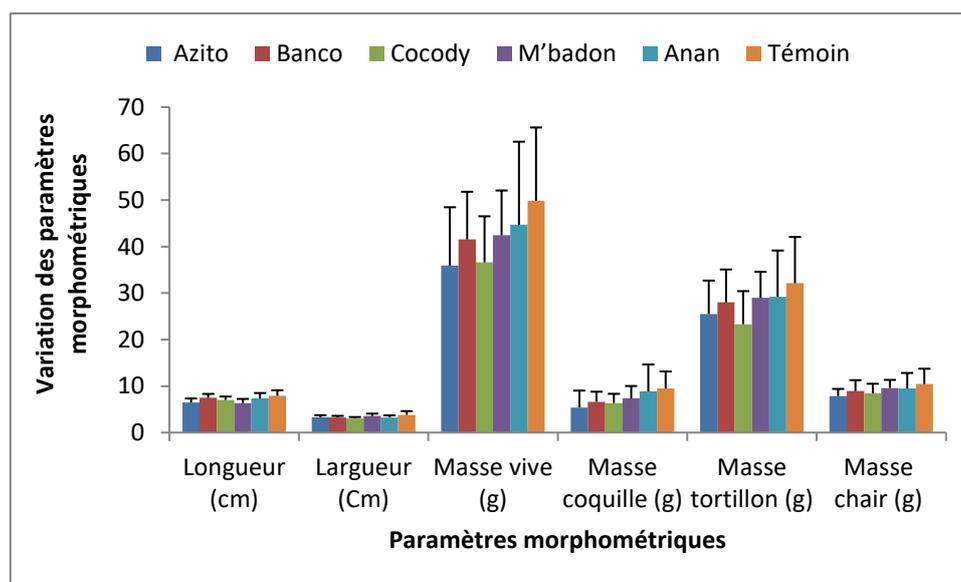


Figure 2: Morphometric data of *Buyina fulica* specimens (N = 50 for each station).

The lowest values of shell length and shell width are those of the Cocody Bay animals. The snails taken from the bays of Cocody, M'badon, Azito and Banco present statically the same living masses. However, the empty shell mass of Azito snails is significantly lower than that of those of M'badon. Also, no significant difference is observed between the shell masses and the visceral masses of the control animals and those of Anan; however, these animals have statistically different flesh masses.

Pollution of the soil by metallic trace elements: The metal trace element contents of the various snail collection sites are recorded in **Table 2**. The copper content of the soil of the control site is $114.77 \pm 32.76 \mu\text{g} / \text{g}$ against a content varying between $101.4 \pm 34.33 \mu\text{g} / \text{g}$ and $444.7 \pm 178.9 \mu\text{g} / \text{g}$ for lagoon

berry soils. Sites can be divided into two groups based on the copper content of their soils. We have the group consisting of the control sites, Azito, Anan and M'badon with a low copper content than the group consisting of the Banco and Azito sites. As for the zinc concentration in the different soils, it is relatively higher than those of the other metallic trace elements (**Figure 3**). The soil of Cocody Bay is more concentrated in this element than the soils of other sites. The concentration of chromium in the soil of Azito is the highest. No significant difference is observed between the other sites for this element. The berries of Cocody, M'badon, Azito and Anan are identical in terms of the lead content of their soil. Only the control soil is less concentrated in lead than that of cocody berry.

Table II: Concentration of metallic trace elements in the soils of the different sites.

	Cu	Zn	Cr	Pb	Cd	ICM
Cocody	129,13 ± 50,71 ^b	2752,76 ± 568,37 ^a	191,45 ± 12 ,55 ^b	288,69 ± 139,43 ^a	20,55 ± 4,13 ^b	143,30
M'badon	101,4 ± 34,33 ^b	1338,2 ± 573,71 ^b	160 ± 24,74 ^B	163,33 ± 45,82 ^{ab}	22,56 ± 13,72 ^b	62,12
Azito	425,75 ± 50,71 ^a	1713,83 ± 630,37 ^b	478,8 ± 226,41 ^a	200,96 ± 18,68 ^{ab}	18,00 ± 4,38 ^b	113,49
Anan	130,19 ± 74,07 ^b	1066,1 ± 339,26 ^b	220,49 ± 12,03 ^b	124,85 ± 40,31 ^{ab}	14,36 ± 2,37 ^b	62,20
Banco	444,7 ± 178,9 ^a	1250,8 ± 351,01 ^b	312,1 ± 61,52 ^{ab}	199,5 ± 74 ^{ab}	40,38 ± 4,22 ^a	81,89
Témoïn	114,77 ± 32,76 ^b	750,11 ± 128,45 ^b	150 ± 33,05 ^b	76,9 ± 50,71 ^b	11,39± 9,38 ^b	40,94
Afnor 1996	100	300	150	100	2	

For the same metal, the indexed average values of the same letters are not significantly different from the Newman-Keuls test ($p > 0.05$).

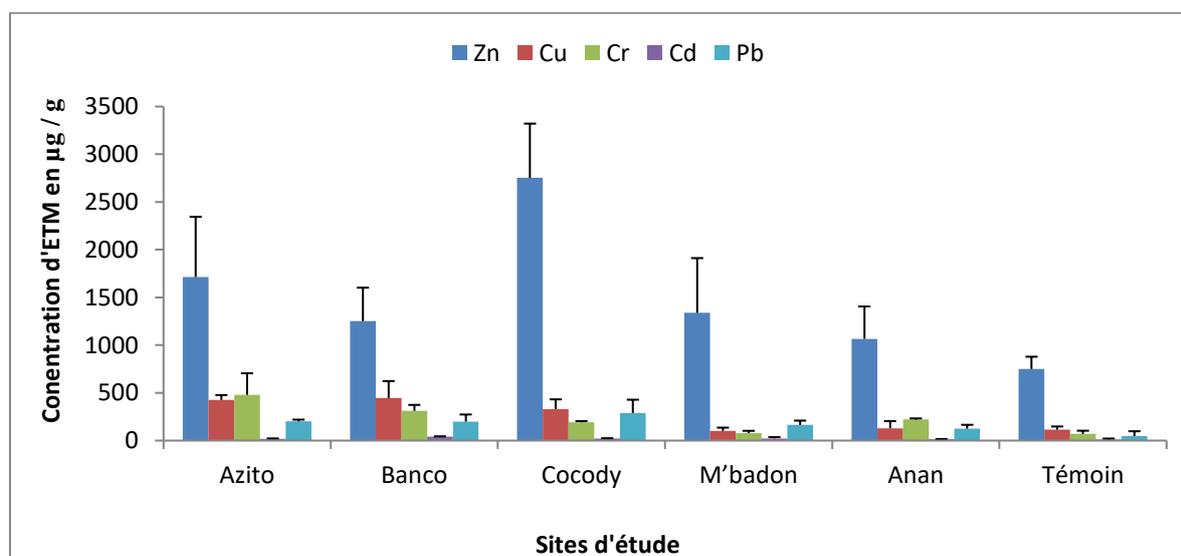


Figure 3: Concentration of MTEs in the different study sites

The mean contamination index (ICM) in metallic trace element of the different snail collection sites varies between 40.94 and 143.30 (**Figure 4**).

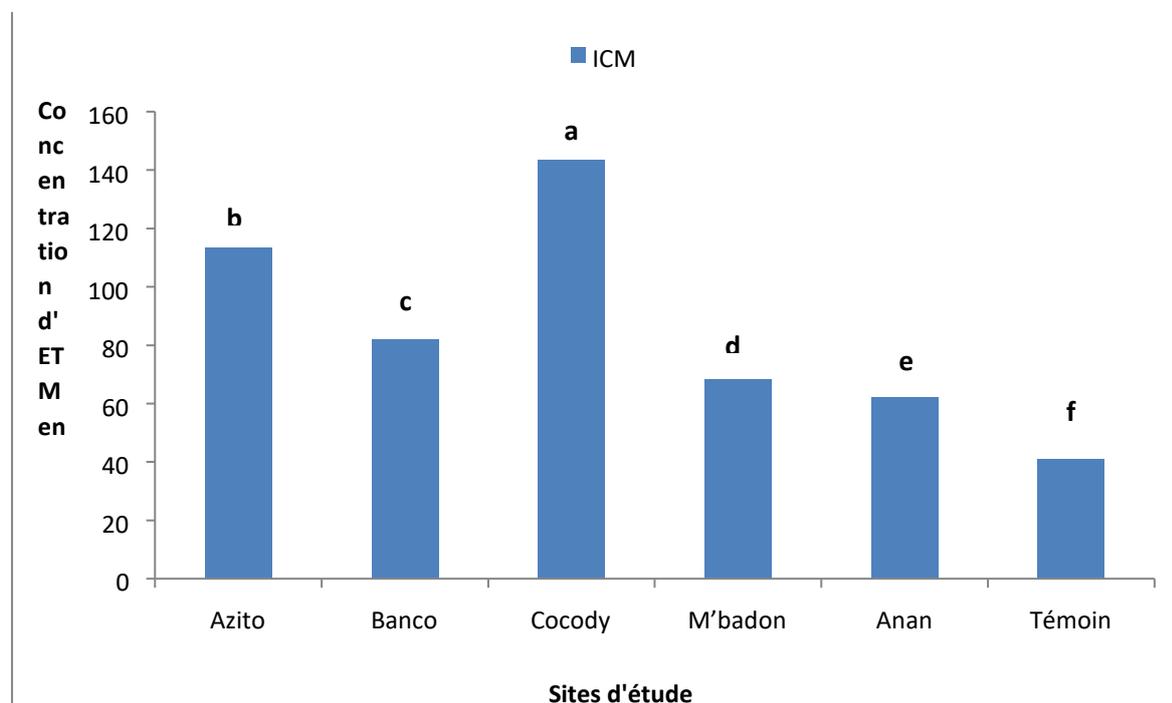


Figure 4: Average concentration indices of HTAs in the different study sites

The comparison of the sites according to the ICM of their soil with the Kolmogorov test ($p < 0.05$) shows a significant difference between them. According to the concentration gradient of their soil in metallic trace elements (ETM), the sites are arranged in order: Witness, M'badon, Anan, Banco, Azito, Cocody.

The correlation analysis between the metallic trace elements, the soil contamination indices and the morphometric parameters of the snails collected is summarized in Table III. With the exception of the lack of correlation between length and height, all growth parameters are interrelated. ICM is strongly correlated with different growth parameters except length. Indeed, a negative and significant correlation is observed between the width, the living mass, the shell mass then the foot mass of the snails and the soil ICM. Which means that general, plus, the soil is contaminated, plus the snails that live there, have weak physical characteristics. Pb and Zn are the soil elements that most influence the biometric parameters of snails. The coefficients of correlation between these elements (Zn and Pb) and the physical characteristics (width, live mass, shell mass and pedal mass) of the animals vary respectively from -0.945 to -0.685 and from -0.958 to -0.822. No significant correlation was observed between cadmium and the morphological parameters of snails. On the other hand, the copper content of the soil has a negative influence on the shellfish and pedaling mass of snails.

DISCUSSION

The soil HME concentrations determined in this study vary from one site to another and are higher than those of Afnor¹⁰. This reflects a contamination of our study sites and therefore a danger to human health.

fauna because of their high molecular weight¹¹. The studied MTEs (Cd, Zn, Pb, Cr and Cu) are of diverse origin. They are related to human activities such as urban waste, practices related to waste incineration such as plastics residues, battery and sludge waste¹².

Cultural practices with the use of fertilizers and pesticides in the treatment of market garden produce and flowers⁴ at the various bays contribute to this pollution.

The situation of urban bays (Banco, Cocody) and their proximity (Azito) to road networks justifies the high Pb level at its sites as mentioned in Maas et al.,¹³ and Modrzewska. Wyszowski¹⁴.

The amount and frequency of precipitation, wind direction and speed, and interception by the environment also influence the deposition rates and consequently the pollution levels of our study sites.

The ETM content of the studied soils varies according to the remoteness of the sites compared to the source of pollution¹⁵⁻¹⁷. The high rate of plant cover at the control and Anan sites also reflects their low level of ETM. Our results are consistent with those of Magiera and Zawadzki,¹⁸ who showed that the quantities of HTAs vary with land use and woodlands.

Snails are in direct contact with the soil from which they are influenced. This would justify the low shell parameters obtained in bays located in urban areas where there is a high rate of pollution. Indeed, several authors have shown the bioaccumulator capacity of terrestrial gastropods. Grara *et al.*⁶, have shown that the Zn²⁺ ion has an inhibitory effect on the shellfish mass. These authors believe that the damage caused by the inhibitory power of this ion on certain enzymes or on the DNA of the aspersa helix is irreversible. It indirectly disrupts important physiological processes by competing with certain essential elements such as calcium (Ca). Laskowski and Hopkin¹⁹ also found that animals in the presence of metals such as Cu and Cd have low living masses. According to these authors, this would be due to a low food consumption of these animals present in the medium with a high copper content.

The strong correlation of height with other parameters shows an adaptive response to the physical and ecological conditions of the environment. Growth in mollusc height minimizes water losses caused by evaporation and / or improving their thermoregulatory capacity^{20, 21}. Good growth in height therefore leads to the growth of other parameters. The correlations of the ICM to the parameters show the negative effect of the MTE on the growth of molluscs. The larger the ICM, the less the molluscs grow. The low correlation of Cadmium to the shellfish mass is explained by the fact that it is a metal whose accumulation in the shell of gastropods is independent of food intake in molluscs²². The morphometric and weight characteristics of *Achatina fulica* collected from polluted berries were significantly ($p < 0.05$) lower than those from the less polluted control site. These differences would be the result of vegetation and a nature of soils unsuitable for the proper growth of snails. In fact, the abundance of vegetation varies from one station to another, whereas the species is abundant in gardens, vegetable gardens, fallow land and around habitats because it prefers sites rich in grasses²³. It is a species that consumes more than 50 plants, crops and by-products of agriculture^{24, 25}. This variation in vegetation could be a hindrance to its diet and therefore a reduction in its growth rate. The low parameters recorded in animals at the Cocody and Banco bays could also be explained by the lighting of the voices of these sites (light pollution) and the numerous noise of vehicles (noise pollution) circulating on these roads. According to ANPCEN²⁶, light causes a massive migration of pigment granules in the center of the retina of the snail, with significant variations in neurosecretions depending on the time of day. These neurosecretions seem to be related to the activity / sleep cycle of these animals. Indeed, the light inhibits the activity of the snail thus causing a strong regression of the weight of these animals. These results corroborate those of Otchoumou²⁷, which showed that a photoperiod of 12 hours of light out of 24 improves growth in

Achatinidae. As for noises, they are one of the causes of animal stress resulting in poor growth^{28, 29}. Noise is therefore a direct and indirect threat to many animal species.

CONCLUSION

The banks of the bays of the Ebrié lagoon located in urban areas are highly concentrated in metallic trace elements (ETM) due to the fact that they suffer more from anthropic pressures. The physical characteristics of the *Achatina fulica* snails living there are strongly influenced by the concentration of soil HME. Thus, snails living in the most concentrated HME soil zones have the lowest living masses, shell size and flesh masses. This study indicates that just like many species of terrestrial gastropod, *Achatina fulica* can be used as a bio-indicator of pollution in Côte d'Ivoire. It can therefore be used in the monitoring of terrestrial environments because it is fond of anthropised environments. These studies must extend throughout the Ivorian territory especially with the advent of gold mulching for identification and remediation of these different polluted sites to preserve these ecosystems and especially the health of populations.

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