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Bioaccumulation of Heavy Metals in the Tissue of the Clam *Galatea paradoxa* and Sediments from the Volta Estuary, Ghana

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ABSTRACT: The concentrations of heavy metals, Mn, Zn, Fe and Hg were determined in sediments and in the whole soft tissue of the clam *Galatea paradoxa* from two clam fishing locations, Ada and Aveglo at the Volta estuary in Ghana from March to September 2008. Thirty clams were obtained from each sampling location monthly and grouped into three size classes of 10 individuals each based on shell lengths as follows: small (25 -40mm), medium (41-55mm), and large (above 55mm). Metal concentrations in the tissues of the different clam size-classes from the two stations were similar and did not vary significantly.A comparative evaluation of the metal concentrations in the clams and sediments however, revealed significant variations in concentrations for Zn, Fe and, Hg. Concentration of Fe in the sediment from Ada for June was 18 times higher than the concentration in the clams similarly, Hg concentrations were approximately 5 times higher in the clam tissues than in the sediments. On the basis of calculated BSAFs the metal enrichment in the tissues of the clams rank in the following order Hg>Mn>Fe. The BSAFs indicated a significant accumulation of Hg in the clam tissues relative to the concentrations of other metals in the sediments. The concentrations of the studied metal in the clam and sediment samples are similar to those observed in areas under low pollution impact and the current the levels of contamination of these metals in the estuary do not exceed the clams' capacity of regulation.

Key words: Bioaccumulation, Heavy metals, Sediments, Galatea *paradoxa*

INTRODUCTION

Growing social concern about environmental quality has been observed in recent years, both on a global and local scale. This is connected with convincing evidence that environmental pollution results in the degradation of ecosystems. Emissions of harmful substances have negative effects on the natural environment and human health (Gadzała-Kopciuch *et al*., 2004). When the consequences of environmental pollution become visible, it is often too late to prevent and chronic toxic effects, impossible to notice at the initial stage of the process, may manifest themselves after many years (Alloway and Ayres, 1998). That is the main reason why it is imperative to conduct periodic pollution monitoring of aquatic environments. Initially, most monitoring programmes were based on the chemical analysis of major contaminants within the environment, until a number of difficulties became apparent (Jamile, 2001). Many authors found that by simply monitoring contaminants in natural waters, they were unable to integrate the overall environmental conditions and their impacts on aquatic life and further found difficulty in quantifying very low contaminant concentrations commonly found in natural waters (Phillips and Rainbow, 1994; Narbonne, 2000).

Bivalves have instead been used by several authors as indicators of aquatic pollution (Otchere, 2003, Ferreira *et al*., 2004, Kljaković-Gašpić *et al*., 2007) mainly because they are widely distributed globally, easy to handle, sessile, filter feeders that have the ability to accumulate high metal concentrations without metabolising the metals appreciably (Gunther *et al*., 1999; Nasci *et al*., 1999; Olivier *et al*., 2002), provide a time-integrated indication of environmental contamination (Regoli, 1998) and can concentrate pollutants in their tissues at concentrations greater than the ambient water (El-Shenawy, 2002). Sediments on the other hand are an important sink of a variety of pollutants, particularly heavy metals and may serve as an enriched source for benthic organisms (Wang *et al*., 2002) especially in estuarine ecosystems. Metals may be present in the estuarine system as dissolved species, as free ions or forming organic complexes with humic and fulvic acids. Additionally, many met-

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als e.g. Pb associate readily with particulates and become adsorbed or co-precipitated with carbonates, oxyhydroxides, sulphides and clay minerals. Consequently, sediments accumulate contaminants and may act as long-term stores for metals in the environment (Spencer and MacLeod, 2002). Exposure of sediment-dwelling organisms to metals may then occur via uptake of interstitial waters, ingestion of sediment particles and via the food chain. The occurrence of elevated concentrations of trace metals in sediments found at the bottom of the water column can be a good indicator of man-induced pollution rather than natural enrichment of the sediment by geological weathering (Davies *et al*. 1991, Chang *et al*. 1998; Opuene and Agbozu, 2008).

Anecdotal information suggests that the Volta basin might be receiving a considerable range of polluting effluents, particularly heavy metals from metal fabrication, the galvanized iron sheets which are principal roofing material in the settlements surrounding the Volta Estuary and agricultural industries along the Volta basin. It is in the light of the above reasons that this research evaluated the sediments and clams from the Volta estuary in Ghana as indicators of heavy metal pollution. This study investigated the levels of the metals in clam and sediment samples as well as relationship between the concentrations in sediments and in the clams.

MATERIALS & METHODS

The study was carried out at Ada and Aveglo, at the Volta Estuary, in Ghana, from March 2008 to September 2008. Ada located on Latitude 05°49' 18.6" N and longitude 000°38.46' 1"E and Aveglo latitude 05°53 28.2" N and longitude 000° 38' 24.7"E represent the most active clam fishing grounds of the Volta estuary (Fig. 1).Riverbed sediment samples were collected on a monthly interval for 7 months using an Ekman grab at the two locations from March to September 2008. Sediment samples were collected from each sampling sites according to the standard procedures described in USEPA's sediment sampling guide (USEPA, 1994) and were kept in LDPE bottles pre-washed with 10% HC1 and stored in insulated iced chests for analysis in the laboratory. In the laboratory the sediment subsamples of 500g from each sampling location were placed in ceramic mortars for drying at 80ºC for 48hrs to a constant weight (Phillips and Yim, 1981).The dried

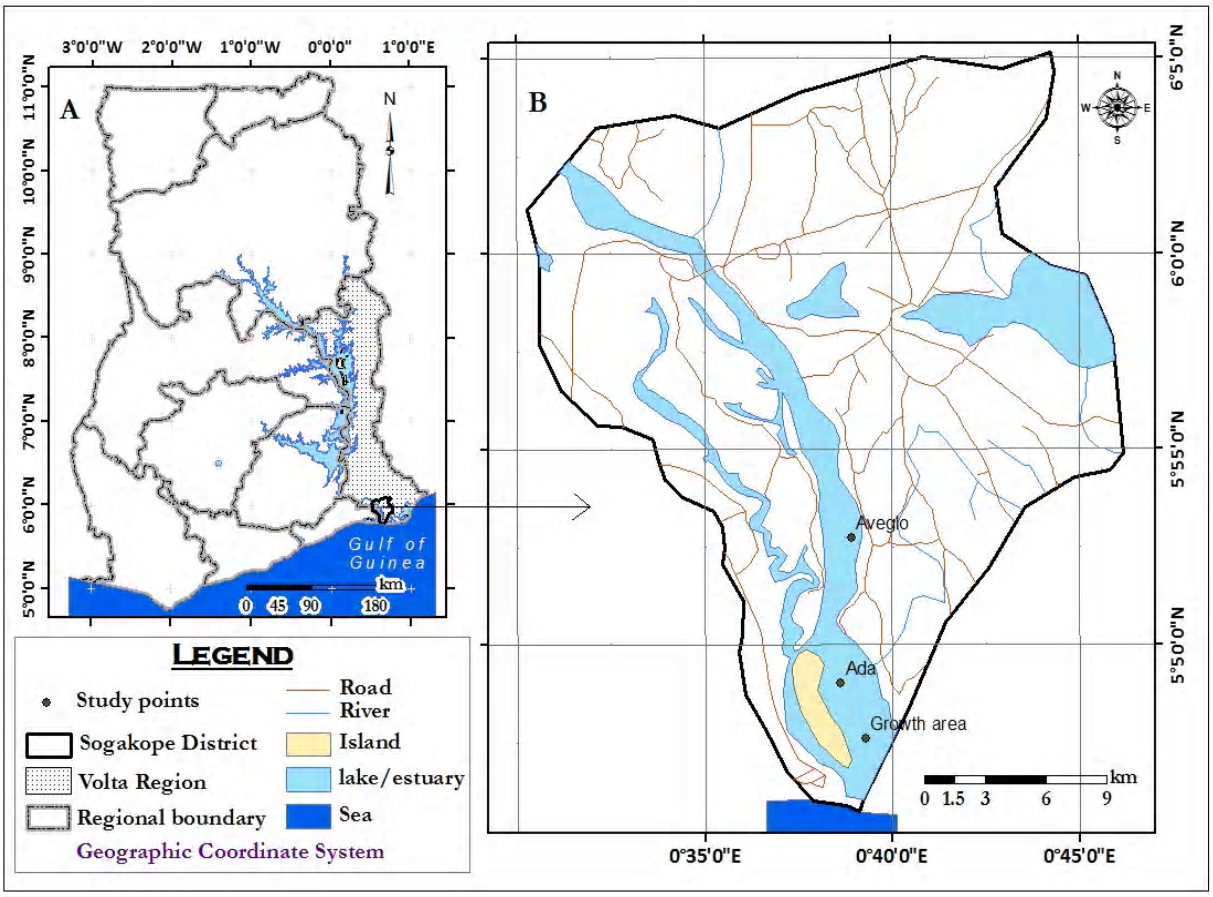

Fig. 1. Map showing the clam sampling locations at Ada and Aveglo in the Volta estuary in Ghana

samples were then gently disaggregated and ample quantities of each sample were finely ground in ceramic mortars and stored in 250 ml acid-washed LDPE bottles. The ground samples were kept at 4ºC in a refrigerator for heavy metal (USEPA, 1994).

Clam samples were obtained from fishermen's catch form the two sampling stations at monthly intervals for 7 months and transported to the laboratory, submerged in river water, in insulated chests within 12 hours for processing and storage for heavy metal analyses. In the laboratory, clam samples were cleansed to remove the mud and any debris and then washed with double distilled water. For each site, clams of various sizes were obtained and grouped into three size classes of 10 individuals each based on shell lengths. The categorization was as follows: small (25-40mm), medium (41-55mm), and large (above 55mm). The various clam size classes were purged of ingested organic and inorganic particles before being analysed for heavy metal accumulation by keeping each size class in distilled water for a 24-hour depuration. After the depuration process, a sterile stainless steel knife was used to dislodge and remove the soft tissue of each clam from the shell (Chiu *et al.,* 2000). The flesh of each subsample was oven-dried to a constant weight at 60ºC for 72 hours (Ferreira *et al*., 2004). Each dry clam sample was weighed on a Sartorius BP 210 S micro balance to the nearest 0.0001 g. Individuals of each size class were ground together into fine powder using a porcelain pestle and mortar. Homogenised subsamples were stored in air-tight, acid-washed (0.1 M HCl) snap-top glass vials for heavy metals analyses (Environmental Agency, 2008).

About 0.5g of the homogenized clam subsamples and the sediment samples were weighed into a 50 ml digestion tube and 1ml of distilled water, 2.0 ml perchloric acid (HNO_3-$HClO_4$) (1:1 vv) and 5.0 ml sulphuric acid (H_2SO_4) were added. Each mixture was refluxed at 200ºC for 30 minutes in a clean fume chamber. The completely digested subsamples were allowed to cool at room temperature, and the undigested portion of the sediments filtered off through a Whatmann Glass Microfibre filter paper (GF/C) to obtain a clear solution and diluted to 50 ml in volumetric flasks with double distilled water (Jin *et. al*., 1999; Otchere, 2003).

Concentrations of Zn, Fe and Mn were determined using a Buck Scientific Model VGP flame Atomic Absorption Spectrophotometer (AAS). All tissue and sediment analytical batches were accompanied by blanks at a minimum rate of one blank per 20 samples. Replicate analyses were conducted on 10% of the samples to evaluate the precision of the analytical techniques. The data were expressed as total concentrations (µg/g dry weight (dw).The Atomic Mercury Analyzer (Model HG 5000) equipped with a mercury lamp at a wavelength 253.7nm was used for the determination of total mercury in the clam soft tissue and sediment samples. Responses were recorded on strip chart recorders as sharp peaks. The peak heights were used for computation of the total mercury concentrations in the clam and expressed as microgram per gram dry weight (µg/g dw). Total mercury concentrations were validated according to standard procedures described for Mercury analyser Model HG 5000 to check for precision and accuracy.Monthly measurements of temperature, salinity, pH, Total Dissolved Solids (TDS), conductivity and Dissolved Oxygen (DO) were taken *in-situ* at both sampling sites over the 7-month period using a Hanna (HI 9028) multi-parameter probe.

Biota Sediment Accumulation Factor (BSAF**)** was calculated for each site and clam size class to evaluate the efficiency of metal bioaccumulation in the tissues of the organisms. BSAFs were calculated for each analyte for each month using the equation:

$$BSAF = \frac{\text{Concentration of heavy metal in the organism}}{\text{Concentration of the heavy metal in sediment}}$$ (Thomann *et al*.,1995)

Spatial patterns of heavy metal concentrations in clams from the two sampling stations, between the sediments of both sampling stations and between the clams and sediments of the two sampling stations were investigated using both the Kruskall-Wallis non-parametric test for independent samples ($p<0.05$) Descriptive statistics were executed using the GraphPad Prism 5 Software.

RESULTS & DISCUSSION

The water quality variables monitored at the two sites were fairly constant without much variation. At Ada**,** pH declined marginally from 6.99 in April to 6.48 in September. Temperature remained fairly stable at 29.22°C in May and 27.28°C in September. Dissolved Oxygen (DO) levels were relatively high in March (8.76 mg/L) but dropped to 2.48 mg/L in September. Salinity however remained constant at 0.03 Practical Salinity Units (PSU) throughout the sampling period. Total Dissolved Solids (TDS) remained fairly constant with values between 31 and 35 mg/L over the study period. Conductivity values were low, ranging between 60 and 70µS cm^{-1}. At Aveglo, pH was between 6.89 and 7.08 during the sampling period. Temperature remained fairly constant during the sampling period ranging between 27.19°C and 28.49°C. Dissolved Oxygen levels ranged from of 2.38 mg l^{-1} to 6.78 mg/L whilst TDS values were between 32 and 42 mg/L during the period. Conductivity values were between 63 and 84µS cm^{-1} whilst salin-

ity remained constant at 0.03 PSU.Concentrations of trace metals in whole soft tissue of the clams and sediments from the two stations at the Volta estuary, collected from March to September 2008, are given in Tables 1 and 2.

The peak Mn concentration of 867µg/g was recorded in the whole soft tissue of the small-sized clams at the Ada in July 2008. The medium-sized clams recorded Mn concentrations between 68µg/g in May and 336µg/g in August 2008. Manganese concentration in the tissues of the large-sized clams recorded a peak concentration of 212µg/g in July 2008. Zinc concentrations were relatively lower in the clams from both stations, with a peak value of 49 µg/g recorded in the medium-size clams from the Aveglo station in May. Results from the analysis of Fe in the tissues of the medium-sized clams registered the highest value of 539µg/g in March although most of the concentration fell within the range of 79 and 307µg/g. Total Mercury concentrations in the clam tissues were low for the two stations varying narrowly between 0.029 and 0.074µg/g (Table 1). The concentrations of the studied metal in the clam and sediment samples are similar to those observed in areas under low pollution impact.

Manganese concentrations in the estuarine sediments ranged between 39 and 390µg/g during the sampling period. Zinc concentrations in the sediments from the Aveglo sampling station were very low, similar to the concentrations recorded at Ada. Iron concentrations were relatively very high in the estuarine sediments, with the lowest concentration of 696µg/g recorded in May 2008 and the highest value of 3476µg/g in August of 2008. Total mercury concentrations in the sediments from the two sampling stations were far lower than the concentrations observed in the tissues of the clams of all the three size classes. The lowest concentration of Total mercury in estuarine sediments of 0.0069µg/g was observed in May 2008 at Ada and the highest of 0.0240 µg/g at Aveglo in April 2008.

Examination of the spatial patterns of trace metals in the different clam size classes (small vs. small, medium vs. medium and large vs. large) from the two stations using the Kruskall-Wallis non-parametric test for independent samples ($p<0.05$) showed that there were no statistically significant differences between the two stations at the Volta estuary regarding all the studied trace metals except variations in Fe concentration in the large-sized clams from Ada and Aveglo.No significant differences ($p>0.05$) were found between the concentrations of Mn, Zn, Fe and Hg in the sediments from the two sampling sites during the study period, indicating a similar bioavailability of the heavy metals in the sediments of the two sampling stations.

Differences in heavy metal concentrations between each clam size group and sediment samples from the two sampling stations were carried out using the Kruskall-Wallis non-parametric test for independent samples ($p<0.05$). No significant differences ($p>0.05$) were observed in Manganese concentrations between the small-sized clams and sediment samples over the study period. Mn concentrations were higher in the sediment samples except during July and August. Significant variations were however observed in the Mn concentrations between the medium and large size clams and the sediment samples. Mn concentrations in the sediments were consistently higher than in the

Table 1. Concentrations of Mn, Fe, Hg and Zn in the tissues of *G. paradoxa* collected from Ada and Aveglo at the Volta estuary from March to September 2008

Month	Mn (µg/g)			Fe (µg/g)			Hg (µg/g)			Zn (µg/g)		
	S	M	L	S	M	L	S	M	L	S	M	L
Ada												
March	109	102	118	194	187	166	0.029	0.049	0.049	23	13	16
April	129	72	103	179	79	133	0.028	0.035	0.051	36	21	30
May	123	68	91	197	102	121	0.043	0.040	0.049	42	26	30
June	73	97	49	139	233	118	0.049	0.045	0.048	42	27	26
July	867	120	212	197	123	142	0.039	0.042	0.044	26	22	43
Aug	629	335	120	209	156	121	0.042	0.041	0.049	19	20	33
Sept	98	197	145	163	316	154	0.040	0.049	0.059	23	19	31
Aveglo												
March	120	206	116	139	539	136	0.055	0.047	0.037	21	41	41
April	59	123	101	143	161	152	0.042	0.045	0.047	25	45	32
May	123	73	115	187	157	178	0.037	0.054	0.040	31	49	34
June	79	73	140	149	170	230	0.046	0.047	0.074	28	16	32
July	164	96	103	195	123	160	0.045	0.042	0.042	35	28	16
Aug	95	125	190	172	307	252	0.053	0.056	0.064	30	32	31
Sept	154	87	130	214	142	151	0.047	0.051	0.045	42	24	48

S- *Small-sized clams (n=10);* **M**- *Medium-sized clams (n=10);* **L**- *Large-sized clams (n=10)*

Table 2. Concentrations in sediment samples collected from Ada and Aveglo at the Volta estuary from March to September 2008

Month	Mn (µg/g)	Fe (µg/g)	Hg (µg/g)	Zn (µg/g)
Ada Sampling Station				
March	180	2758	0.0079	3
April	192	2532	0.0082	3
May	302	2008	0.0078	ND*
June	189	2541	0.0080	3
July	354	1749	0.0078	1
Aug	370	696	0.0140	3
Sept	197	978	0.0069	ND*
Aveglo Sampling Station				
March	160	1564	0.0210	3
April	187	1214	0.0240	2
May	300	780	0.0078	ND*
June	66	1114	0.0230	2
July	39	1683	0.0080	3
Aug	390	3476	0.0100	5
Sept	274	3244	0.0119	6

ND* - *Not Detectable (concentrations were in trace amounts below the detection limit of the AAS.)*

medium and large size clams over the sampling period. Zinc concentrations were significantly higher ($p<0.0001$) in all the clam size classes compared to the sediment samples. Iron concentrations in the sediment samples were 10 and 18 times higher than the concentrations in the small-sized clams in May and June respectively. Similar trends were observed between the sediments and the other size classes. Highly significant differences ($p<0.0001$) were observed in all the size-classes and the sediment samples for Iron. Total mercury concentrations showed highly significant variations (($p<0.0001$) between all the clam size classes and the sediment samples. THg concentrations were approximately five times higher in the clam tissues.

Results from the Aveglo sampling station portrayed a trend similar to Ada. Differences in Mn concentrations between the clam and sediment samples were not significant ($p>0.05$) for all the clam size classes. Zn showed highly significant variations ($p<0.0001$) between the all the size classes and the sediment. Differences in concentration were similar to the trend observed at the Ada sampling station. Highly significant differences ($p<0.001$) existed between all the clam size classes and sediment samples for Fe. Iron concentrations in sediments were significantly higher than in the clam samples; twenty times higher than the concentration in the small-sized clams in August 2008. Highly significant differences ($p<0.0001$) were recorded for total Mercury concentrations in the clam and sediment samples. The differences in concentrations ranged between two to five times more in the clam tissues. The Biosediment Accumulation Factors (BSAFs), Table 3 for each site were calculated to evaluate the efficiency of metal uptake by the clams and to describe the accumulation of studied metals. Zinc was however excluded because it was not detected in the sediment samples for certain months at both sampling stations. On the basis of the calculated BSAFs metal enrichment in the tissues of the clams was rank in the following order Hg>Mn>Fe. The average BSAF values reveals mercury as having the highest BSAF values. Hg contamination levels were found to be higher in the clams than in the sediments, suggesting a higher rate of accumulation of Hg by *G. paradoxa.* This could be as a result of the water acting as an additional source of Hg accumulation in *G. paradoxa*. Fe and Mn concentrations were generally lower in the clam tissues than in the sediments, suggesting that the levels of contamination of these metals in the estuary do not exceed the clams' capacity to regulate them. The interactions between metal geochemistry and animal physiology determine the differences in the bioavailability among heavy metals (Wang *et al*., 2002). The relationship between the concentrations of the studied contaminants in the clam tissues and the sediments was not clear-cut, supporting the fact that several variables control both the bioavailability and accumulation of heavy metals in individuals exposed to contamination (Ansari *et al.*, 2004, Martin-Diaz *et al*., 2006).

Heavy metal concentrations in the clam tissues did not vary significantly between the two sampling stations during the study period. This could be due to similarities in the bioavailability of the heavy metals to the clams (Ferreira *et al*., 2004); suspended particulate matter, food sources, and the homogeneity in environmental and hydro-graphic parameters at the two sampling stations.

With the exception of Mercury all the heavy metals examined in this study are essential metals and have

Table 3. Biota-sediment accumulation factors (BSAFs) for *G. paradoxa* from Ada and Aveglo at the Volta estuary from March to September 2008

Month	Mn (µg/g)	Fe (µg/g)	Hg (µg/g)
Ada Sampling Station			
March	0.61	0.066	5.43
April	0.53	0.051	4.65
May	0.31	0.066	5.64
June	0.39	0.064	5.92
July	1.13	0.085	5.34
Aug	0.97	0.14	3.14
Sept	0.74	0.21	7.00
Aveglo Sampling Station			
March	0.92	0.20	2.20
April	0.57	0.13	1.86
May	0.32	0.22	4.41
June	1.46	0.16	2.42
July	3.07	0.096	5.37
Aug	0.35	0.069	5.77
Sept	0.45	0.052	4.01

intracellular regulatory mechanisms to keep their concentrations in equilibrium in the organisms (Ferreira *et al.*, 2004). This could also explain the absence of any significant spatial variations in metal concentration between the two sampling sites. The similarity in metal concentrations in the sediments could also be attributed to similarities in important factors such as mineralogy and grain size (Trefry and Priestly, 1976).Analyses of the heavy metal concentrations in the clam and sediment samples revealed no distinct relationship between heavy metal levels in clam tissues and sediments in which they thrive. Heavy metal accumulation in the clams may not be directly or solely derived from sediments as observed by Huanxin *et al*., (2000). Other sources of heavy metals in bivalve tissues are derived from living or dead suspended particles and from dissolved metals in the water (Huanxin *et al*., 2000).

The relatively consistent monthly concentrations of Mn, Fe and Zn in whole soft tissues of *G. paradoxa* may well represent efficient metabolism and detoxifying processes that include transportation, transformation, sequestration and/or excretion of excess metals (Connell *et al*., 1999). The results further suggest that the levels of contamination of these metals do not exceed the clam's capacity of regulation (Amiard *et al*., 1985; Durou *et al*., 2005; Mensi et al. 2008).The relatively higher concentrations of Zn in the clam tissues compared to the concentrations in the sediments suggests a high rate of accumulation by the clams, a physiological mechanism induced by exposure or even a high relevance of the water as an additional source of contamination (Cardoso *et. al*., 2008). Although monthly concentrations of Fe in the sediments from both stations were generally higher than Mn, their biosediment accumulation factors (BSAFs) were generally lower than that of Mn (Table 4). This phenomenon occurs because Fe is deposited much more quickly than Mn but is strongly bound to the sediments under estuarine conditions (Huanxin *et al*., 2000). It is, thus, not readily available to the clams. Mn on the other hand can be said to be released much more easily from sediments than Fe and thus more available to the clams accounting for the higher BSAFs for Mn. Hg has much higher monthly BSAF values probably because it is a non-essential trace metal, which is not metabolised in the tissues of the clam and thus accumulates in the clam tissues. Peak metal concentrations and BSAF values for most of the heavy metals were recorded just prior to or at the beginning of the spawning season lending credence to accumulation of heavy metals prior to spawning. Before the spawning period, proteins and carbohydrates contents, which have a high affinity for heavy metals, are accumulated for gonad tissue production, energetic storage and consumption (Latouche and Mix, 1982; Páez-Osuna *et. al.,* 1995).The release of heavy metals from sediments is controlled by the complex dynamics of the heavy metals and the physical and chemical conditions of the environment. Hence, there was no clearly defined relationship between the heavy metal concentrations in the clam tissues and in the sediments. Other factors of the environment are certainly implicated in this observation.

At the two sampling stations (Ada and Aveglo), very intense clam fishing commences at the onset of rainy season in March and ends at the start of the dry season in December each year. Introduction of heavy metals into the estuary during the intense fishing activities could come from sources such as fuels leakages and fumes from outboard motors and from the motorized air compressors used by the divers in the their clam fishing activities. Metals could also be introduced from sources such as the paint cover of the boats used in the fishing activities. In Tunisia, Chouba *et al.*, (2007) also found higher levels of heavy metals in the mullet, *Mugil cephalus* during high rainfall peri-

ods and the times for most intense fishing activities. The elevated concentrations of heavy metals in this period might also be attributed partly to surface water run-off from the surrounding agricultural lands into the Volta estuary. The study did not observe any known point source of pollution. This provides evidence that even clams from areas with no known point sources of contamination may have measurable body burdens of heavy metals. This may probably be due to the processes of natural weathering and supply from locations further upstream.

The relatively high concentration of essential heavy metals in the clam and sediment samples, particularly Manganese and Iron might be attributed to local hydrological conditions, weathering and the intensive leaching of mineralised rocks in the catchment area during rainstorms. The use of galvanized iron sheets as the principal roofing material in the settlements surrounding the Volta estuary could also account for the high levels of Fe in the clams and sediments. According to Otchere (2003), higher wet season levels of Fe and Zn might as well be due to import from surrounding settlements as most roofing in Ghana are made of galvanized iron sheets, most of which are presently rusty. Many metals are also found in agricultural products such as fertilisers. Those present in fertilisers include Mn and Zn which eventually accumulate in agricultural soils and become exposed to water bodies and the organisms present in them through run-off during the rainy season (Otchere, 2003).

CONCLUSION

Analyses of the clam and sediment samples revealed no distinct relationship between heavy metal concentrations in clam tissues and sediments in which they thrive indicating that heavy metal accumulation in clams may not be directly or solely derived from sediments but from other sources such as living or dead suspended particles and from dissolved metals in the water Concentrations of the studied metals varied significantly between the clams and sediments for both stations though both samples showed different affinities for the studied metals. The results further suggest that the levels of contamination of these metals in the estuary do not exceed the clams' capacity of regulation.

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