Some Metal Elements Concentration in *Malletia cumingii* (Hanley, 1860) (Bivalvia) in Okpon River, Cross River State, Nigeria

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Abstract Molluscs are important food item all over the globe. They have been harvested from the wild and some cultured for food; such that 20% of aquatic animal protein comes from molluscs. They have also been widely used as indicator of pollution. On the other hand, many are intermediate hosts of parasitic disease of many animals and man. *Malletia cumingii* is usually harvested in Okpon River, a tributary of Cross River in Cross River State of Nigeria. Because of the value of Molluscs as indicator of pollution, this species was analyzed to ascertain the metal elements concentration levels for reason that some of these metals are toxic to man. Samples were collected from three locations along the River. Sampling was done two times to include the two main seasons of the year (dry and wet seasons). The samples were oven-dried (MAMMART) at 100°C and were pounded in mortar to powder form. Metal concentrations were measured using flame photometer (RP 7) and atomic absorption spectro-photometer. Metals analyzed included Copper, Cadmium, Chromium, Iron, Manganese, Nickel, Lead, Zinc, Calcium and Magnesium at different wavelengths. Results showed that metal concentration was increasing from the upper reaches of the river downward except zinc. It was suggested that zinc have a point source in the upper reaches while others metals were added to the water body by anthropogenic activities. However, the concentration of the metals did not reach toxic level in the molluscs for human consumption.

Keywords Metal elements; *Malletia cumingii*; Okpon River; Concentration; Toxicity; Cross River State

1 Background

The study of molluscs is always an important one as molluscs are used as human, aquatic and many terrestrial animals’ food (Gupta and Gupta, 2006). Because of their importance in human food, they are used in Aquaculture to create a net gain in protein to help alleviate the fishing pressure on feral fin fish. Nine million, one hundred Metric tonnes (22 percent) of Aquaculture products in 2014 A.D. were molluscs (FAO, 2016). Unlike carnivorous fish, which need a fish-based food, molluscs feed on diet based on plant materials. Molluscs feed both in natural and artificial environments. They are less expensive because they are of low trophic level (Simpkins and Williams, 1989; Okon and Ibom, 2012). Sequel to this, species like Pacific oyster (*Crassostrea gigas*), Japanese carpet shell (*Ruditapes philippinarum*), Yesso scallop (*Patinopecten yessoensis*), blue mussel (*Mytilus edulis*) and the blood cockle (*Anadara granosa* have been cultured). Contribution of molluscs to world food fish Aquaculture in 2013 was 20.10% by weight (15.5 million metric tons) and 13.80 % by money value (20.7 USD) (FAO, 2015).

Many serve as intermediate hosts of parasitic helminthes (David, 1994; Ntonifor and Ajayi, 2007; Abe, 2010; Kalu et al., 2012). They are also identified as the most appropriate group of organisms to be used as environmental bio indicators because of the following qualities. They are cosmopolitan in distribution, individuals have limited mobility, exhibit wide variation in life cycle strategies such that a particular strategy can be monitored, their sizes are large enough, absence of external and internal skeleton bring them closer to their environment and have limited ability to excrete pollutants directly via their kidney or other excretory tissues, they are sensitive to environmental chemicals, simple and well and well known internal organisation an quantities of the pollutants are measurable in their tissues, organs or whole organism (Oehlman and Schutle-Oehlman, 2003).

Modification or changes in environment is a continuous phenomenon. Altering their habitat affects survival (Ayotunde and Ada, 2013). Direct destruction of some of these habitats because of agricultural and urban
development and habitat transformation resulting from dam construction and human settlements are important causes of molluscs’ population declines in many areas (Hunters et al., 1976; Yang et al., 2013). Most freshwater molluscs species are highly sensitive to water quality because of their permeable skins and because they need a good oxygen supply. Larval stages of unionids require fish as a host for development. As fish stock become scanty, the parasitic larval stages of the molluscs lack host to complete their development. It therefore means that loss of fin fish stock leads to loss of bivalve requiring the fin fish their development in an area. Continuing loss of molluscs diversity is detrimental, not only to ecosystems around the world, but in the long run, to the welfare of mankind itself (Svobodová et al., 1993). Indeed, molluscs are crucial to the integrity of ecosystems, the evaluation of environmental health, and human well-being.

Some heavy metals have beneficial functions to the bodies of organisms while others are not and can be highly toxic. They accumulate in the body tissue of organisms because they are rarely broken down (Ekpo et al., 2008). However, large quantity of heavy metals and derivatives are discharged by industrial plants and municipal sewage treatment plants into the aquatic ecosystems. They may also get into the water from polluted runoff in urban and agricultural areas (Ayotunde et al., 2012).

Okpon River serves as a major source of drinking water and site of fishing activities. There are many fishing landing sites along the river. These include: Ekurri beach, Okpon beach and Nsin beach. Where the river transverse villages, there are sand mining activities. This forms a big industry in Ochon where the river transverses Calabar-Ikom high way, English Sand beach and Men sand beach are equally found in Ochon. Other sand beaches include those in Ekurri (Ekurri sand beach) and Nsin (Nsin sand beach), Onyen Okpon (Monkey Agwu Okpa sand beaches). This equally provides livelihood for the inhabitants. There is relatively a high population density along the river due to its reach resources (Ako and Salihu, 2004; Study.com, 2016). Logging activities have been going on. As Rainforest Movement (2016) wrote ‘Logging has significantly reduced animal habitat, shrinking the animal populations that serve as a traditional source of protein in Cross River State. Meat obtained from feral animals commonly called bush meat that was once plentiful is now scarce (pers. observ.). The plundering of trees which provide shelter has left whole areas without windbreakers or sufficient trees to check the devastating rainstorms. Thus, the roofs of houses are often blown off by the slightest rainstorm.’ Because of high anthropogenic activities, natural phenomenon like weathering and seapage of materials from surrounding basements, there is likely to be inflow of metals into the river. In addition to indirect feeding of aquatic systems with contaminants, humans feed the system with metal containing items like personal care products (Schueler, 2000; Burton and Pitt, 2007). Apart from metal pollution, climate change which is presently much talked about is likely to affect the ecology of the river.

Due to the toxicity level of heavy metals concentrations in aquatic food items such as fish species, they have received considerable attention in different places (Mohamed and Gad, 2008; Klavins et al., 2009; Ayotunde et al., 2012; Ekpenyong et al., 2015). Many research interests have been generated on the integrity of aquatic environment especially as it concerns heavy metal pollution (Rashed, 2001; Tawari-Fufeyin and Ekaye, 2007; Yilmag, 2007; Yilmag et al., 2009). As Elder and Collins (1991) pointed concentration of metals in an organisms cannot be extrapolated from place to place due to differences in individual organisms and environmental characters, we work to investigate the concentration of some metals in Malletia cumingii in Okpon River.

2 Materials and Methods

2.1 Study area

Okpon River is a tributary of Cross River. It has its source from Etung Local Government Area of Cross River State Hills. Okpon River flows from Ekurri in Etung Local Government Area and empties into Cross River. The Okpon River is located in the southern part of Nigeria latitude 4°25”-7°0” North and longitude 7°15”-9°30” East. The climate of the area is characterized by two seasons dry and raining. The raining season is (May-October) with higher precipitation, while the dry season is (November-April) with low precipitation (Freund et al., 1995).
The vegetation of the river is made up of dense growth of tall trees made of thick foliage. The foliage reduces the amount of sunlight that gets into the river that in turn plays a part in the distribution of types of organisms in the river ecosystem. Due to the fertility of the Okpon River Basin; there is a high concentration of human population along the River. Fishing activity includes the harvesting of *Mellitia cumingii* which is a source of animal protein for the local population.

Samples were collected from three locations along the River as indicated in the map (Figure 1). Sampling was done two times (in January and July of 2006) to include the two main seasons of the year (dry and wet seasons). Concentrations were analyzed and average of both the dry and wet seasons found. The specimens were dissected according to guides from Olayemi et al. (2012) to remove the tissues. These were preserved in pure H₂SO₄ waiting for laboratory analysis of the metals (Khadse, 2010).

![Figure 1 Map of Cross River State showing the location of the sampling stations as bold crosses (Ekurri, Nsin and Ochon) along the river](image)

The tissues were removed from preservative and soaked in water for four hours. These were washed in deionized water repeatedly to rinse off the preservative. The samples were oven-dried (MAMMART) at 100°C and were pounded in mortar to powder form. One gram of each sample was digested in 15 ml of nitric acid and 5 ml of perchloric acid. The mixture was heated on a hot plate until it became solid. This was digested to a clear liquid and made up to 100 ml using deionized water. Metal concentrations were measure using flame photometer (RP 7) and atomic absorption spectro-photometer. Metals analyzed included Copper, Cadmium, Chromium, Iron, Manganese, Nickel, Lead, Zinc, Calcium and Magnesium at different wavelengths as shown in Table 1.
Table 1: Wavelengths used in measuring light absorbance for various metals at different lamp apertures and instrument used

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Cu</th>
<th>Cd</th>
<th>Cr</th>
<th>Fe</th>
<th>Mn</th>
<th>Ni</th>
<th>Pb</th>
<th>Zn</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>324.8</td>
<td>326.1</td>
<td>357.9</td>
<td>248.4</td>
<td>279.5</td>
<td>341.5</td>
<td>283.3</td>
<td>213.9</td>
<td>422.7</td>
<td>202.6</td>
</tr>
<tr>
<td>Lamp aperture (nm)</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Instrument used</td>
<td>AAS</td>
<td>AAS</td>
<td>AAS</td>
<td>AAS</td>
<td>AAS</td>
<td>AAS</td>
<td>AAS</td>
<td>AAS</td>
<td>FP</td>
<td>FP</td>
</tr>
</tbody>
</table>

Note: AAS = Atomic absorption spectrophotometer; FP = Flame photometer

3 Results

Concentrations of metals in all tissues combined for a particular station were displayed in Table 2. Metals that were different in their concentration among the stations included Manganese (Mn), Lead (Pb), Zinc (Zn) and Calcium (Ca). Iron (Fe), Cadmium (Cd), Copper (Cu) and Magnesium (Mg) were not significantly different in concentration among stations. Means which carry the same letters were statistically the same while those with different letters were statistically different.

Table 2: Show the mean values of the metals at the various stations in Malletia cumingii from Okpon River

<table>
<thead>
<tr>
<th>Location</th>
<th>Fe</th>
<th>Mn</th>
<th>Pb</th>
<th>Cd</th>
<th>Cu</th>
<th>Zn</th>
<th>Ca</th>
<th>Mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Upper reaches</td>
<td>0.062</td>
<td>0.066</td>
<td>0.00</td>
<td>0.043</td>
<td>0.046</td>
<td>0.047</td>
<td>1.079</td>
<td>0.409</td>
</tr>
<tr>
<td></td>
<td>±0.014 a</td>
<td>±0.013b</td>
<td>±0.000c</td>
<td>±0.010a</td>
<td>±0.011a</td>
<td>±0.016a</td>
<td>±0.088a</td>
<td>±1.107a</td>
</tr>
<tr>
<td>B Middle reaches</td>
<td>0.069</td>
<td>0.077</td>
<td>0.063</td>
<td>0.046</td>
<td>0.041</td>
<td>0.034</td>
<td>0.818</td>
<td>0.323</td>
</tr>
<tr>
<td></td>
<td>±0.012a</td>
<td>±0.012a</td>
<td>±0.009a</td>
<td>±0.014a</td>
<td>±0.012a</td>
<td>±0.014b</td>
<td>±0.051b</td>
<td>±0.130a</td>
</tr>
<tr>
<td>C Lower reaches</td>
<td>0.064</td>
<td>0.071</td>
<td>0.071</td>
<td>0.042</td>
<td>0.045</td>
<td>0.028</td>
<td>0.870</td>
<td>0.339</td>
</tr>
<tr>
<td></td>
<td>±0.008a</td>
<td>±0.010ab</td>
<td>±0.042b</td>
<td>±0.011a</td>
<td>±0.013a</td>
<td>±0.014b</td>
<td>±0.143b</td>
<td>±0.106a</td>
</tr>
</tbody>
</table>

Figure 2; Figure 3; Figure 4 show the concentrations of the different elements in the various tissues namely mantle, abductor muscle foot and gills in different stations. In Figure 2 it was observed that manganese had a high absolute concentration value in the mantle compared to other elements. Manganese and zinc were comparatively higher than other elements in the mantle in the different stations, while calcium was higher than other elements in different tissues and stations. Figure 5 is a four axis graph which shows that calcium was the most highly concentrated as it is furthest apart from the origin of the graph. This was followed by magnesium and manganese.

![Figure 2 Metals elements concentrations in the tissues of Malletia cumingii in station A (Ekurri). All the elements were not significantly different ($\alpha = 0.05$) in concentration except Magnesium whose concentration was lower in the mantle compared to other tissues. It is also observed that Calcium was higher than any other metal followed by Magnesium.](image-url)
Figure 3 Metals elements concentrations in the tissues of *Malletia cumingii* in station B (Ochon). Manganese, Cadmium, Zinc, Calcium and Magnesium were not statistically different between one organ and the other at (α = 0.05), but Iron, Lead and Copper were differentially concentrated among the tissues.

Figure 4 Metals elements concentrations in the tissues of *Malletia cumingii* in station B (Nsin). All the elements except lead and copper were not significantly different in concentration in the various tissues; Lead and Copper were less concentrated in the abductor muscle. Copper was least concentrated in the foot compared to all other tissues (α = 0.05).
Figure 5 A graph of metal elements concentration in the tissues *Malletia cumingii* obtained from the three sample stations of Okpon River, Cross River State, Nigeria. Calcium was the most highly concentrated followed by Lead.

4 Discussion

Magnesium and Calcium were higher in concentration than other elements in station A. All others were not statistically different in concentration. Magnesium and Calcium are usually part of biological systems. These are usually not grouped as heavy metals as their relative mass is not greater than five times the weight of water (Glanze, 1996; Duruibe et al., 2007). Magnesium is known to regulate the functions of enzymes. It is co-factor of enzymes that break down fat and glucose and functions in the production of proteins. Since it is important in the formation of energy currency, ATP (Adenosine Triphosphate) it could be regarded as a source of fuel. Its role in the proper functioning of sodium-potassium pump makes it important in maintenance of electrolyte balance in the bodies of organisms (Byjus, 2015). Calcium is abundant in bones of organisms. Bivalves have no bones. Its exoskeleton (the shell) is highly impregnated with calcium (Smith, 1986). Its high concentration was not a surprise therefore because it is needed for further formation (growth) of the shell. Apart from shell formation, it is important in muscle contraction and nervous coordination (Pleshchitser, 1958; Byjus, 2015). Oehlman and Schulte-Oehlman (2003) pointed that molluscs are good indicators of metal availability as they are one group of animals with metallothioneins, which are low-weight molecule protein with high cysteine content and having high affinity for absorbing trace elements.

Metals concentrations above normal concentrations constitute pollution. Apart from pollutants entering the water systems through weathering (Idodo and Oronseye, 2006), anthropogenic sources are the reasons for entry of pollutants into water (Ogbeibu and Ezeunara, 2002). For instance, the concentration of Lead, increased from station A to C. Such increases could be attributed to increasing anthropogenic impact. For instance, Lead is a component of fuel used in automobiles and light generators. The number of light generators and automobiles are adding as one more from the upper reaches of the river to the lower reaches. If the Lead is not biodegraded or removed by chemical reaction, or these processes do not remove it more than it is added, the concentration is expected to increase downward as observed in Table 2. We may argue that it is because settlement increases and adds downward from the upper part of the river downwards. It is not every pollutant or element that increases in concentration down the river. Some elements could be released from a source and may be consumed by organics or even chemical reactions as it moves down the river. Such was observed in Table 2 for Manganese. There may
be a point source pollution for this element in the upper reaches of the river, which may be absorbed, or removed by chemical binding to other substances. Many organisms have been used as bioindicators of aquatic environmental pollution. As Oehlman and Schulte-Oehlman (2003) posited, organisms which have limited range of mobility are good bioindicators. *Malletia cumingii* is a benthic bivalve with proximity to bottom sand. Metal elements load or metal element concentrations in the environment can be estimated from this kind of species. Similarly, it is the opinion of Khatri and Tyagi (2015) that through the application of Bioindicators we can predict the natural state of a certain region or the level/degree of contamination. It is also pointed out that the concentration of metals in an organism is not necessarily an indication of the level of concentration in the water column. According to Elder and Collins (1991), bioaccumulation and toxicity are extremely situation dependent; therefore, concentration levels should not be extrapolated from one particular area to other situations where the biological species or environmental conditions are different. That even within one species, individual characteristics such as size, life stage, sex, and genotype can have significant effects on responses to contaminants.

The quantity of metals absorbed and maintained in an organisms’ body depends on a lot of factors as was explained by Cheng (1987), Elder and Collins (1991), Ayotunde et al (2012) and Oehlman and Schulte-Oehlman (2003) to include; concentrations and chemical characteristics of water, ecological needs, metabolism and feeding patterns of organism tolerance, size, life stages, species, organic substances, pH, temperature, alkalinity, hardness, inorganic ligands, interactions, sediments and season. These factors can equally alter metal toxicity in the aquatic environment substantially by causing attenuating effect.

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