Environmental pre-disposition of Organisms to Contaminants with references to Heavy Metals Residues in the Brackish and Marines Gastropods

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ABSTRACT:
Environmental influences on organisms were studied on most common mollusks in the mangrove swamp and lower intertidal zone of Atlantic Ocean, Nigeria. Samples were collected between January and December, 2016. Five samples each of common whelk and Littorina littorea of relatively equal size and weight were collected at night from different stations and transported to the laboratory in a well ventilated container. Heavy metals in the tissues were digested with 5mL of HNO3 and 3mL of concentrated hydrochloric acid in 5:1 ratio and analyzed using Atomic Absorption Spectrophotometer. Marine environment had higher metals residue than the brackish water in both organisms and both seasons. The order of accumulation of metals in the soft tissues of L.littorea and B.undatum from both sampling environments were; Fe > Zn >Mn >Pb > Cd. The concentrations of heavy metals in the soft tissues of L.littorea and B.undatum revealed that, the geochemical composition had great effects on metal accumulation, with Fe, the most dominant elements in the Earth’s Crust, having the highest concentrations. Cadmium the least metal reported in this study has no known biological functions. The adverse human health effects associated with exposure to heavy metals, even at low concentrations are diverse, hence the needs for thorough investigation of the water qualities in this region to ascertain the degree of the toxicity of heavy metals pollution and the response of the environment.

Keyword: Metals, Mangrove swamp, Ocean, Common whelk, Littorina littorea

INTRODUCTION
Over the years, the elemental composition in the world’s oceans is being updated with highly specialized oceanographic vessels, new analytical instrumentation, contamination-free sampling protocols and large scale data inter-comparisons to measure reliable trace concentrations of many dissolved constituents in seawater. Brackish and marine ecosystem are very reach in biodiversity, thus their contamination will adversely affects the balance of aquatic ecosystem and the human health. Increased population, rapid urbanization, oil and gas production, illegal (local) refineries in the Niger-Delta Creeks, heavy rainfall throughout the year, abuse of chemical fertilizers and misuse of pesticides especially in the rural populists have created numerous environmental and ecological problems in Nigeria’s coastal area and marine environment [1,2].

Excess of metals such as Fe, Mn, Zn are harmful and are readily bioaccumulate along the food chain [3, 4]. Similarly, nonessential metals such as Cd and Pb are very toxic even at a low level of exposure and are not important for metabolic activities [5]. Bioaccumulation is the net build-up of substances from water in an aquatic organism as a result of enhanced uptake and slow elimination of such substances [6].

Terrestrial and aquatic molluscs are widely used as bioindicators due to their seeming reflection of environmental trace metal contamination [7]. Gastropods, accumulate metals to high concentrations than any other group of invertebrate, and are potential bioindicator of aquatic health [8]. Bioaccumulation of metals in gastropods had been reported by several investigators, [9], studied bioaccumulation of metals in snail Helix aspersa. [10] studied bioaccumulation of heavy metals (Cu, Zn, Fe) in freshwater snail (Pliaovota; Oliver 1804) from Ikpoba River of south Nigeria. [11] studied heavy metals accumulation in Oxyloma hirasei from the Upo wetland. [12] studied metal accumulation in pond snail Lymnaea stagnalis in freshwater. [13] studied bioaccumulation of heavy metals in soft body tissues of gastropods Thais mutabilis and sediments from intertidal zone of Bandar Abbas. B. undatum and L. littorea are benthic filter feeders. They ingest metal-enriched particles directly, thereby giving an indication of their bioaccumulation ability of metals [14]. They are dominant gastropods species that serves as source of food to populate in Niger Delta region, Nigeria. It becomes imperative that the metal levels in these organisms need to be analyzed for the safety of mankind.

MATERIALS AND METHODS
Study area: The Ikebiri River drains into the Atlantic Ocean at Ebidoughene (Station 1). The stations submerged with water during high tide and exposed to the air during low tide. The area is sandy and has rocky cliffs. Upstream to this station are the other stations; Ngbokolo (Station 2) and Obomikorogbene (station 3). These are fluctuating environment. The salinity is variable depending on the tide, the amount of freshwater entering from rivers or as rain, and the rate of evaporation. The salinity of the water in this environment fluctuates widely between negligible to 35 ppt. The habitat is the mangrove swamp. These stations do not experience tide and the water are slightly stratified, the saline water circulates in at the bottom, mixes with fresh water, and then flows out at the top (salinity thus increases
with depth and out toward the ocean). Agricultural activities, domestic and industrial effluents containing various organic and inorganic pollutants, like heavy metals, pesticides, oils, and fertilizers etc are regularly discharged into this river consciously and unconsciously, thereby affecting its water quality.

**Samples Collection:** Samples were collected between January and December, 2016. Five samples each of common whelk and *L. littorea* of relatively equal size and weight were collected at night (they are more of nocturnal than diurnal) from different stations (Figure 1) and transported to the laboratory in a well ventilated container. The sample stations were visited monthly for 12 months and a total of sixty specimens of each of the specimen were analyzed for the present of pesticides and heavy metals.

**Samples preparation:** In the laboratory, the body tissues were removed, washed in distilled water and dried separately in oven at about 110ºC to constant weight. After complete drying the tissues were powdered in mortar and pestle and stored separately by labeling the specimen with date and species name.

**Sample Extraction and Analysis:** Heavy metal was digested by weighing 50mg dry powder of whole soft body tissue and transferred to the glass tube and mixed with 5mL of HNO3 (analytical grade) and 3mL of concentrated hydrochloric acid (HCl) acid in 5:1 ratio. After 30 minutes, the samples were stirred and left overnight. On the next day, samples were dried on hot plate till the clear white fumes appeared. 10mL volume of solution was maintained by adding mixture of HNO3 and concentrated hydrochloric acid (HCl) in a drop wise. After allowing the flask to cool, double glass distilled water was added to bring the volume to 50 mL by using volumetric flask and then solution was filtered through Whatman filter paper number 41. The analysis was carried out using Atomic Absorption Spectrophotometer (AAS).

**Data Analysis**
The data were summarized separately for each sampled station using descriptive statistics (means, range, and histogram). Statistical differences between the seasons were analyzed using Student’s *t* test, while the differences between the marine and brackish stations were analyzed using one-way analysis of variance with confidence range of *p*<0.05 with SPSS (16.0 version), SPSS Inc, USA.

**Results**
Accumulation of heavy metals in dominant gastropods at Ngbokolo and Obomikorogbene along the brackish Ikebiri River and at Ebidoughene in the intertidal zone of the lower Atlantic Ocean is shown Table 1 and 2 with further illustration in figure 2 and 3.

### Table 1: Occurrences of metals (ug/gdw) during the dry and wet seasons in soft tissue of *L. littorea* from brackish water and intertidal zone of the lower Atlantic Ocean, sampled monthly from January to December, 2016. Means are based on the monthly observations.

<table>
<thead>
<tr>
<th></th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Range</td>
<td>Mean ± SE</td>
<td>Range</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Dry season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ebidoughene</td>
<td>506 ± 3.1</td>
<td>610 –315</td>
<td>312 ± 5.20</td>
<td>320 –302</td>
<td>18 ±1.10</td>
</tr>
<tr>
<td>Ngbokolo</td>
<td>430 ± 2.3</td>
<td>446 –412</td>
<td>216 ± 3.10</td>
<td>228 –200</td>
<td>08 ±0.10</td>
</tr>
<tr>
<td>Obomikorogbene</td>
<td>417 ± 1.2</td>
<td>430 –390</td>
<td>210 ± 2.20</td>
<td>219 –201</td>
<td>05 ±0.10</td>
</tr>
<tr>
<td>Wet season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ebidoughene</td>
<td>420 ± 2.3</td>
<td>480 –320</td>
<td>300 ± 0.30</td>
<td>306 –290</td>
<td>13±0.30</td>
</tr>
<tr>
<td>Ngbokolo</td>
<td>280 ± 1.3</td>
<td>300 –210</td>
<td>200 ± 2.20</td>
<td>217 –189</td>
<td>03±0.10</td>
</tr>
<tr>
<td>Obomikorogbene</td>
<td>220 ± 0.7</td>
<td>240 –196</td>
<td>142 ± 1.30</td>
<td>160 –130</td>
<td>02±0.10</td>
</tr>
</tbody>
</table>

### Table 2: Occurrences of metals (ug/gdw) during the dry and wet seasons in soft tissue of *B. undatum* from brackish water and intertidal zone of the lower Atlantic Ocean, sampled monthly from January to December, 2016. Means are based on the monthly observations.

<table>
<thead>
<tr>
<th></th>
<th>Fe</th>
<th>Zn</th>
<th>Mn</th>
<th>Pb</th>
<th>Cd</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Range</td>
<td>Mean ± SE</td>
<td>Range</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>Dry season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ebidoughene</td>
<td>733 ± 4.2</td>
<td>810 –689</td>
<td>410 ± 2.10</td>
<td>440 –420</td>
<td>31±2.20</td>
</tr>
<tr>
<td>Ngbokolo</td>
<td>610 ± 3.1</td>
<td>650 –590</td>
<td>400 ± 2.00</td>
<td>418 –402</td>
<td>19±0.30</td>
</tr>
<tr>
<td>Obomikorogbene</td>
<td>518 ± 2.4</td>
<td>602 –410</td>
<td>360 ± 1.20</td>
<td>386 –315</td>
<td>12±0.20</td>
</tr>
<tr>
<td>Wet season</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ebidoughene</td>
<td>610±1.2</td>
<td>680 –520</td>
<td>490 ± 2.10</td>
<td>502 –490</td>
<td>23±1.20</td>
</tr>
<tr>
<td>Ngbokolo</td>
<td>410 ± 1.3</td>
<td>450 –390</td>
<td>374 ± 1.20</td>
<td>417 –299</td>
<td>13±0.40</td>
</tr>
<tr>
<td>Obomikorogbene</td>
<td>320 ± 1.2</td>
<td>340 –280</td>
<td>280 ± 2.30</td>
<td>299 –230</td>
<td>10±0.10</td>
</tr>
</tbody>
</table>
Iron has the highest value of $506 \pm 3.1\mu g/gdw$ and $733 \pm 4.2\mu g/gdw$ in *L.littorea* and *B.undatum* respectively, while Cadmium, with the concentrations; $0.4 \pm 0.1\mu g/gdw$ and $1.40 \pm 0.1\mu g/gdw$, was the least metal observed. The order of accumulation of metals in both organisms is Fe > Zn > Mn > Pb > Cd. In *L. littorea*, Fe concentrations varies significantly ($p < 0.05$, F= 7.20) among the three the stations, both in dry and wet seasons. The concentrations of Zn varies significantly ($p < 0.05$, F= 5.60) only during the dry season. No significant difference ($p > 0.05$, F= 9.20) in Mn level in dry season, but there was variation among the three stations during the wet season ($p < 0.05$, F = 8.90). No significant differences ($p > 0.05$, F = 7.60) in Pb concentrations for both seasons. Similarly, no significant difference ($p > 0.05$, F = 5.10) in Cd concentrations for both seasons. In *B.undatum*, Fe concentrations was highly significance ($p < 0.01$, F= 9.00) among the three the stations, both in dry and wet seasons. The Zn concentrations varied significantly ($p < 0.05$, F= 7.50) only during the wet season. There was significant difference between the Ebidougbene and Ngbokolo stations ($p < 0.05$, F = 6.30) and between Ebidougbene and Obomikorogbene stations ($p < 0.05$, F= 8.50). But no significant difference ($p > 0.05$, F= 4.90) between the two brackish water’ gastropods Zn’s concentrations during the wet season. No significant differences among the three during the wet season ($p > 0.05$, F= 7.70). Mn concentrations varies significantly between Ebidougbene and Ngbokolo stations ($p < 0.05$, F= 7.10), and between Ebidougbene and Obomikorogbene stations ($p < 0.05$, F= 6.70). No significant difference between Ngbokolo and Obomikorogbene stations ($p > 0.05$, F = 4.40) in Mn concentrations. No significant differences ($p > 0.05$, F = 7.50) in Pb concentrations in both seasons. Similarly, Cd concentrations did not varies significantly ($p > 0.05$, F = 5.10) in both seasons. The gastropods from intertidal zone had higher metals residues than the brackish environment (Fig 2 and 3). Regarding organisms, *B.undatum* accumulated the metals than the *L.littorea*. The average annual concentrations of the metals in station 1, 2, 3 were; in *B.undatum*, Fe (610.506,390) µg/gdw, Zn (512,313,116) µg/gdw, Pb (10, 8, 3) µg/gdw, Cd (2, 2, 1.7) µg/gdw, while in *L.littorea*, they were; Fe (476.348,208) µg/gdw, Zn(218,109,102)µg/gdw, Mn (103,88,64 µg/gdw), Pb (10,8,8.1) µg/gdw, Cd (1,2,1.5, 1.3) µg/gdw.

### DISCUSSION

The occurrence of contaminants in the most common mollusks (*L.littorea* and *B.undatum*) in the mangrove swamp and lower intertidal zone of Atlantic Ocean, Nigeria were studied and described for the first time. The concentrations of heavy metals (Fe, Zn, Mn, Pb and Cd) in the soft tissues of *L.littorea* and *B.undatum*, revealed that, the geochemical composition had great effects on metal accumulation, with Fe, the most dominant elements in the Earth’s Crust, having the highest concentrations.
The order of accumulation of metals in both organisms was; Fe > Zn > Mn > Pb > Cd. The high concentrations of iron in this study could be as a result of its abundance nature [15]. Fe is a vital constituent of plant and animal life and its deficiency leads to a sort of anaemia, causing fatigue, headache and anorexia. However, intake of more than 0.5 g of soluble iron salts may cause grave injury to the alimentary canal followed by a series of serious effects such as hepatitis. Continuous intake of excessively high amounts of iron causes haemochromatosis which eventually leads to liver cirrhosis [16,17]

Zinc is generally regarded as one of the essential metals for animals, and is needed in trace amounts. It is known cofactor of iron and manganese. These metals are essential because they form an integral part of one or more enzymes involved in a metabolic or biochemical process. The biologically active form of manganese is the Mn$^{2+}$ ion. Many enzymes have been shown to be activated by manganese. It is widely distributed throughout the body tissues and fluids. Manganese participates in mucopolysaccharide metabolism and is connected with superoxide dismutase. Chronic manganese poisoning has been known to occur among miners. Manganese is toxic if inhaled and excess results in neurological disorders [18].

Lead and cadmium, have no known function in the body and are referred to as toxic metals. When the lead content in algae goes beyond 0.5μg/gdw, enzymes required for photosynthesis become inactive. Consequently, the rate of photosynthesis diminishes; the algae will manufacture less food and therefore will not grow less healthily. The decreased in the algal growth leads to an inadequate quantity of food for animals and this may affect the whole food chain. Fish are more susceptible than algae to lead. When lead concentrations exceed the level of 0.5μg/gdw, gill function is affected [19]. Embryos and fry are more sensitive to the toxic effects of lead than are adult fish. Lead is more toxic at lower pH and in soft water [20]. The toxicity of lead to fish vary from one species to another, for instant, Goldfish are relatively resistant to lead since they have the ability to eliminated lead via their gills [21].

Cadmium has no known essential biological functions and it is considered as one of the most toxic heavy metals. Though, the concentrations reported in this study is quite low, but detrimental. In fish, cadmium can cause various structural and pathomorphological modification in various organs at low concentrations. The kidneys and liver are the preferred sites of accumulation of cadmium. Skeletal deformities in fish influence greatly the ability of the fish to search food and to escape predators [22]. The most toxic form of cadmium is divalent ion (Cd$^{2+}$). When cadmium is present in the human or fish body, production of metallothionein is stimulated. The resulting low molecular weight protein binds with Cd, consequently diminishing the toxicity of cadmium. The process usually occurs in the liver of fish and human. However, in case where the cadmium concentration is too high, the metallothionein detoxification system cannot decrease the toxicity of cadmium effectively. Therefore, the excess cadmium can produce its hazardous effects [20, 21, 23]. The monitoring of the cadmium content in fish species is important since the consumption of fish is the major source of intake of cadmium for the general population. The major cadmium constituent of fish or other seafood is absorbed mainly in CdCl$_2$ form and in human being; the gastrointestinal absorption of cadmium is approximately 3-8% of the ingested load. In humans and other mammals, the absorption of cadmium is enhanced by dietary deficiencies of calcium and iron and by low protein diets. Once cadmium is present in the body; it is transported by the blood and distributed primarily to liver and kidney, which are the principal sites of accumulation of Cd in the body. The elimination of cadmium by the body is an extremely slow process and it has a biological half-life of 20 to 30 years [21].

However, essential metals become toxic when they reach a threshold value and their presence become first inhibitory and afterwards toxic and even lethal for the organism [24]. The intracellular levels of essential metals are regulated by transporters (which translocate metal across the plasma membrane) as well as by metallothionein and other metal binding proteins [25]. The relatively high concentration of heavy metals during the dry season coincides principally with decreasing rate of organic matter decomposition, due to low water temperature. Similar observation was reported in marine organisms investigated at different periods in the Gulf of Chabahar, Oman Sea [26].

The disparity in the accumulation of the metal by these gastropods could be attributed to their different affinity for absorption. This is because metal entering a given environment are distributed among different phases depending on their specific chemical characteristics, the nature and availability of binding site. Similarly, differentially exposed to metals in a given environment and varying degree of accumulation of metal. Equally, the differences in metal uptake, detoxification or metabolism and elimination kinetics of the organisms is vital in accumulation of metals in an organism. Also, the rate of uptake of metal by an organism may depend on the chemical form of the metal which may vary from site to site. Other chemical factors such as pH, temperature, hardness and salinity may affect the rate of metal uptake as well [27].

In aquatic systems, zinc, cadmium and lead are some of the heavy metals of greatest concern. These elements are toxic to organisms above specific threshold concentrations but many of them are essential for metabolism at lower concentrations. Lead and cadmium have no biological function. They have contributed to serious problems in freshwater, estuarine and coastal ecosystems. The concentration of a trace metal in a given organism results from the net balance between the processes of metal uptake and metal loss. The different accumulation strategies go from a strong net accumulation through weak net accumulation to regulation. Net accumulation is the balance of absolute uptake and excretion of metals. Strong 'accumulators' show no metal excretion. The term 'regulator', however, defines an organism which shows no significant change in body metal content over time on exposure to a raised metal concentration. This regulating response is difficult to maintain as a long-term strategy although it is possible at short-term exposures. Therefore, environmental metal level is not the only factor affecting the metal content of aquatic organisms. The accumulated amount of metals also depends upon abiotic factors (metal solubility, metal speciation, complexation etc) and biotic factors (growth, biochemical composition, reproductive condition, metabolism, excretion etc).

**CONCLUSION**

The ingestion of metals by benthos is an obvious means of exposure to metals because they accumulate substantial amounts of metals in their tissues, especially in the muscles, and thus they represent a major dietary source of metals for general population. The adverse human health effects associated with exposure to heavy metals, even at low concentrations are diverse. There is needs for thorough investigation of the water qualities in this region, especial the potential ecological risk index (PERI), to ascertain the degree of the toxicity of heavy metals pollution and the response of the environment.
REFERENCES


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