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Full Length Research Paper

Comparison of trace element contamination levels (Cu, Zn, Fe, Cd and Pb) in the soft tissues of the gastropods *Tympanotonus fuscatus fuscatus* and *Tympanotonus fuscatus radula* collected in the Ebrié Lagoon (Côte d'Ivoire): Evidence of the risks linked to lead and cadmium.

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A comparative survey of the levels of contamination of the two gastropods, of the Potomidae family, has been done in the lagoon Ebrié close to the city of Abidjan. It revealed that *Tympanotonus fuscatus radula* (TFR), adapt better to the conditions of the lagoon environment than *Tympanotonus fuscatus fuscatus* (TFF). Besides, *T. fuscatus radula* shows a higher bioaccumulation capacity of the trace elements than *T. fuscatus fuscatus*: the ratios of contamination index of *T. fuscatus radula* to those of *T. fuscatus fuscatus* are in the order of 1.5 for copper (Cu), 4.9 for iron (Fe), 3.2 for zinc (Zn), 95.9 for lead (Pb) and 6.7 for cadmium (Cd). Therefore *T. fuscatus radula* could be a better metallic pollution indicator than *T. fuscatus fuscatus*. A hierarchical classification analysis permitted, on the basis of trace elements contents in the soft tissues of *T. fuscatus radula* higher than 10%, to determine the polymetallic character of the pollution in the different sampling stations (with the exception of Biét 3) as well as the differences in the bioavailability of the trace elements. Besides, possible health risks linked to the consumption of *T. fuscatus radula* exist because of the lead concentrations which, in 35.57% of the samples, are higher than the consumption standards of the European Union (EU) and the World Health Organisation (WHO). Even if the contents in cadmium are lower than the consumption standards of the EU and the WHO, the health risks linked to this trace element still remains because of its cumulative character in some vital organs of human.

Key words: Trace element, gastropod, *Tympanotonus fuscatus*, bioaccumulation, Ebrié lagoon, Côte d'Ivoire.

INTRODUCTION

According to Guyonnet (2003), coastal zones are exposed to increasing anthropogenic pressures of both industrial and urban origin and which are often not clearly assessed. Thus, appropriate biological indicators of the sanitary aspect of these ecosystems are necessary in order to protect and, if necessary, to restore the natural environment. It was reported that a biological indicator must be a non migrant species, have a long life span, be

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Table 1. GPS coordinates of the sampling stations.

Collection zone	Sampling station	GPS Coordinates	
		Longitude (W)	Latitude (N)
Attécoubé	Atté 1	004°02'16.8''	05°20'00.2
	Atté 2	004°02'35.9''	05°20'25.0
Biétri	Biétri 1	003°58'33.5''	05°16'01.9''
	Biét 2	003°58'25.9''	05°16'10.3''
	Biét 3	003°58'18.1''	05°16'13.3''
	Biét 4	003°57'53.4''	05°16'08.8''
Cocody	Blokos	004°00'12.7''	05°19'18.9''

Table 2. Correspondences between classes of size and length of the shells of the molluscs.

Length of the shells (cm)	Classes of size
<2	Class 1
[2 ; 2.5[	Class 2
[2.5 ; 3[	Class 3
[3 ; 3.5[	Class 4
[3.5 ; 4[	Class 5
≥ 4	Class 6

wide-spread, have a reasonable size, be easy to sample and be able to accumulate pollutants (Hamed and Emara, 2006).

In terms of abundance, among the gastropods of West Africa, the Potamididae are prominent but present only one species, *Tympanotonus Fuscatus* in the region (Zabi and Le Loeuf, 1992). *T. Fuscatus* is important because of its wide distribution and abundance. *Fuscatus* and *Radula* are its two physical forms; they are microphagous species (they lick the upper part of the organic substrate). They live in great numbers in substrates rich in organic debris. They are found in the brackish waters of the lagoons and lakes along the coasts of the Golf of Guinea, from Senegal to Congo (Pauly, 1975; Davies et al., 2006; Zabi and Le Loeuf, 1993)

In Côte d'Ivoire, these molluscs are consumed by the inhabitants of the lagoon zones as in Nigeria (Davies et a., 2006). Furthermore, in their early life stages they constitute an important food source for malacophagous fishes (Fagade, 1969). According to Davies et al. (2006), the ingestion of contaminants by these species could eventually affect the health of the inhabitants. It is therefore vital to determine the levels of concentration of these contaminants in order to assess the potential risks to which these inhabitants are exposed through the consumption of the species.

The aims of this study are to: compare the relative capacities of adaptation and bioaccumulation of the two sub-species of *T. fuscatus* in the polluted environment of different sampling stations based on the concentrations in trace elements of the gastropods' soft tissues, and assess the potential risks to which the inhabitants consuming these gastropods are exposed in relation to their state of contamination by lead and cadmium.

MATERIALS AND METHODS

Choice and location of the sampling stations

The locations of the sampling stations are presented in Figure 1 and Table 1. Each of them was chosen in the part of the Ebrié lagoon close to the city of Abidjan, because of the proximity to important socio-economic activities:

- Cocody zone (Blokos station): It receives mainly domestic wastes of the communities of Cocody, Adjamé (Williamsville and 220 Logements) and Treichville. Due to internal currents, it is also subjected to the influence of the dumpings from the General de Gaulle bridge, whose contents include waste oils (Marchand et Martin, 1985).
- ·Biétri zone, bay of Biétri, (Biét1, Biét2, Biét3 and Biét4 stations): It is situated at the lower part of a zone dominated by industries (food, petrochemical, painting, agrochemical, metallurgical). It also receives the inflows of the slaughterhouse and the artisanal tanneries.
- Attécoubé zone (Atté1 and Atté2 stations): Maritime circulation zone, situated at the lower end of a repair docks and facing CARENA (ship repair and construction site). It receives the inflows of the metallurgical shops, oils wastes, wastes of domestic origin from the communities of Attécoubé, and a part of those of Adjamé and Yopougon.

Collection, treatment and analysis of the samples

Living molluscs were collected by hand and sent to the laboratory in aereted flasks containing some lagoon water after cleaning and getting rid of the mineral remnants. The species of *T. fuscatus radula* and *T. fuscatus fuscatus* (Figure 2) were isolated and their gastric contents were removed after remaining in de-onised water for 48 h.

For comparison analysis, the molluscs were divided into five classes of sizes (Table 2). These were defined after measuring the length of the shells of each specimen (distance separating the two extreme tips of the shell). Once the molluscs were removed from their shells, the soft tissues (flesh of the molluscs) were regrouped to make a homogenate mass according to their class and sampling station; then they were frozen at -20°C until they got mineralized.

Digestion of the soft tissues

All the reagents used were of analytic purity. All the material used

Figure 1. Map of the sampling stations in the Ebrié lagoon. A1 = ATTE1; A2 = ATTE2; C = BLOKOS; B1 = BIET1; B2 =BIET2; B3 = BIET3; B4 = BIET4.

(a) *T. fuscatus radula* (b) *T. fuscatus fuscatus*

Figure 2. Photographs of the two subspecies of *Tympanotonus fuscatus*.

for the digestion was soaked in a 5% nitric acid solution for 24 h, rinsed with demineralised water, air-dried, then maintain plugged and wrapped before being used. The protocol of digestion of soft tissues is the one proposed by the Centre d'Expertise en Analyse Environnementale du Québec (2003).

Chemical analysis of the trace elements in the soft tissues

The trace elements of interest (Cu, Zn, Fe, Pb, Cd) were then determined in the digested solutions, using Thermoelemental type M6 brand of an atomic absorption Spectrometer equipped with a flame operated atomisation system and a deuterium compensator. The wave-lengths of metal detection were respectively 248.3 nm; 324.8 nm; 213.9 nm; 217.0 nm; 228.8 nm for Fe, Cu, Zn, Pb and Cd. The instrumental limits of detection for Fe, Cu, Zn, Pb, Cd were respectively of 6; 3; 1.0; 10; 1.5 ppb. The standardization curve is only validated if the coefficient of determination of the line $r^2 \geq 0.995$. For each sample, the metallic concentration retained is the average of three determinations made within four seconds each. Such an average is only kept if the three differ from each over by less than 5%. The recovery rates between 90 and 110% were determined using doped solutions.

The contamination index (CI) of an organism is expressed by the ratio between the maximal and minimal concentration of the element (Mouvet, 1984). It enables one to compare the levels of bioaccumulation of a pollutant at a given station.

Statistical analysis of the data

All the statistical analysis of the data as well as the drawing of curves were made using STATA 8 Intercooled software (STATA Corporation) and EXCEL 2003 except the hierarchical classification analysis which was done with Statistica 4.5 software. Parametric tests were applied after modifying the variables using type log(x+1) to ascertain the normality of the distributions (test of Shapiro and Wilk) and to verify the homogeneity of the variances (Fisher-Snedecor F test).

The Student t-test was applied up to 5% confidence level to assess the differences in the metallic concentrations of the soft tissues: for the same species sampled from the two stations where both species were present; of the two species for the same metal in the same sampling station.

Oneway ANOVA test applied up to 5% confidence level followed by the Bonferroni's post hoc test was used to compare the levels of different trace elements in the soft tissues of each species.

In order to get an appropriate scale for the representation of the concentrations of all trace elements and to use parametric tests (anova and Student t test), we proceeded to a change of variable: the averages used are those of log(1+[ETM]), with [ETM] = concentration of given trace elements expressed in mg/kg wet weight.

In order to have a typology of the bioavailability of the pollutants in the sampling stations, an analysis of ascending hierarchical classification was done on the basis of trace element contents in soft tissues of the gastropods collected at the different sampling stations.

RESULTS AND DISCUSSION

Distribution of the two gastropods

Identification of the taxa revealed the absence of *T. fuscafus fuscafus* in five of the seven collection stations: four stations of Biétri zone and one station of Atté 2 zone. *T. fuscafus radula* was found in all the collection stations of the different zones.

Several authors determined the parameters able to affect the distribution of molluscs in aquatic environment (Lodge et al., 1987; PIP, 2006; Guerra-Garcia and Garcia-Gomez, 2004; Guiral, 1992; Ellingsen, 2002). According to PIP (1988), there are some species tolerant to a large range of ecological conditions; these constitute the most common species or even specific to a given area. Thus, the presence of *T. fuscatus radula* in all the collection stations is due to its remarkable adaptation capacity, as observed by Pauly (1975) in the coastal lagoon of Sakumo. This is confirmed by the results of Monteillet (1979) who showed, using marking techniques, that the physical appearance of *T. fuscatus* rugged shell depends effectively on its environmental conditions. It is therefore possible that the disappearance of the *fuscatus* form from the Biétri zone could be attributed to its poor tolerance.

Metallic concentrations in the soft tissues of the two species

The metallic concentration levels in the soft tissues of the same gastropod are compared according to class of sizes as well as their sampling stations, considering both species were present at the sampling stations.

Influence of the class of sizes on the contamination levels of the gastropods

Figure 3 shows the average concentrations of the trace elements in the soft tissues of *T. fuscatus radula* and *T. fuscatus fuscatus*. It was observed for both gastropods, irrespective of trace element and sampling station, that the average concentration of the soft tissues increased with the class of sizes in the order of class 3 < class 4 < class 5 < class 6 and hence with the size. One can conclude that the two gastropods undergo bio-accumulation of trace elements.

Relative bioaccumulation of the trace elements by the gastropods

Irrespective of the sampling station and the classes of size, the essential elements (Fe, Cu, Zn) were more present than the toxic ones (Pb, Cd) in soft tissues of all species (Figure 4).

Besides, the relative order of the concentrations in Zn and Cu seemed to depend both on the species and the sampling station. The order of the concentrations in the soft tissues of *T. fuscatus fuscatus* was:

- Fe> Cu> Zn> Pb> Cd for the samples of the station of Blokos,
- Fe> Zn> Cu> Pb> Cd for the samples of the station of Atté1.

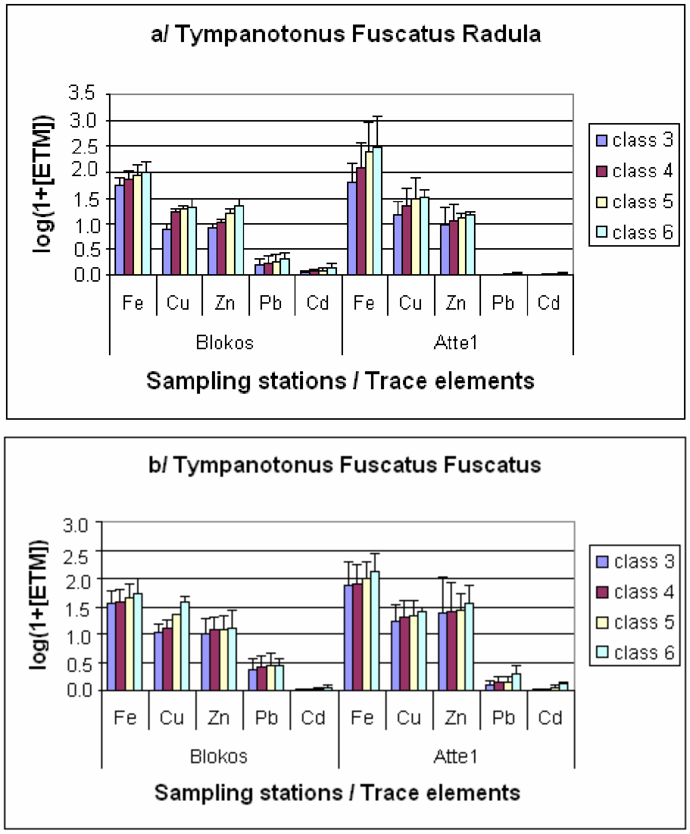


Figure 3. Mean metallic concentrations in the soft tissues for the four classes of size per trace element at Blokos and Atté1 stations: a) for TFR, b) for TFF.

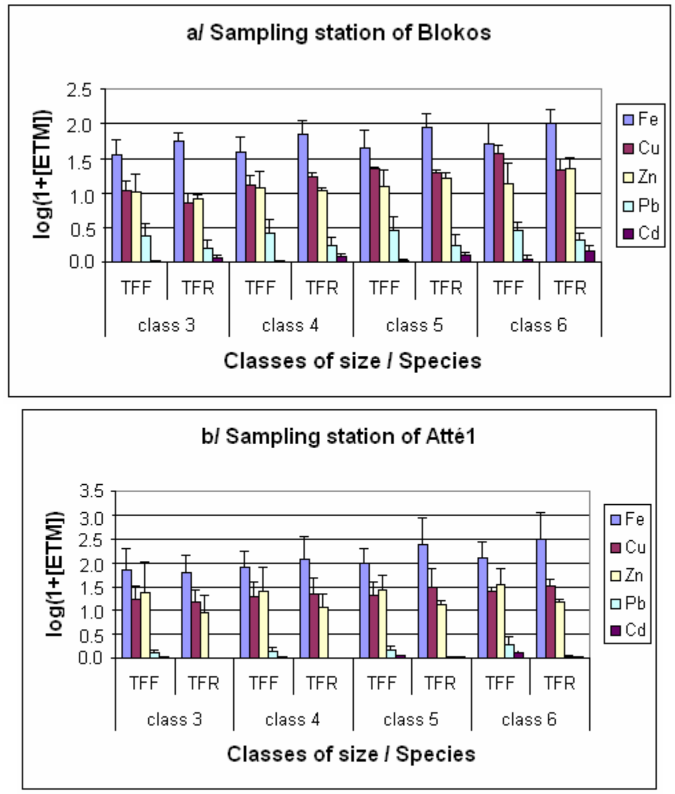


Figure 4. Mean concentrations of trace elements (Fe, Cu, Zn, Pb, Cd) in the soft tissues of each subspecies (TFR and TFF) per class of size: a) at Blokos b) at Atté1.

However, the sole order of Fe> Cu> Zn> Pb> Cd was observed in the soft tissues of *T. fuscatus radula*

The one way ANOVA and followed-up of the post hoc test of Bonferroni revealed that the differences were meaningful except those observed between the concentrations of Cu and Zn and between those of Pb and Cd (except one case). In the last case, the differences between concentrations of Pb and Cd in soft tissues were meaningful for *T. fuscatus fuscatus* in Blokos.

According to Hamed and Emara (2006), the relative availability of the trace elements can be attributed to the differences in the incoming loads of these at the different stations or to the chemical transformations that they underwent before their absorption by the organisms.

The most contaminated species by the different metals

Figure 5 shows the average concentrations of the trace elements [wet weight (mg/kg)] in the soft tissues of the two species by class and by station. The relative behaviour of the two gastropods, as far as a trace element was concerned, seemed to depend both on the metal and the sampling station. In fact, the Student t-test revealed that, in the stations where the two gastropods were present (stations of Atté1 and Blokos), Pb concentrations in the soft tissues of *T. fuscatus radula* were lower than those of *T. fuscatus fuscatus*. Elsewhere, the concentrations of Fe in the soft tissues of *T. fuscatus fuscatus* were lower than those of *T. fuscatus radula* in a same station. It seemed that the relative contaminations by Cu, Zn and Cd depended on the sampling stations. While *T. fuscatus radula* was more contaminated by Cd and Zn than *T. fuscatus fuscatus* in the Blokos station, *T. fuscatus fuscatus* was more contaminated than *T. fuscatus radula* by these metals at the Atté 1 station for the same classes of size. The observations of the relative contamination levels of *T. fuscatus radula* and *T. fuscatus fuscatus* by Cu were opposite to those of Cd and Zn.

The Student t-test, applied to the concentrations in trace elements of the soft tissues of *T. fuscatus radula*, revealed that those of Atté1 were significantly less contaminated in toxic elements (Pb and Cd) than those of Blokos but no meaningful difference was observed between the concentrations in essential elements (Fe, Cu, Zn). Besides, the analysis of the metallic concentrations of the soft tissues of *T. fuscatus fuscatus* revealed that the samples of Atté1 were more contaminated in Fe and Zn than those of Blokos; but for Pb, the observations were the opposite to those of Fe and Zn. No significant

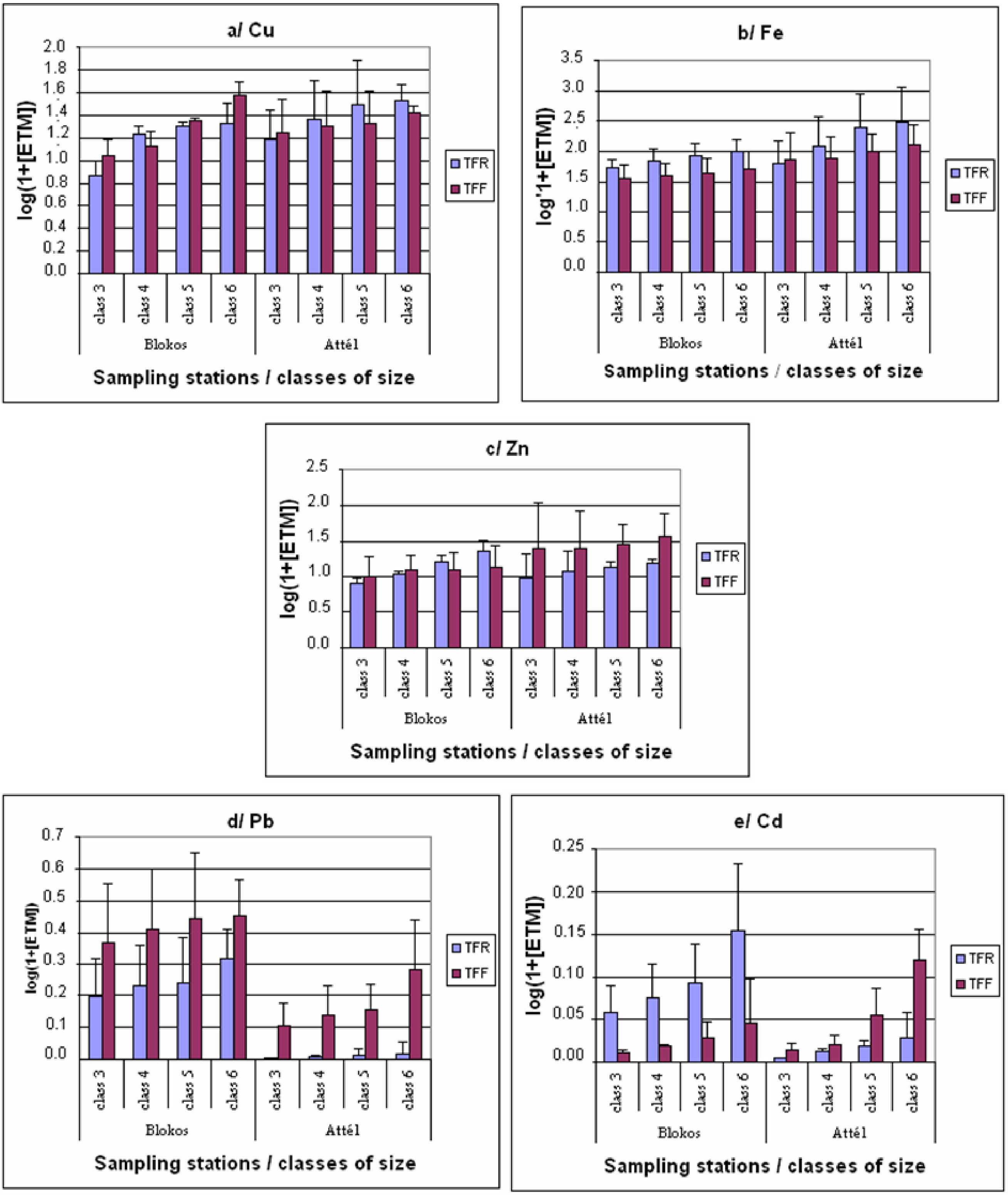
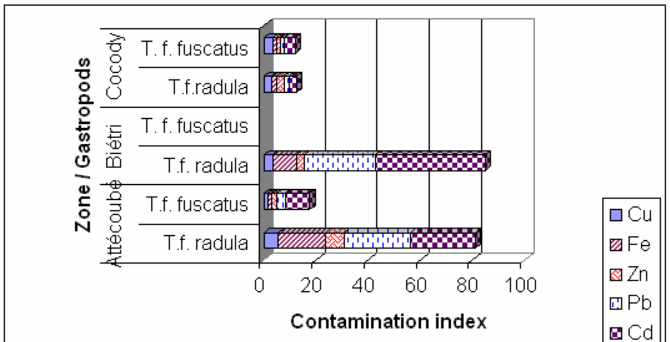


Figure 5. Mean concentrations of each trace element in the soft tissues of TFR and TFF per class of size at Blokos and Atté1 stations: a) Cu, b) Fe, c) Zn, d) Pb, e) Cd.

difference was observed considering the concentrations in Cu and Cd at the level of 5%.

According to Hamed and Emara (2006), some physiological and environmental factors could be responsible



	Attécoubé		Biétri		Cocody	
	T.f. radula	T.f. fuscatus	T.f. radula	T. f. fuscatus	T.f.radula	T. f. fuscatus
Cd	24.714	9.382	42.000		3.000	4.192
Pb	25.428	3.400	27.473		1.820	1.361
Zn	7.142	1.491	2.764		2.968	1.349
Fe	18.171	1.798	9.340		1.818	1.465
Cu	5.589	1.523	3.327		3.130	3.623

Figure 6. contamination index of TFR and TFF per collection zone.

for the observed differences: they could be due to the difference in availability of the metals to the biological organisms or to the fact that different mechanisms of absorption and retention of a same metal are developed by these, at the different stations.

Bioaccumulation ability of the two gastropods

Figure 6 shows the contamination index (CI) of *T. fuscatus radula* and those of *T. fuscatus fuscatus* calculated from the metallic concentrations in their soft tissues for each collection zone. It is observed that *T. fuscatus radula* presented indices of metallic contamination higher than those of *T. fuscatus fuscatus* in all zones, except for Cu and the Cd in Cocody. This result seemed to show that *T. fuscatus fuscatus* could be less capable to reflect the conditions of the environment than *T. fuscatus radula*. Moreover, the highest indices of contamination were observed for the toxic elements in the zones of Attécoubé and Biétri, while the opposite was observed in Cocody. So the zones of strong anthropogenic activities were the sources of the highest concentrations in toxic elements.

Metallic contamination index ratios

Case of survey zone as a whole

As *T. fuscatus fuscatus* had disappeared from the zone of Biétri, only data for *T. fuscatus radula* was taken into account from this zone to calculate the contamination index ratios of the two gastropods for the whole survey zone. They were added to those of the two gastropods in Attékoubé and Cocody before the maximal and minimal values of the soft tissues' metallic concentrations were determined for all three zones (Table 3).

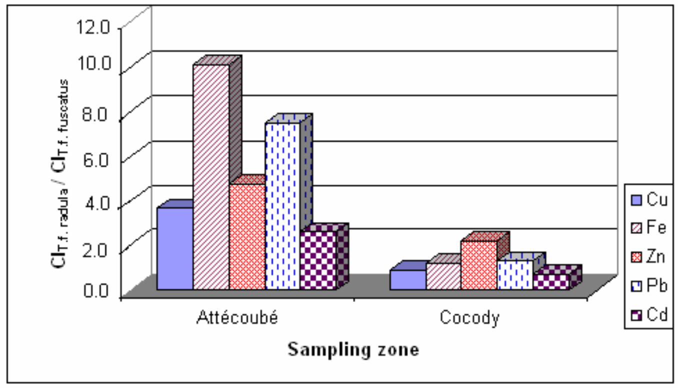


Figure 7. Ratio of contamination index of TFR to those of TFF in the collection zones of Attécoubé and Cocody.

The observed ratios of contamination index showed that *T. fuscatus radula* had the highest bioaccumulation capacity irrespective of the metal considered. Besides, the toxic traces elements were always more bio-accumulated by the two gastropods than the essential ones; the orders of bioaccumulation were Pb>Cd>Fe>Zn>Cu and Cd>Pb>Zn~Fe~Cu for *T. fuscatus radula* and *T. fuscatus fuscatus*, respectively. If the contamination index varied from one metal to the other in the case of *T. fuscatus radula*, these were in the same order of magnitude for *T. fuscatus fuscatus* with regard to the essential elements. The values obtained indicate that *T. fuscatus radula* can accumulate more than 4, 3, 6, 95 or 1.5 times Fe, Zn, Cd, Pb, Cu respectively than *T. fuscatus fuscatus*.

Case of each sampling zone

Figure 7 shows the ratios of the metallic contamination index of *T. fuscatus radula* with respect to those of *T. fuscatus fuscatus* in the zones where the two species were present. It was observed that the ratios of the contamination index were higher in Attécoubé than Cocody irrespective of the metal. Besides, this ratio followed the order Fe>Pb>Zn> Cu>Cd and Zn> Pb>Fe>Cu>Cd in the zones of Attécoubé and Cocody, respectively. The values obtained showed that, *T. fuscatus radula* could accumulate more than 9, 4, 3, 2 or 7 times Fe, Zn, Cu, Cd, Pb respectively than *T. fuscatus fuscatus*.

On the whole, whichever approach was considered, it was evident that the radula form (*T. fuscatus radula*) will permit to discover levels of less important pollution than *T. fuscatus fuscatus*.

Characterization of the trace elements bioavailability in the different stations of the survey zone

The dendrogram resulting from hierarchical classification analysis on the basis of the trace elements contents of the soft tissues of *T. fuscatus radula* at the different sta-

Table 3. Contamination index (CI) of the two species in the whole of the survey zone.

TRACE ELEMENT	$IC_{T.\ fuscatus\ radula}$	$IC_{T.\ fuscatus\ fuscatus}$	$IC_{T.\ radula}$ / $IC_{T.\ fuscatus}$
Cu	5.59	3.62	1.50
Fe	18.17	3.73	4.90
Zn	12.07	3.77	3.20
Pb	636.05	6.63	95.90
Cd	80.62	12.08	6.70

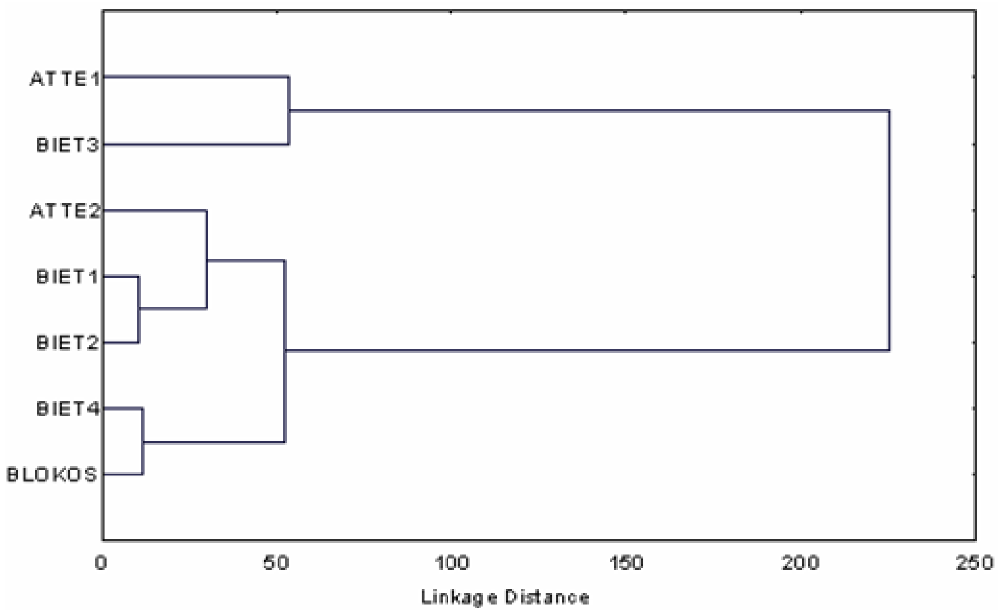


Figure 8. Dendrogram of the sampling stations with respect to the availability of trace elements to *T. fuscatus radula*.

tions is shown in Figure 8. It can be seen this figure helps one to distinguish two main types of stations: 1. The first type regrouped the stations of Atté1 and Biét3 which were characterized by the highest concentrations of iron in the gastropods' soft tissues as opposed to those of other stations. On the other hand Atté1 and Biét3 distinguish themselves by the highest concentrations of copper in the gastropods' soft tissues for the sampling station of Atté1 whereas those of Biét3 were among the lowest of the whole survey zone. 2. The second type regrouped stations Atté2, Biét1, Biét2, Biét4 and Blokos which could be placed into two subtypes based on their Cd concentration differences. The gastropods of Atté2, Biét1 and Biét2, the first subtype, had the lowest concentrations while those of Biét4 and Blokos, the second subtype, presented the highest concentrations of Cd in their soft tissues. Within these two subtypes some features made possible to characterize metal bioavailability in the stations. In the first subtype: 1. gastropods of Atté2 station distinguished themselves from those of Biét1 and Biét2 stations by the concentrations of lead in their soft tissues: these lead concentrations were the highest of the survey zone for the Biét1 and Biét2 stations; 2.Gastropods of Biét1 and Biét2 distinguished themselves by the concentrations of Cd in their soft tissues, the highest being in Biét2.

In the second subtype, the soft tissues of gastropods of Biét4 and Blokos had the highest concentrations in cadmium. Those of Biét4 distinguished themselves by having much lower levels in Zn and in Cu than those of the Blokos.

On the whole, the zone of Biétri appeared to be the place where the trace elements investigated were the most available. However, for every trace element, particular stations seemed to exist where higher quantities were available as indicated above. Indeed, the bays are the most polluted parts of the lagoon because of the low renewal rates of waters contained therein, which do not permit the good dilution and the evacuation of the pollutants (Dufour et al., 1994).

The bay of Bétri, (semi - closed area), is the most isolated of the bays of the urban region (Arfi et al., 1981). It is here that the industrial activity is most intense and therefore receives the largest chunks of pollutants

Table 4. Comparison of Cd and Pb contents of soft tissues with consumption standards.

Metal	Size Class	*T. fuscatus fuscatus* metallic concentration (mg/kg ww)	*T. fuscatus radula* metallic concentration (mg/kg ww)	Standards of EU/WHO/FAO (mg/kg ww)
Cd	3	0.0264 – 0.0340	0.0073-0.1427	1.0
	4	0.0452 – 0.0495	0.0142-0.2450	Règlement (CE) N°466/2001 (*)
	5	0.0665 – 0.1342	0.0210-0.4072	CX/FAC 05/37/19 April 2005 (**)
	6	0.1087 – 0.3190	0.0487-0.5885	
Pb	3	0.2750 – 1.3389	0.0073-2.8580	1.5
	4	0.3741 - 1.5641	0.0095-3.3798	Règlement (CE) N°221/2002 (***)
	5	0.4345 – 1.7892	0.0246-4.3158	
	6	0.9347 - 1.8233	0.0397-4.6432	

(Dufour et al., 1994); the currents are weak (Lemasson et al., 1981) and the renewal of waters limited. It stands out therefore as the most polluted as several authors have indicated (Dufour and Seploukha,1975; Marchand and Martin, 1985; Dufour et al., 1994; Adingra and Arfi, 1998).

Marchand and Martin (1985) pointed out earlier excess levels of different trace elements including Zn, Cu, Pb, Cd, in the sediments of the bays of Bétri, Banco (Attécoubé) and Cocody notably. The presence of the site of repair dock as well as the CARENA workshops explain the Fe pollution dominating in Attécoubé.

Assessment of risks linked to the gastropods' soft tissues contamination

The assessment of risks to which the inhabitants consuming the two gastropods are exposed has been done (Table 4) by comparing the levels of the metallic concentrations of the toxic elements (Pb and Cd) in their soft tissues with the consumption standards of the FAO and the WHO (1982)] or of the EU (2001, 2002). 10 samples out of 28, that is, 35.57%, all collected in the zone of Bétri (mainly at the stations of Biét1, Biét2 and Biét4) and covering the different classes of size from *T. fuscatus radula*, were unfit for consumption because of their concentrations of soft tissues in Pb superior to European Union standards (EU); this proportion concerned 3 samples out of 14 (21%) for *T. fuscatus fuscatus* also covering the different classes of size, all collected in the zone of Cocody.

Besides, one also noted that no surpassing of the consumption standards of the EU or the FAO was observed for the concentrations of Cd in the soft tissues of the two species; however, the long term danger exists, considering the cumulative character of cadmium (Lauwerys, 2003; INERIS, 2005). Besides, synergistic actions of the metallic elements are not to be excluded due the possibility of increasing their respective toxicities.

Conclusion and perspectives

Of the two species of gastropods, *T. fuscatus radula* seems more adapted to the lagoon environment which has diverse pollution levels. It is a better bio-accumulator of trace elements than its ecotype *T. fuscatus fuscatus.* This allows it to be used to detect lower metallic pollution levels. These results designate *T. fuscatus radula* as a biological indicator of pollution by trace elements. However, these results have to be confirmed and the survey zone extended to cover the whole lagoon, investigate the influence of different seasons while trying to establish an interrelationship between the metallic concentration of its soft tissues and that of the ecosystem (water, sediments, etc).

Elsewhere, because of its adaptation to its environment and its capacity to accumulate trace elements, *T. fuscatus radula* creates risks for inhabitants consuming it. A campaign to increase public awareness should be organized for consumers of these gastropods as well as for the fishermen in order to minimize these risks.

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