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Bioaccumulated trace metal profiles of *Tympanotonus fuscatus*, *Periophthalmus barbarous* and *Guinearma* (*Sesarma*) *alberti* collected from a perturbed freshwater mangrove swamp in Warri, Nigeria

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ABSTRACT: Bio –concentrated levels of trace metals; Fe, Zn, Cu, Cd and Pb in three (3) freshwater mangrove fauna; *Tympanotonus fuscatus* (periwinkle), *Periophthalmus barbarous* (mudskipper) and *Guinearma* (*Sesarma*) *alberti* (West African Sesarmid Crab) was investigated utilizng atomic absorbance spectrophotometery. Faunal sampling was conducted at five (5) sites in the Falcorp wetland area of Warri, Delta State, once monthly for an eighteen (18) month period. Maximal mean Fe and Zn values; 349.7 9 µg/g, dried wt ±15.64 and 16.92 µg/g, dried wt ±1.65 were recorded for the respective mudskipper and crab samples while minimal mean Fe and Zn values; 124.14 µg/g, dried wt ±7.85 and 7.71 µg/g, dried wt ± 0.96 were documented for the digested periwinkle tissues. The differences in the mean Fe, Zn, Cu and Cd values was siginificantly different at different levels (*P<0.001*, *P <0.01* and *P<0.05*). The detection of significant bioconcentration of these metals especially Cd, is a possible indication of the wide reaching deletrious ecological effects of anthropogenic activities in the affected area.

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There has been increased research activity into heavy metal pollution in the different environmental compartments, i.e., aquatic niches, within our natural environments. This is apparently due to their toxicity and persistence especially of some trace metals grouped as non essential; e.g., Pb, Hg and Cd which even at trace levels can be toxic , and have unknown functions within the existing trophic levels in these aquatic ecosystems (Mehana *et al.*, 2020). Benthic invertebrate fauna in aquatic ecosystems play an important role in the transformation of the organic matter sediment on the bottom to its base elements and subsequently contribute to the basic nutrition of fish. The composition of the benthic fauna has largely been regarded as a good indicator of water quality because, unlike planktonic species, they form relatively stable communities in the sediments which do not change over long time periods (El- Shabrawy and Khalil, 2003). Heavy metals emnanting from either direct discharges or surface run off upon entering the aquatic environment are known to rapidly aggregrate with circulating particulate materials and eventualy settling in the aquatic bottom sediments (Abu and Nwokoma, 2016). Trace metal accumulation from the overlying water to the sediment has been known to be reliant on some extrinsic environmental factors which include; pH, electrical conductivity and surface area availability for adsorption as a result of the difference in sediment particle size distribution (Abu and Nwokoma, 2016). Sediment linked digenetic mechanisms has been known to cause changes and redistribution of these metal pollutant between the solid and the dissolved phases, but a consequence of sedimentation process is the immobilization of majority of the metallic pollutants are normally rendered immobilized through sedimentation, which therein makes these pollutant bioavailable for a host of micro and marco benthic invertebrates which include mudskippers, molluscs and gastropods (Abu and Nwokoma , 2016). Heavy metal bioaccumulation in the body tissues of wildlife fauna is largely dependent on the type of heavy metal, and its concentration in the environment. The usage of benthic invertebrates as bio-indicators could help to quantify the level of bioavailabilty of these metals in different aquatic ecosystems. Benthic invertebrates take up these metal pollutants from the water, sediment litter and from food, and the pollutant concentrations in their tissues (or sometimes the changes in such concentrations) can serve as a time-integrated measurement of pollutant bioavailability (Zulkifli *et al.*, 2012). The respective swamp faunal bio indicators utilized in this study; *Tympanotonus fuscatus* (periwinkle), *Periophthalmus barbarous* (mudskipper) and *Guinearma* (*Sesarma*) *alberti* (West African Sesarmid Crab) are native faunal species present in the fresh water mangrove wetlands of the Niger Delta region. The Falcorp mangrove swamp is a part of this wetland belt and the water channel within this swamp interphases with other water bodies of neighbouring creeks. The swamp has been and is currently been impacted negatively as a consequence of several human activities within its vicinity which include farming and fishing activities, dumping of petrochemical wastes, accurring from the

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activities of the nearby Warri petroleum refinery and an upsurge in bunkering (crude oil theft) and illegal refining activities within the swamp and neighbouring creeks. Several authors have reported the suitablility and effectiveness of utilizing these representative benthic fauna and mudskipper as bio indicators with respect to aquatic niches exposed to varying levels of heavy metal pollution (Ikram *et al.*, 2010; Chaiyaraa *et al.*, 2013; Ansari *et al.*, 2014; Abu and Nwokoma, 2016; Moslen *et al.*, 2017; Aigberua and Izah, 2018; Numbere, 2019; Abiaobo *et al.*, 2020). Earlier publications by Odigie and Olomukoro (2020a; 2020b) profiled the heavy metal content of surface water and sediments collected from various sampled points within the perturbed Falcorp swamp and an adjoing creek; Ifie. However, it has been observed that sole chemical evaluation of water and sediments provides insufficient data on heavy metal bioavailability in the affected environment (Chaiyaraa *et al*., 2013). This study attempts to evaluate the bio accumulated heavy metal profiles of respective faunal specimens; *T. fuscatus* (periwinkle), *P. barbarous* (mudskipper) and *G.* (*Sesarma*) *alberti* (West African Sesarmid Crab) collected from same sampled locations as previously described by Odigie and Olomukoro (2020b).

MATERIALS AND METHODS

The Study Area: As earlier described by Odigie and Olomukoro (2020a; 2020b), the Falccorp mangrove swamp is located within the jurisdiction of Warri south Local Government Area of Delta State, Southern Nigeria. The swamp area is behind the Warri refinery and petrochemical company complex. The water channel in the mangrove swamp is continually fed by precipitation during the wet season and also by water flow from surrounding creeks, rivers and surface runoffs from neighboring communities within the surrounding watershed catchment area.

Sampling Stations: The five (5) sampling locations visited in the course of this research had earlier been described by Odigie and Olomukoro (2020a; 2020b) (Fig. 1). Representative specimens of *T. fuscatus* (periwinkle), *P. barbarous* (mudskipper) and *G.* (*Sesarma*) *alberti* (West African Sesarmid Crab) were collected from the sample locations with the aid of very experienced indigent fishermen. The animal specimens were obtained at a frequency of once monthly for a period of eighteen (18) months as previously stated by Odigie and Olomukoro (2020a; 2020b).

Collection and preparation of bio-indicator specimens: At each sampling period, a total of twenty (20) periwinkles, five (5) mudskippers and five (5) crabs were collected from sampling points. The periwinkles were handpicked, while the mudskippers and crabs were collected with the aid of fishing nets and native crab traps. The handpicked periwinkles were treated, dried, marcerated and digested in accordance with procedures described by Moslen *et al.* (2017). The derivation of filtrate from the digested periwinkle tissue was also conducted in accordance with the method reported by Moslen *et al.* (2017). The collected mudskippers were prepared and treated for acid digestion with reference to procedures described by Ikram *et al.* (2010). Also the digested mudskipper tissue filtrate were filtered and stored in accordance with procedures reported by Ikram *et al.* (2010). The trapped crabs were retrived and washed with distilled water. The tissue and the carapace of the respective crabs were separated, treated and digested in accordance with methods detailed by Chaiyara *et al.* (2013). Pure analytical grade Nitric acid was utilized in the digestion of the marcerated crabs and mudskippers respectively

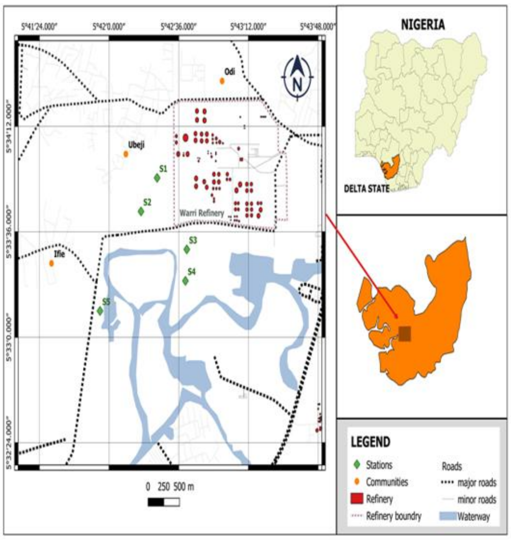

Fig. 1: Map indicating the respective sampling sites within the swamp area

Determination of selected heavy metal content of the faunal derived filtrates: The selected trace metal profiles; Cu, Zn, Pb, Cd and Fe of the tissue filtrate derived from the periwinkles, West African Sesarmid crabs and mudskippers was evaluated using an air–acetylene flame Atomic Absorption Spectrophotometer (AAS model GPC A932 ver. 1.1). Prior to usage, all glassware and plastic materials were acid washed in 10% nitric acid and later rinsed with distilled water to prevent metal contamination. Quality control samples prepared from standard solutions of Fe, Zn, Cu, Cd and Pb were also evaluated at an interval of once for every five (5) filtrate samples to ascertain metal accuracy. Procedural blanks; digested distilled water without the sample were also prepared and evaluated after every five (5) samples. The obtained values were expressed as µg/g wet weight. The instrumental detection limit of all the metal electrodes was 0.001µg/g.

Determination of the BAF and BASF values: The bioacculumation factor (BAF) and Biota Sediment Accumulation Factor (BSAF) heavy metal values for the respective bio indicators was evaluated using formula as described by Bu-Olayan and Thomas (2008) and Ziyaadini *et al.* (2017).

Statistical Analysis: Statistical analysis was carried out on the data generated from each sampling station using general descriptive statistics. The student T – test and Kruskel Wallis Test was also used to test for significance at the 0.05 level of probability for the seasons and the different stations respectively. Multivariate analysis of the available data was done using the SPSS (version 16.0) and Duncan's Multiple Range test (DMR) was utilized to locate significant difference(s) at 95% confidence interval where one existed.

RESULTS AND DISCUSSION

The summarized mean heavy metal values obtained for the bio-indicator samples is shown in Table 1 . Maximal mean Fe and Zn values; 349.7 9 µg/g, dried wt ±15.64 and 16.92 µg/g, dried wt ±1.65 were recorded for mudskipper and crab samples respectively (Table 1). Minimal mean Fe and Zn values; 124.14 µg/g, dried wt ±7.85 and 7.71 µg/g, dried wt ± 0.96 were documented for digested periwinkle tissues respectively (Table 1). The highest mean Cu, Cd and Pb values; 3.46 µg/g, dried wt ±1.21, 2.09 µg/g, dried wt ± 0.96 and 0.41 µg/g, dried wt ±0.17 were all recorded for the sampled mudskippers while the lowest mean Cu, Cd and Pb values; 0.12 µg/g, dried wt ±0.06, 0.03 µg/g, dried wt ± 0.01 and 0.01 µg/g, dried wt ±0.01 were detected in the respective periwinkle, crab and periwinkle samples (Table 1). The observed differences in the mean Pb and Cr values across the respective biota samples was statistically insignificant ($P>0.05$) (Table 1). The differences in the mean Fe, Zn, Cu and Cd values was siginificantly different at different levels ($P<0.001$, $P<0.01$ and $P<0.05$) (Table 1).

The results for the seasonal variation for the respective bio concentrated heavy metals in *T. fuscatus* (periwinkle) is presented in Table 2. The observed differences between the seasonal mean trace metal values was statistically insignificant ($P>0.05$) (Table 2). Amongst the mean bio accumulated heavy metals detected in the perwinkle tissues, Fe; 138.14 µg/g, dried wt ±9.51 was the highest, while nil values were recorded in respect of Cr for both dry and wet seasons (Table 2). Summarized seasonal variation for the respective bio concentrated heavy metals present in *P. barbarous* (mudskipper) tissues is shown in Table 3. The differences in the mean zinc, copper, cadmium, lead and chromium readings was statistically insignificant ($P>0.05$) (Table 3). However, the difference between the seasonal mean Fe values detected in the mudskipper tissues was statistically significant ($P<0.05$) (Table 3). With respect to the mean bio accumulated heavy metals detected in the mudskipper tissues, Fe; 402.48 µg/g, dried wt ± 20.97 was the highest, while a dry season minimal nil value was detected in respect of Cr (Table 3). Summarized seasonal variations for the mean bio concentrated heavy metals recorded for *G. alberti* (West African Sesarmid crab) tissues was presented in Table 4. The detected variations between the seasonal mean trace metal values was statistically insignificant ($P>0.05$) (Table 4). For the mean bio accumulated heavy metals detected in the compounded West African Sesarmid crab tissues, Fe; 217.95 µg/g, dried wt ± 21.92 was the highest, while nil values were recorded in respect of Cr for both dry and wet seasons (Table 4). Significant BAF values for Fe and Zn with respect to all the examined bio –indicators were recorded (Table 5).Aside from Cd, all the BAF values for the other heavy metals; Fe, Zn, Pb and Cr was significant in respect to the examined mudskipper tissues (Table 5).BASF values for Fe, Zn and Cu were insignificant for all the bio-indicators (Table 6). Significant BASF values were recorded for cadmium with respect to all the bio indicators (Table 6). Significant BASF values for Cr and Pb with respect to West African Sesarmid crab tissues was also recorded (Table 6). In this study, there were significant differences ($p<0.05$, 0.01, 0.001) in the bio concentrated heavy metal levels in all studied biota (Periwinkle, Crab, Mudskipper) with the exception of Pb and Cr (Table 1). Higher concentrations of heavy metals were obtained in the dry season than the wet season with *P. barbarous* (mudskipper) having more comparative bio accumulated levels of Fe, Cd, Cu and Cr respectively (Table 3). The detection of varying levels of Zn, Pb, Fe, Cu and Cd in all the examined benthic bioindcators collaborated previously documented findings by Odigie and Olomukoro (2020b) which indicated the presence of these trace metals in sediments obtained from the same sampled sites. However, aside from mudskipper specimens collected in the wet season, all the periwinkle and crab specimens did not bioaccumulate Cr (Table 1-4), despite the presence of this metal in the surrounding sediments as earlier reported by Odigie and Olomukoro (2020b)..

Table 1: Summarized mean heavy metal profiles of the different bio-indicator specimens collected from Falcorp Mangrove

Parameter	Periwinkle	Mudskipper	West African Sesarmid crab	P-value	Significant-Level
	$\bar{X}\pm$S.E	$\bar{X}\pm$S.E	$\bar{X}\pm$S.E		
Iron (µg/g, dried wt) (Min-Max)	124.14 ± 7.85^{c} (72.12-175.23)	349.79 ± 15.64^{a} (201.02-498.77)	187.78 ± 13.31^{b} (106.33-281.08)	0.00	*P<0.001****
Zinc (µg/g, dried wt) (Min-Max)	7.71 ± 0.96^{c} (1.99-13.07)	12.69 ± 1.20^{b} (2.44-19.39)	16.92 ± 1.65^{a} (3.94-27.03)	0.00	*P<0.001****
Copper (µg/g, dried wt) (Min-Max)	0.12 ± 0.06^{b} (0.01-1.07)	3.46 ± 1.21^{a} (0.03-16.14)	0.26 ± 0.07^{b} (0.01-0.92)	0.00	*P<0.01***
Cadmium (µg/g, dried wt) (Min-Max)	0.49 ± 0.48^{b} (0.001-8.70)	2.09 ± 0.96^{a} (0.00-10.81)	0.03 ± 0.01^{c} (0.00-0.07)	0.06	*P<0.05**
Lead (µg/g, dried wt) (Min-Max)	0.01±0.01 (0.00-0.09)	0.41±0.17 (0.00-2.09)	0.37±0.25 (0.00-3.56)	0.21	*P>0.05*
Chromium (µg/g, dried wt) (Min-Max)	0.00±0.00 (0.00-0.00)	0.19±0.18 (0.00-3.18)	0.00±0.000 (0.00-0.00)	0.33	*P>0.05*

Note: *P<0.001-There is very highly significant difference, P<0.01-There is highly significant difference**, P<0.05-There is significant difference*, P>0.05-There is no significant difference*

Table 2: Summarized seasonal mean bio accumulated trace metal values for *T. Fuscatus* (periwinkle) samples collected from Falcorp Mangrove swamp

Parameter	Dry season	Wet season	P-value	Significance level
	$\bar{X}\pm$S.E	$\bar{X}\pm$S.E		
Fe (µg/g, dried wt)	138.14±9.51	115.23±10.80	0.16	*P>0.05*
Zn (µg/g, dried wt)	7.35±1.92	7.93±1.07	0.78	*P>0.05*
Cu (µg/g, dried wt)	0.04±0.02	0.17±0.09	0.28	*P>0.05*
Cd (µg/g, dried wt)	0.02±0.00	0.81±0.79	0.44	*P>0.05*
Pb (µg/g, dried wt)	0.00±0.00	0.01±0.01	0.36	*P>0.05*
Cr (µg/g, dried wt)	0.00±0.00	0.00±0.00	0.29	*P>0.05*

Note: *P>0.05*-There is no significant difference amongst the mean values

Table 3: Summarized seasonal mean bio accumulated trace metal values for P. *barbarous* (mudskipper) samples collected from Falcorp Mangrove swamp

Parameter	Dry season	Wet season	P-value	Significance level
	$\bar{X}\pm$S.E	$\bar{X}\pm$S.E		
Fe (µg/g, dried wt)	402.48±20.97	316.26±14.95	0.00	*P<0.01**
Zn (µg/g, dried wt)	11.94±1.92	13.18±1.59	0.63	*P>0.05*
Cu (µg/g, dried wt)	2.98±0.99	3.77±1.92	0.76	*P>0.05*
Cd (µg/g, dried wt)	0.72±0.28	2.96±1.52	0.26	*P>0.05*
Pb (µg/g, dried wt)	0.61±0.38	0.29±0.15	0.38	*P>0.05*
Cr (µg/g, dried wt)	0.00±0.00	0.31±0.29	0.41	*P>0.05*

Note: *P<0.01-There is highly significant difference*, P>0.05-There is no significant difference*

Table 4: Summarized seasonal mean bio accumulated trace metal values for *G. alberti* (West African Sesarmid crab) samples collected from Falcorp Mangrove swamp

Parameter	Dry season	Wet season	P-value	Significance level
	$\bar{X}\pm$S.E	$\bar{X}\pm$S.E		
Fe (µg/g, dried wt)	217.95±21.92	168.58±14.64	0.07	*P>0.05*
Zn (µg/g, dried wt)	15.20±1.94	18.01±2.42	0.42	*P>0.05*
Cu (µg/g, dried wt)	0.24±0.12	0.27±0.09	0.85	*P>0.05*
Cd (µg/g, dried wt)	0.03±0.01	0.02±0.01	0.39	*P>0.05*
Pb (µg/g, dried wt)	0.00±0.00	0.61±0.40	0.25	*P>0.05*
Cr (µg/g, dried wt)	0.00±0.00	0.00±0.00	0.58	*P>0.05*

Note: *P>0.05*-There is no significant difference

Table 5: BAF values for the bio-concentrated heavy metals

Heavy metal	Mudskipper	West African Sesarmid crab	Periwinkle
Fe (µg/g, dried wt)	**3.44**	**1.22**	**2.13**
Zn (µg/g, dried wt)	**3.59**	**1.85**	**1.36**
Cu (µg/g, dried wt)	**10.45**	0.18	**2.11**
Cd (µg/g, dried wt)	0.02	0.01	0.25
Pb (µg/g, dried wt)	**6.21**	0.15	0.05
Cr (µg/g, dried wt)	**12.04**	0.05	11.92

NB*: BAF >1 is significant and BAF < 1 is insignificant. Boldned values exceeded threshold limits.*

Table 6: BSAF values for the bio-concentrated heavy metals

BSAF	Mudskipper	West African Sesarmid crab	Periwinkle
Fe (µg/g, dried wt)	0.29	0.82	0.47
Zn (µg/g, dried wt)	0.28	0.54	0.73
Cu (µg/g, dried wt)	0.10	5.65	0.47
Cd (µg/g, dried wt)	**43.75**	**222.41**	**4.05**
Pb (µg/g, dried wt)	0.16	**6.91**	**20.76**
Cr (µg/g, dried wt)	0.08	**20.55**	0.08

NB*: BSAF >1 is significant and BSAF < 1 is insignificant. Boldned values exceeded threshold limits*

This trend would indicate the non suitability of utilizing *T. fuscatus* (periwinkle) and *G.* (*Sesarma*) *alberti* (West African Sesarmid crabs) as potential bio-indicators of Cr contamination of estuarine niches. Periwinkles and mudskippers are a common part of the daily diet of some human communities residing in the Niger-Delta region of Nigeria.

Goyer, (1991) reported that cadmium can bioaccumulate in mussels, oysters, prawns, lobsters and fish and documented that several related health risks such as high blood-pressure, liver disease and nerve or brain damage can occur if these heavy metal tainted foods are consumed beyond threshold limits. Several studies have also reported the bioconcentration of heavy metals in several types of aquatic biota. Ayenimo *et al.,* (2005) reported appreciable quantities of heavy metals such as iron, copper, barium, lead, cadmium and nickel in periwinkles purchased from public markets in Warri River, Nigeria. Also, Falusi and Olanipekun (2007), also documented high bioconcentration of heavy metals in tropical crab (*Carcinus* sp.) harvested from River Aponwe, Ado-Ekiti. Olalode *et al*. (2008) reported high concentrations of manganese and zinc in crabs, while elevated levels of cobalt, copper and iron were detected in periwinkles. Ikejimba and Sakpa (2014) reported higher bioaccumulation of some heavy metals in periwinkles collected from Egboko River.

Aside from the associated BAF value for Cd, the BAF values for all the trace metals with respect to the collected mudskipper specimens were higher than threshold value of 1(Table 5). This trend might be reflective of their feeding habit as *Periophthalmus* species are carnivorous mudskippers known to prey on little crabs and other arthropods (Ansari *et al*., 2014). The BAF values recorded for the examined mudskippers also indicated the strong possibility of bioaccumulation of trace metals in several internal tissues of the mudskippers such as the gills, muscles and the digestive system respectively (Ansari *et al*., 2014). Long term exposure to cadmium is known to primarily affect the kidneys, resulting in tubular proteinosis (Goyer, 1991). As Cd and Zn are known to possess identical oxidation states, Cd can replace Zn present in metallothionein, thereby inhibiting Zn from performing its role as a cellular scavenger of free radicals (Jaishankar *et al*., 2014). Worryingly, high BASF levels of Cd were deduced with respect to all the faunal specimens and Pb levels were significantly higher for crab and periwinkle samples respectively (Table 6). This trend would indicate the non suitablility of the aquatic fauna as food source for indigent human communities which are known to depend on these mangrove fauna as source of protein. This trend also buttress the urgent need for further investigations into the bio-concentrated levels of heavy metals in other aquatic fauna such as fishes, prawns, molluscs and crayfish popularly consumed by residents within the study area and other parts of the freshwater ecosystems within Delta State, Southern Nigeria. Lead has been documented to have deleterious effe cts on human health (Jaishankar *et al*., 2014). Pb has also been regarded as a carcinogenic metal and its toxicity can be either acute or chronic (Jaishankar *et al*., 2014). Acute Pb toxicity has been known to cause symptoms which vary from appetite loss, headache, hypertension, abdominal pain, renal dysfunction, fatigue, and sleeplessness to arthritis respectively (Jaishankar *et al*., 2014). . Chronic exposure to Pb can culminate in symptoms which range from mental retardation, birth defects, psychosis, autism, allergies, dyslexia, weight loss, brain damage, kidney damage and in some situations mortality (Jaishankar *et al*., 2014). Chaiyaraa *et al*. (2013) outlined the importance of investigating trace metal concentrations in marine organisms as these studies can reveal the likely public health risks, these aquatic organisms may constitute for unsuspecting consumers.

Conclusion: Significant BAF and BSAF values with respect to Zn and Cd were recorded for the examined benthic faunal indicators.Although, currently there is a dearth of appropriate Nigerian standards on permissible trace metal levels in marine and aquatic fauna meant for human consumption, the detection of significant bioacumulated levels of these metals especially Cd, might be indicative of the wide reaching deletrious ecological effects of anthropogenic activities in the affected area.

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