Journal of Biology, Agriculture and Healthcare
ISSN 2224-3208 (Paper) ISSN 2225-093X (Online)
Vol.11, No.4, 2021

Heavy Metal Contamination Levels in Clams (Galatea paradoxa, Born 1778) and Surface Sediments from Mono River Estuary, Togo, and its Health Implications

Kamarou Faré KONDO[1] Komlan Mawuli AFIADEMANYO[2*] Kamilou OURO-SAMA[1]
Hodabalo Dheoulaba SOLITOKE[1] Kissao GNANDI[1]
1.Laboratoire de Gestion, Traitement et Valorisation des Déchets (GTVD), Faculté des Sciences, Université de Lomé, BP. 1515, Lomé, Togo
2.Laboratoire d'Ecologie Animale et d'Ecotoxicologie, Département de Zoologie et Biologie Animale. Faculté des Sciences, Université de Lomé, BP.1515, Lomé-Togo

Abstract
Heavy metal pollution in aquatic ecosystems is one of the most important threats of human health and food chain. Thus, in this study, the level of Cd, Fe, Hg, Mn, Pb and Zn accumulation in surface sediments and in *Galatea paradoxa*, were investigated to estimate their potential health risks via consumption to residents alongside the Mono river, Togo-Benin border. Samples were collected at two fishing areas and analyzed using flame atomic absorption spectrophotometry Moreover, the likely health risks developed as a result of human contamination by heavy metal through clam consumption was also assessed using target hazard quotient (THQ) and hazard index (HI). The mean maximal metal concentrations (µg/g dw) were as follows: Fe (25624) > Mn (1176.0) > Zn (23.29) > Pb (4.67) > Hg (0.038) in silty sediments and Fe (15692) > Mn (654.78) > Zn (12.53) > Pb (2.42) > Hg (0.034) in sandy sediments. Cd content were all below the detectable limit. When compared against some consensus-based sediment quality guidelines (TEL, ERL, AFNOR), it was observed that all the concentrations, excluding Fe levels in the samples, were lower than reference values. Similarly, computed PLI was less than 1 indicating that the lower tidal parts of the Mono were unpolluted. The accumulation pattern of studied trace metals in the clams followed a slightly different trend from that found in the sediments (Fe>Zn>Mn>Hg>Pb>Cd). Fe (1353 ± 162 µg/g dw) and Zn (51,8 ± 8.9 µg/g dw) content in whole tissues of *G. paradoxa* were well above the maximum concentration allowable by food safety criteria. In contrast, Cd (0.097 mg/kg dw), Hg (0.24 mg/kg dw) and Pb (0.18 mg/kg dw) content were very low. Computed THQ values were all below 1, with highest results found in the small size clams regardless of the heavy metals. The HI values indicate that none of the analyzed heavy metals may pose serious risk to consumers through the clams studied. The highest risk was posed by small clams, followed in decreasing order by medium and large clams. We therefore suggest that the clams taken from main fishing locations at Mono river are safe to eat.
Keywords: Heavy metals, Sediment pollution, *Galatea paradoxa*, Human health, Mono River
DOI: 10.7176/JBAH/11-4-06
Publication date: February 28th 2021

1. Introduction

Pollution of coastal waters has increased dramatically in recent years and has led to an increase in environmental problems in many developing countries (Kumar *et al.* 2013). In Togo, since the second half of the 1900s, rivers, estuaries and coastal lagoons have received high inputs of pollutants due to the escalation of the activities of palm oil plantations, the misuse of pesticides in cotton cultivation, the cross-border traffic in adulterated fuel and above all, the discharge of untreated waste from the treatment of phosphates at the Kpémé plant (Gnandi & Tobschall, 2001; Gnandi, 2003; Rezaie-Boroon, 2011; Tanouayi *et al*. 2015). Although metals such as Zn, Cu, Mn, Co and Fe are known to be essential elements, which play important roles in biological metabolism at very low concentrations (Saeed *et al.* 2008), all trace metals whether essential or not, are potentially toxic at a threshold bioavailability. Existing research has demonstrated that living organisms surviving in highly polluted environments are subjected to severe oxidative stress potentially leading to cell death (Padmini *et al.* 2005; Yahya *et al.* 2018). Furthermore, heavy metals entering aquatic ecosystems subsequently enter the food chain and accordingly a frequent consumption of seafood contaminated by trace metals, namely As, Cd, Hg, Pb, Cd and Cu, can cause hazards to human health (Jensin & Jernelov, 1969; Mance, 1987).

Benthic molluscs are widely used in a given ecosystem to detect positive and negative changes. They have proved as useful bio monitors in freshwater and marine ecosystems due to their natural capacity to accumulate elevated concentration of trace metals and their persistence in the same place. (Elder & Collins, 1991; Richardson & Lam, 2004; Weng & Lu, 2017). Bivalves have also the ability to detoxify themselves (Connell *et al.* 1999). The balance between these two processes is not fixed but depends on many factors. Factors known to affect the uptake of trace metals and their accumulation in bivalves include metal bioavailability, characteristics of the physical environment, season of sampling, size and physiological state of the organism (Boening, 1999; Ravera, 2001).

The freshwater clam, *Galatea paradoxa* (Born, 1778) [Order, Veneroidea, Superfamily, Tellinoidea and the Family Donaciade] is a bivalve mollusc confined to estuaries and lower tidal parts of large rivers in West Africa including Mono River (Lawson, 1963; Kondakov *et al.* 2020). The species is the basis of an artisanal fishery throughout it range (Etim, 1994; Adjei-Boateng *et al.* 2012). Individuals are harvested from their natural growing beds and serves as a means of livelihood to young men and women from nearby communities who fish, process and market the species. Its nutritional properties make it valuable to consumers, so its consumption and market value have increased significantly in recent years. Regrettably, most *Galatea* populations are heavily threatening by anthropogenic impacts such as dam construction, riverbed substrate mining and river pollution (Adjei-Boateng *et al.* 2012; Obirikorang *et al.* 2013). Therefore, the safety of clams has been a matter of concern for human health and has been attracting more attention (Etim, 1991; Obirikorang *et al.* 2009, 2010; Amissah *et al.* 2010; Nwabueze & Oghenevwairhe, 2012; Asare-Donkor *et al.* 2015; Leizou & Muhammad, 2018).

In Togo, research on metal contamination in aquatic environments has increased in recent years but most of them focused on concentration of pollutants in water, marine organisms and lagoon's fish species (Gnandi *et al.*, 2006; Bawa, 2007; Ouro-Sama *et al.*, 2014; Ouro-Sama, 2019). Consequently, despite their economic importance, bivalve shellfish, notably freshwater clams and mangrove oysters have received very little attention (Solitoke, 2019). Thus, our study aimed to (i) to determine the concentration of some heavy metal (Cd, Fe, Hg, Mn, Pb and Zn) in *G. paradoxa* a bivalve mollusk harvested from the Mono river (ii) to investigate the pollution levels of two important fishing sites. Frequently, fried or boiled clams are sorted in lot of different sizes (small, medium and large) before being sold. It would then be interesting to investigate the influence. In addition, since *G. paradoxa* is an important component of the human diet in the zone, the risk of consuming the clam was also assessed.

2. Materials and Methods

2.1 Study area

Samples were obtained in December, 2017 and July, 2018 (wet and dry seasons, respectively) from stationary fishing points located between Kpondavé, latitude 6°19'60" N and longitude 1°43'0" E and Avévé, latitude 6°24' N and longitude, 1° 45' E in the District of the Grand Lakes (Figure 1). The two sites are within the active clam fishing zones on the Mono River.

The Mono River is the most prominent river in Togo. Approximately 400 km long, this river drains into the Bight of Benin through an extensive system of brackish water lagoons. Along the southern portion towards its mouth, it forms the international boundaries between Togo and Benin. Most of the river's basin on the upper tableland is cultivated for maize, cassava and cotton.

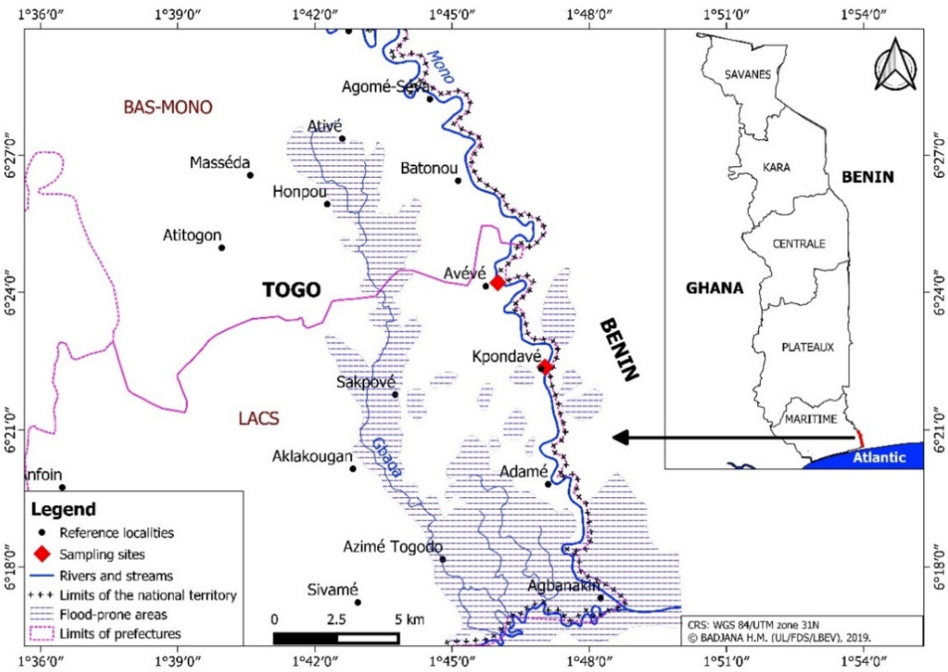

Figure 1. Map showing the study area and sampling sites of the Mono river estuary

The fluvial sediments in the clam distribution areas are mainly sand particles. However, depending on the site, there may be significant differences in the particle size distribution of the beds. Thus, muddier sediments are found in areas where bank erosion is very high. This is the case of the Kpondavé site where the proportions of fine particles (<63 μm) vary around 40% depending on the season. On the other hand, the sediments of the Aveve site are essentially sandy with a proportion of medium size particles exceeding 80%.

2.2 Samples collection and analytical methods

The Clams were collected by hand picking at low tide. For each sampling site, 30 individual clams for each size were obtained and categorized based on shell length as: small (25 - 40 mm), medium (41 - 55 mm) and large (>55 mm). There were three replicates for each size class. Back to the laboratory, the bivalves were kept for 24 h in river water from the same sampling station to allow depuration of gut contents and of particulate material present in the mantle cavity. A sterile stainless steel knife was used to quickly dislodge and remove the flesh of each clam from the shell as described by Chiu *et al.* (2000). These fleshes were oven-dried to a constant weight at 60°C (Ferreira *et al.* 2004). The clams of each size class were ground together using pestle and mortar. Homogenised subsamples were digested with nitric acid (HNO_3 68%) in Teflon vessels at 90°C for Fe, Mn, Pb, Zn analysis and at room temperature for Hg one.

Sediments were dried at 80°C to a constant weight. After grinding then sieving, 2 g of the fraction inferior to 63 µm was digested using aqua regia method ($1HNO_3$:3HCl) according to the French standard NF ISO 11466 at 120°C for Fe, Mn, Pb, Zn analysis and at room temperature for Hg analysis. Analyses were carried out using Atomic Absorption Spectrophotometer (AAS) Thermo Electron S Series type for Fe, Mn, Pb, Zn. As for Hg it was analyzed using a hydride and cold vapour generator, Thermo Scientific VP 100 type coupled to the AAS Thermo Electron S Series. These analyses were carried out according to French standards (NF T90-112, NF EN ISO 5961, NF EN 1233, NF EN 1483, NF EN ISO 11969) in the "Gestion, Traitement et Valorisation des Déchets (GTVD)" laboratory of the Faculty of Sciences, University of Lomé. All solvent/reagents were of analytical grade (SIGMA-Aldrich and Merck).

2.3 Quality control and accuracy

The quality of the analytical methods has been verified by internal control. For that, a blank sample prepared simultaneously with the same reagents and under the same experimental conditions. It allowed zeroing the spectrometer. It permitted to determine possible contaminations and to eliminate the quantization errors. Also, standard solutions of each element were analysed at regular intervals to verify the accuracy of the results.

2.4 Assessment of sediments contamination

2.4.1 Contamination factor (CF)

The contamination factor (CF) was calculated with the following equation (Häkanson, 1980):

$$CF = \frac{Cm \quad Sample}{Cm \quad Background} \qquad (1)$$

where Cm Sample refers to the concentration of a given metal in the sample studied and Cm Background refers to the value of the same metal in the reference sample. The CF values were interpreted as follows: CF<1 indicates low contamination, 1≤CF<3 is moderate contamination, 3≤CF<6 is considerable contamination, and CF>6 is very high contamination. In this study, average shale (Turekian & Wedepohl, 1961) was used as a background value for heavy metals (Zn, Cr, Cu, Ni, Mn, Co, and Fe) since there were no existing background values for these heavy metals in the study area.

2.4.2 Pollution load index (PLI)

Originally proposed by Tomlinson *et al.* (1980) and thereafter widely used (Li et al., 2013; Sahli *et al.* 2014), the PLI provides valuable information and advice for policy and decision makers on the pollution level of an aquatic ecosystem. The PLI of a single site is obtained using the equation below:

$$PLI = \sqrt[n]{CF1 \times CF2 \times \cdots \times CFn} \qquad (2)$$

where n is the number of metals. The PLI value of 0 indicates perfection, a value of one (1) indicates the presence of only baseline levels of pollutants, and values above one (1) would indicate progressive deterioration of the site and estuarine quality.

2.5 Health risk analysis

2.5.1. Calculation of daily intake rates (EDI)

Daily intake of contaminated food is a general pathway of heavy metal exposure for local inhabitants. The estimated daily intake was calculated to assess the average daily loading of metal into the body system of a specified body weight of a consumer. It was calculated based on the formulas below (Chien *et al.* 2002; Rattan *et al.* 2005; USDA, 2016):

$$EDI = \frac{Chm \times Ef \times Ed \times IR}{B_W \times TA} \qquad (3)$$

$$IR = ADI \times Cf \quad (4)$$
$$Cfactor = IRww - IRdw \quad (5)$$
$$IRdw = IRww[(100 - Wac)] \div 100 \quad (6)$$

From which Chm is the heavy metal concentration in clams (mg/kg dw), Ef, is the exposure frequency (365 days per year); Ed is, the exposure duration, equivalent to average lifetime (61,1 years for an adult in Togo) [United Nation, 2019]; IR is the ingestion rate (kg/person/day); Cf is the conversion factor (0.265) for fresh weight (ww) to dry weight (dw); Bw is the average body weight (average adult body weight was considered to be 67,64 kg) (Aduayi-Akue, 2015) and TA is, the average exposure time for non-carcinogens (given by the product of Ed and Ef).

IRdw is the dry weight intake rate, IRww is the wet weight intake rate, and Wac is the percent of water content in the raw clam which was 76.7% in this study.

The consumption rate (ADI) of fresh clam for Togolese is not available in the literature. Therefore, the average daily intake rate (ADI) was calculated by conducting a survey where 100 adults within the fishing communities were asked for their daily intake of clam (Wang *et al.* 2005; Khan *et al.* 2009). The average daily clam intake for adults and children were calculated to be 95g/day/person (expressed as fresh weight).

2.5.2 Target hazard quotient and Health index

Risk of intake of metal-contaminated clam to human health was characterized by Target Hazard Quotient (THQ) and Health Index (HI). THQ is a ratio between exposure to a potentially hazardous element and its reference dose (RfD. This risk assessment method has been applied by many researchers on bivalves (Denil et al., 2017; Liu et al., 2018) and proved to be valid. The calculation method was as follows:

$$THQ = \frac{EDI}{RfD} \quad (7)$$

The RfD values used in this study for Cd, Fe, Hg, Mn, Pb and Zn were 1×10^{-3}, 7×10^{-3}, $1,4 \times 10^{-3}$; $3,6 \times 10^{-3}$ and 3×10^{-3} (mg/kg body weight/day) respectively (US EPA, 2013; RAIS, 2020). Assuming additive effects, HI (TTHQ) is a measure of the potential risk of adverse health effects from more than 1 element. HI greater than 1 suggests likelihood of adverse effects on human health and the necessity for further action (US EPA, 1989).

$$HI = \sum_{i=1}^{n} THQi \quad (8)$$

2.6 Statistical Analysis

The STATISTICA 6.1 software was used for all statistical analyses. All data were tested for normality by Shapiro-test and homogeneity of variances by Levene's test. Since data did not respect the former assumptions of parametric analysis, non-parametric tests were applied. Kruskal-Wallis test (K-W test) followed by a multiple comparison test with Holm adjustment method was performed to detect differences between geographical areas and between clam's size classes. The level of significance for statistical analyses was always set at $\alpha = 0.05$.

3. Results

3.1 Heavy metals in sediments

The heavy metal concentrations in the sediment samples collected during the dry and rainy seasons are summarized in Table 1. Fe was by far the main metal contaminant of the sediments of the river. Indeed, the Fe content was very high with a maximum value of 25624 ± 2843 µg/g recorded during the rainy season and a minimum value of 15692± 1260 µg/g measured during the dry season. Next to Fe, the Mn concentration were also very high with a maximum value that amounted to 1176 ± 111 µg/g measured in the rainy season in the mud sediment. The sediment content of these two heavy metals were well above the allowed limit values. Contrariwise, Zn, Hg and Pb were present in the sediments but in small quantities compared to the reference values. In fact, the maximum levels measured reached only 23.29 ± 1.8 µg/g, 0.038 ± 0.003 µg/g and 4.67 ± 0.31 µg/g, respectively. The overall trend of heavy metal concentrations in the sediments was Fe > Mn > Zn > Pb > Hg

Results of ANOVA and post-hoc testing (Table 1) revealed seasonal differences in metal levels except for Hg and Pb (Kruskall-W, $p < 0.05$). Likewise, data compiled in the Table 1 also show that the concentrations of the heavy metals investigated were generally higher in the silty sediments (Kpondavé) than in the sandy sediments (Avévé) whatever the season.

Table 1. Heavy metal concentrations in sediment samples (mg/kg) from Mono River

Sampling sites	Season	Heavy metals concentrations (µg g-1)					
		Cd	Fe	Hg	Mn	Pb	Zn
Avévé (sandy substratum)	Dry	BDL	15692±126[a]	0.028±0.004[a]	387.17 ± 14.2[a]	2.42±0.39[a]	12.53±0.5[a]
	Rainy	BDL	19588±157[b]	0.034±0.004[a]	654.78 ±69[b]	1.5±0.5[a]	8.4±0.55[b]
	Mean		17640	0.031	520.98	1.96	10.47
Kpondavé (silty substratum)	Dry	BDL	22805 ±106[c]	0.038±0.003[a]	673.84±13.7[c]	4.67±0.31[b]	23.29±1.8[c]
	Rainy	BDL	25624 ±284[d]	0.033±0.004[a]	1176±111[d]	3.72±0.88[b]	15.79±1.4[d]
	Mean		23214.5	0.036	924.92	4.60	19.54
Reference values	ERL			0.15		46.7	150
	TEL			0.17		35	123
	AFNOR standards		1000	0.4	300	100	
	ASV		47200	0.4	850	20	95

a,b,c: Means carrying different superscript in each row are significantly different ($p<0.05$); BDL (Below Detection Limit). ASV-(Turekian and Wedepohl, 1961); ERL and TEL (Long *et al.* 1995)

The heavy metal contents were compared to French (AFNOR) and North American (ERL for USA and TEL for Canada) standards. The threshold effects level (TEL) and Effects Range-Low (ERL) (Long *et al*. 1995) for a given sediment parameter are the concentrations below which adverse biological effects are expected to occur only rarely. Our results show that the heavy metal contents were almost all below the standards used. Only contents of Fe were much higher than the French standard (up to 260%).

Table 2. Contamination factors (CF) and Pollution Load Index (PLI) of heavy metals

Site	Season	Contamination factors (CF)					PLI
		Fe	Hg	Mn	Pb	Zn	
Avévé	Dry	0.45	0.13	0.65	0.14	0.18	0.23
	Wet	0.56	0.16	1.09	0.09	0.12	0.27
Kpondavé	Dry	0.65	0.18	1.12	0.27	0.33	0.41
	Wet	0.73	0.16	1.96	0.27	0.22	0.42

The average contamination factor (CF) of Fe, Hg, Mn, Pb and Zn were 0.56, 0.16, 1.20, 0.19 and 0.21 (Table 2). Overall, the CF for all metals were in the descending order of Mn>Fe>Zn>Pb>Hg. It follows that pollution load index was below 1 (PLI < 1).

3.2 Heavy metals in clams

Concentrations of six metals in whole tissue of clams depending on the season and type of sediment are shown in Figure 2.

Cadmium (Cd) levels in clams collected in dry season were in concentrations below the detection limits and were very low in those collected during the rainy season. The highest concentration of Cd (0.097 µg/g) was found in small-size clams. Iron (Fe) levels in the whole clam tissue were all very high in the dry season whatever the substrate (silt or sand) in which the molluscs were collected. Fe concentration during that season decreased from 1560 µg/g in small individuals to 1186 µg/g in large-size clams. Conversely, the Fe concentrations measured in the rainy season were all low. Mean Mn content also decreased with the mollusc size class. At Kpondavé, the contents of Mn in small-sized clams were 62,5 µg/g in dry season and 30,54 µg/g in rainy season while those in large size clams were 47,78 µg/g and 18, 77 µg/g respectively. Zinc (Zn) levels are relatively low regardless of the sampling site, when compared to the WHO standard of 1000 µg/g. In the dry season, mean Zn concentrations in the whole soft tissue decreased from 62,7 µg/g (in small-size) to 48,75 µg /g (in large-sized clams). In the rainy season, Zn concentrations varied little with values around 48 µg/g.

The tendency to decrease the metal concentrations with an increase in body size class is not significant in all cases. Regarding Hg, concentrations were low and had changed very little in the dry season whereas on the contrary, during the rainy season, the metal level increases with the size of the mollusc. The small individuals contained 0.01 and 0,015 µg/g of Hg respectively while the larger ones contained 0.22 and 0,16 µg/g. Lead (Pb) concentrations exhibited an irregular pattern for different sized clams at the two sites. At Kpondavé, metal levels in medium-size clams of both season and large-size clams of the rainy season were below the detection limit while small and large size clams recorded high Pb concentration (0,39 and 0,2 µg/g respectively).

The different clam size classes (small vs. small, medium vs. medium, and large vs. large) are compared using Kruskal–Wallis one-way analysis of variance. Results shows that metal concentrations were generally higher in the dry season than during the rainy season. Exception were observed at Avévé in the case of Hg contents in small size class (K-W, p-value = 0.06) and Zn levels in medium-size class (K-W, p-value= 0.66). It should also be noted that for Zn, the trends were contradictory; it is rather the clams harvested in the rainy season which contained higher contents. Likewise, slight geographic variations related to the nature of riverbed were also found in Fe, Hg, Mn and Zn concentrations. Indeed, the heavy metals content of clams collected at Avévé (mainly sand particle) had lower metal concentrations than those of Kpondavé (muddier sediments). However, observed differences were statistically significant only in the case of Mn and Zn ($p<0.05$).

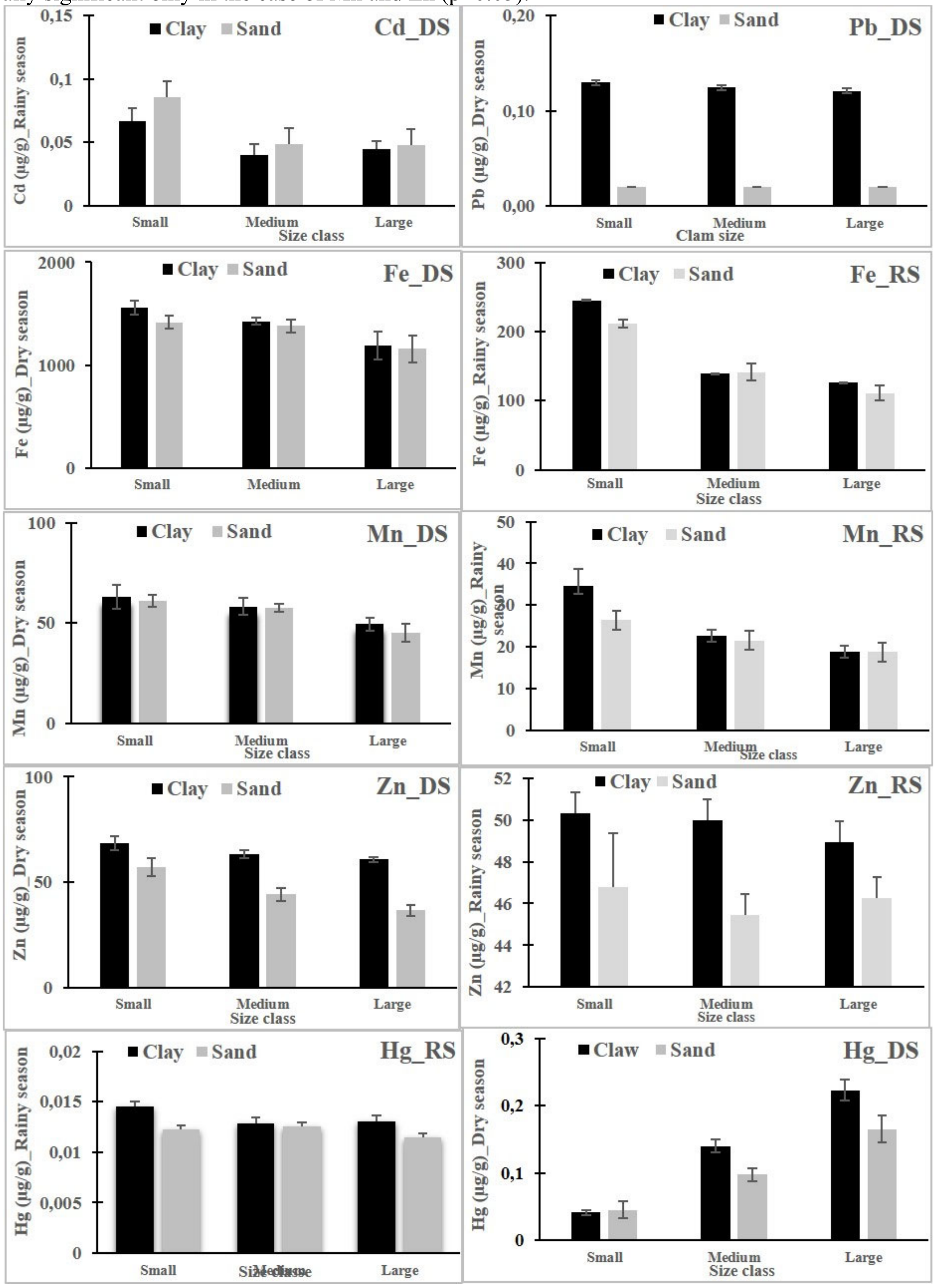

Figure 2. Mean concentrations of Cd, Fe, Hg, Mn, Zn in clam tissues are presented (DR: Dry season; RS: Rainy season)

3.3. Health risk assessment of clams

As shown in Table 3, highest daily intake was calculated for iron (0.330 mg/kg bw/day), while the lowest values were recorded for cadmium (0-2.49x10-5 mg/kg bw/day). Meanwhile, computed target hazard quotient (THQ) values and hazard indexes (HI) following the consumption of different sizes class clams are given in Table 4. The THQ varied from 0.357-0.541 for Mn, 0.337-0.441 for Fe, 0.053-0.068 for Zn, 0.001-0.016 for Pb, 0.001-0.003 for Hg and 0-0.017 for Cd, with highest values found with the small clams' category regardless of the heavy metal. As seen from the table, THQ of the studied metals were all much lower than 1, especially for Cd, Hg, Pb and Zn. The overall trend of health risk of heavy metals for the consumption of clams from Mono River is Mn > Fe>Zn > Cd > Pb > Hg and small size clams > medium size > large size. After summation, it appears that, hazard indexes (total THQs) were close to 1 for medium (0.96) and large (0.84) clams and slightly above the unity for small clams.

Table 3. Estimated daily intake (EDI) of metals via clam consumption (mg/kg/day)

Clam category		EDI					
		Cd	Fe	Hg	Mn	Pb	Zn
Kpondavé	Small	$2.49x10^{-5}$	0.308	$1.03x10^{-5}$	0.018	$6.35x10^{-5}$	0.019
	Medium	$1.49x10^{-5}$	0.291	$2.85x10^{-5}$	0.015	$5.56x10^{-5}$	0.020
	Large	$1.67x10^{-5}$	0.244	$4.4x10^{-5}$	0.012	$6.45x10^{-5}$	0.020
Avévé	Small	0	0.330	$1.03x10^{-5}$	0.016	$6,7 x10^{-5}$	0.019
	Medium	0	0.281	$2.05x10^{-5}$	0.015	$5,9 x10^{-5}$	0.017
	Large	0	0.246	$3.32x10^{-5}$	0.012	$1.7 x10^{-5}$	0.015

Table 4. Non-carcinogenic risk (target hazard quotient, THQ) and overall toxic risk (hazard index, HI) of clams from Mono river

Clam category		THQ						HI
		Cd	Fe	Hg	Mn	Pb	Zn	(∑THQ)
Kpondavé	Small	0.025	0.441	0.001	0.541	0.016	0.062	1.06
	Medium	0.015	0.416	0.002	0.458	0.014	0.068	0.93
	Large	0.017	0.349	0.003	0.381	0.016	0.068	0.84
Avévé	Small	0	0.471	0.001	0.491	0.002	0.064	1.02
	Medium	0	0.402	0.001	0.445	0.001	0.056	0.91
	Large	0	0.337	0.002	0.357	0.004	0.053	0.75

4. Discussion

4.1 Distribution of Metals in riverbed sediments

Previous studies have shown that Mono estuary is subjected to contaminating materials capable of initiating the impairment of the water and sediments quality (Kunkel, 1990; Rezaie-Boroon *et al.* 2011). The present study confirmed the presence of toxic trace elements in surface sediments of main fishing grounds of *G. paradoxa* at lower tidal parts of the river. Of all the heavy metals investigated, Fe was found to be the most abundant metal in the sediments, ranging from 17640 to 23214 µg/g. These values were significantly higher than those observed in most of the sub regional rivers such as Volta in Ghana (Madkour *et al.* 2011), Sombreiro & Ewuru/Rara in Nigeria (Wokoma, 2014; Omorola *et al.* 2014) but are not unprecedented (Table 5); it was established that iron is the most widespread heavy metal in nature, making up 5.08% of the Earth's crust (Greenwood & Earnshaw, 1998).

The study did not observe any significant local pollution sources in the vicinity of the sites. Nonetheless, the occurrence of enhanced concentrations of Fe in Mono River may be related largely to the geology and geochemistry of southern part of Togo. Indeed, the outcropping rocks of the Precambrian-Paleozozoic crystalline bedrock of Togo consist of iron rich itabirites to intermediate granitoids, including enclaves of mafic igneous rocks and gneisses (Agbossoumonde *et al.* 2007). The weathering of this bedrock may have released these minerals. This conclusion is consistent with Thornton and Nduku (1982) who attributed the very high concentration of iron in the Gwebi River (Zimbabwe) to the underlying banded ironstones that traverse much of the northern part of the upper Manyame catchment. Likewise, high iron values in Day river (Morocco) were related in part to its iron calcareous-rich sediments (Barakat *et al.* 2012).

Table 5. Heavy metal concentrations (µg/g) in the sediments from Africa and worldwide

Region	Cd	Fe	Hg	Mn	Pb	Zn	Ref.
Mono River	BDL*	17640-23214	0.031-0.036	520.98-924.92	1.96-4.60	10.47-19.54	This study
Mono River	0.021-0.425		BDL		3.6-7.25	6.45-25.7	Rezaie-Boroon *et al.* 2011
Lac Zowla	0.41-2.91		0.008-0.021	106.15-3426.	7.48-25.56	18.6-98.2	Solitoke, 2019
Lagoon of Aného	0.10-2.24		0.01-0.06	109.82-3426.05	1.31-28.48	8.58-124.25	Ouro-Sama, 2019
Lake Togo	0.96				56	450	Gnandi. 2002
Volta River		693-2758	0.0069-0.024	110-393		01-09	Madkour *et al.* 2011
Lower Volta		56821		318	21.7	391	Biney, 1991
Sombreiro River	0.022	340.7 ± 106.2			0.474± 0.4	5.37±1.9	Wokoma, 2014
Ewuru/Rara	BDL	687.23		52.62	13.23	91.14	Omorola *et al.* 2014
Nakivubo stream	0.88-1.62	30085-58354		363.47-1467.47	64.05-138.64	177.89-442.4	Sekabira *et al.* 2010
Day River	0.6-6.27	15670-36010			72.93-140.36	49.84-149.19	Barakat *et al.* 2012
Turag river	0.2-3.6	10413-14455		2512-7964	13.1-24.6	119.4-548.9	Mohiuddin *et al.* (2016)
Drini Bardhë	0.1-1	14515-24430		220.8-458.8	11.6-30.3	38.1-99.4	Haxhibeqiri *et al.* 2015

BDL= below the detection limits of the Atomic Absorption Spectrophotometer

Similarly, the very high concentrations of Mn in surface sediments could derived from the natural geological processes of rock alteration and particle transport associated with the phenomenon of hydrated oxide precipitation of Fe/Mn in an aerobic environment (Singer and Stum, 1970). However, contamination of anthropogenic origin cannot be excluded, the Mono River catchment being an agricultural beehive, in particular cotton and oil palm cultivation (Mawussi *et al.* 2010). As such, fertilizers, insecticides and herbicides used on these crops are subsequently washed into the river via surface runoff.

On the other hand, Cd, Hg and Pb were either below detection limit or present but in very low concentrations. Higher values of these metals have been found in previous work on sites located downstream from ours (Rezaie-Boroon *et al.* 2011) and in costal lagoons (Gnandi, 2003; Ouro-Sama *et al.* 2014; Tanouayi *et al.* 2015; Solitoke *et al.* 2018). The occurrence of enhanced concentrations of heavy metals in these ecosystems had been attributed mainly to discharge effluents from the phosphorites processing plant situated at Kpémé on the coast. These effluents, rich in arsenic and cadmium, enter the coastal lagoons and the Mono River through the action of tides. The present results therefore suggested that the marine influence in the process of the contamination of the river would be limited to the lower part of the stream.

Furthermore, obtained data showed seasonal differences in the concentrations of Fe, Mn and Zn with higher values in rainy season than in the dry season for the first two metals. This finding is in agreement with previous studies (Chouti *et al.* 2010; Gouin, 2016; Ouro-Sama, 2019). The contributions due to the erosion of the geological matrix and the introduction of leachates and effluents via run-off could explained higher concentrations of chemical elements in rainy season. The low Zn levels of metals observed in the dry season could also be explained by the diffusion of the element in the dissolved phase, due to the physicochemical conditions (high temperatures) and the release of the particulate material under the action of the currents (Van Den Berg, 1993). Also, sediment enrichment may occur during dry season when heavy metals precipitating out of water columns are deposited on bottom sediments (Islam *et al*. 2015). This study observed also that average heavy metal levels in sandy sediments (Avévé site) were lower than those in muddier sediments (Kpondavé site) matching with the fact that organic matter and grain size are important factors affecting the distribution of trace elements in sediments (Liaghati *et al.* 2003; Bastami *et al.* 2012; Ouro-Sama, 2019).

Comparing results of the present study with TEL and ERL values, it was observed that all the concentrations, excluding Fe levels in the samples, were lower than reference values. Similarly, the pollution load index (PLI) calculated for the two studied sites indicate that contrary to the estuary sediments (Rezaie-Boroon *et al.* 2011), the lower tidal parts of the river contain only baseline levels of pollutants. Concentration levels of all studied metals in both samplings locations do not exceed the world average concentration of shale (Turekian & Wedepohl, 1961).

Accordingly, we concluded that the two main *Galatea* fishing grounds are not unpolluted with Cd, Hg, Fe, Mn and Zn. Only baseline levels of pollutants were recorded and thus the metals content are supposed not likely to have adverse effects on the living organisms.

4.2 Distribution of metals in clams

The analyzed concentration of heavy metal in the studied clams gives the following sequence Fe>Zn>Mn>Pb>Hg>Cd, with Fe having significantly higher concentration than all others combined (89%). Obtained heavy metal contents in the clams were compared with data reported in the literature (Table 6). Of course, heavy concentrations in the tissues of *G. paradoxa* vary considerably among studies surely due to differences in metal concentration and chemical characteristics of surface sediment from which clams were sampled and ecological need metabolism and feeding patterns of bivalves (Weng-Xion & Lu, 2017). Nonetheless Cd, Hg, Mn, Pb and Zn concentrations fell within the range of values reported for many other estuarine and freshwater environments from Ghana (Obirikorang *et al.* 2009; Serfor-Armah *et al.* 2010; Sarfo *et al.* 2011) and Nigeria (Etim *et al.* 1991; Nkwabweze *et al.* 2012; Leizou *et al.* 2018). In contrast to our findings, very high content of Cd (2.20±2.39 mg/kg dw) and Zn (4.01±0.69 mg/kg dwt) were reported by Asare-Donkor *et al.* (2015) for *G. paradoxa* sampled at Ada in Volta estuary. Nevertheless, comparing the Cd results with those of Ouro-Sama *et al.* (2014) and Solitoke (2018) who worked on oysters in the coastal area of Togo, very low concentrations were observed in the present study, indicating that the source of Cd in the study area was relatively limited

Table 6. Selected heavy metal concentrations (µg/g) in clams and mussels from West Africa rivers and lagoons

Location	Heavy metals						Reference
	Cd	Fe	Hg	Mn	Pb	Zn	
	Galatea paradoxa **(freshwater clam)**						
Mono River. Togo	0.039-0.097	111.57-1556	0.011-0.239	16.5-69.68	≤ 0.18	32.08-72.17	This study
Volta River. Ghana	0.11	71-539	0.028-0.074	49-867		13-49	Obirikorang *et al.* 2009
Lower Volta. Ghana	BDL		BDL	491.18±7.53		92.29±13.84	Sarfo *et al*. 2011
Volta estuary. Ghana	3.20-4.70	61.45-165.05			0.05-5.35		Asare-Donkor *et al.* 2015
Cross River Nigeria	0.11-0.6				0.3-3.6	96-172	Etim *et al.* 1991
Warri River. Nigeria	0.91-1.349	16.9-49.77	0.153-1.3	0.563-4.149	1.38-5.383		Nkwabweze *et al.* 2012
	Cassostrea gasar **(Mangroves mussels)**						
Zalive lagoon. Togo	40.31		0.31		10.13	908.57	Solitoke, 2019
Zowla lagoon Togo	34.26		0.2		6.43	766.3	Solitoke, 2019
Aneho lake. Togo	0.90				2.9		Ouro-Sama *et al,* 2014
Metal limits in soft tissues	0.2	100	0.5	100	0.2-2		Weng-Xiong & Lu, 2017

The amounts of iron in *G. paradoxa* compared to other studied heavy metals could be related to the abundance of this element in substrate/sediment. This hypothesis is in agreement with Regoli & Orlando (1991) who reported that when the environmental levels of iron are high, the metal may be absorbed in the forms of hydroxide flakes which are partly accumulated within endocytic vesicles and partly in inter tubular spaces. The high level of Fe might be also due to the major role played by this essential metal in maintaining the proper physiological functions of organism (Kamaruzzaman, 2010). However, the very high iron content of clams should attract attention as subsequent consumption of these seafood by humans can pose significant health risks. (Banner, 1986). Powers *et al.* (2003) have reported that that a high intake of Fe combined with Mn is responsible for the deposition of iron oxides in the case of Parkinson's disease. (Powers *et al.* 2003).

In mollusks, size and weight along with age are considered as important factors which determines bioaccumulation. Most authors reported a reciprocal relationship between body size and the accumulation of aquatic contaminants in suspension-feeding bivalves (Amiard *et al.* 1986; Yap *et al.* 2009). Bilos *et al.* (1998) have suggested that the increase in metabolic rates in relation to different body sizes might affect the uptake and elimination of metal. With regard to *G. paradoxa*, Etuk *et al.* (2000) found significant relationships between the age (body size) and metal concentrations in clams: Mg, Ca, Pb and Cd increased with clam age while those of Fe and Mn decreased. Asare-Donkor *et al.* (2015) compared different *G. paradoxa* size classes from Ada (Ghana) sampling station using one-way ANOVA and observed significant differences ($p<0.05$) for cadmium and copper concentrations in the whole tissue. In the present study, it was observed that smallest clam individuals (30-40 mm) contained the highest levels of Fe, Mn and Zn. The opposite result was obtained for mercury: the largest individuals had the highest metal concentration. This would suggest that concentration of studied trace metals in *G. paradoxa* decreased or increased depending on the metal with increasing body size. However, observed variations in the different size classes from both sampling stations were not statistically significant. This result agreed with Amisah *et al.* (2010) who also reported a non significant variations in metal concentrations with regard to body size of clams collected from the Volta River, except for mercury content. It may therefore be concluded may therefore be concluded that metals concentrations in *G. paradoxa* can increase, decrease, or remain constant with body size. The same contradictory results were observed for mussels due to complicating factors influencing the size dependence for some elements (Riget *et al*. 1996)

4.3 Potential health risk of heavy metals through freshwater clam intake

With regard to health risk to man, the mean concentrations of the chemical elements in the clams did not exceed the Federal Environmental Protection Agency (FEPA) maximum allowable limit for these elements in food. The concentrations of these metals in the clam were therefore, within health limits and therefore do not present an immediate health risk to consumers. Similarly, estimated hazard index (HI) of Fe, Mn, Zn, Pb and Cd for adults were found to be below (large and medium size classes) or just equal to the value of 1 (small size class). This means that, overall, there is no concern for potential non-carcinogenic health effects and risks. This indicates that the local inhabitants consuming shellfish, *G. paradoxa* were not exposed to possible adverse health effects. Similar studies conducted in the Volta River and Diebu Creek in Niger Delta have reached the same conclusions (Amissah *et al*. 2009; Obirikorang *et al.* 2010; Leizou *et al*. 2018).

5. Conclusion

The levels of six heavy metals in sediments and *Galatea paradoxa* were analyzed and their potential health risks were estimated. The results show relatively high levels of iron (Fe), and Zinc (Zn) in surface sediments of Mono River while cadmium (Cd), Mercury (Hg) Manganese (Mn) and Lead (Pb) content were either low or lower than the detection limits of the Atomic Absorption Spectrophotometer. The sources of heavy metals in the study area were largely natural (coming from the geological matrix) and secondarily anthropogenic (fertilizers, insecticides and herbicides used on crops via surface runoff). Nevertheless, when compared to a number of sediment quality guidelines, the concentrations of these heavy metals were found to be below the levels considered to have the potential to cause biological effects.

Fe (1186-1560 µg/g) had the highest concentration in soft tissues of *G. paradoxa* followed by Zn (32.08-72.17 µg/g). All other studied metals contents were low and below the maximum allowed limits. The computed hazard indexes for adults via ingestion pathways were found to be less or just equal to 1. It may therefore be concluded that the consumption of *G. paradoxa*, at the rate of 95 g/day/person throughout the year most probably does not pose a health hazard to the local populations. However, clams only accounted for a part of the human diet in the fishing areas. Other seafood, such as fish, crabs and shrimps might contain considerable levels of heavy metals, which could significantly increase the risk of ingestion of heavy metals by the local population. Therefore, the routine biomonitoring of the clam expanded to other shellfish for consumption should be done to ensure continuous food safety.

Competing interest: Authors have declared that no competing interests exist.

Author Contributions

A.K.M. conceived and designed the research proposals; K.F.K performed the experiments and analyzed the data, and the writing of the original draft. A.K.M, K.O., D.H.S. and K.G. contributed to the review and revision guidance of the paper. All authors have read and agreed to the published version of the manuscript.

Acknowledgements: The authors thank Hèou Maléki. BADJANA and Togbe Evegno DZEKOTO for the contribute they has provided.

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