Concentration of Heavy Metals (Zn, Cd, Pb) in Kidney and Gill of Catfish (Clarias spp.) in Ebonyi River, Southeastern Nigeria

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Citation

Abstract
Assay for heavy metals in kidney and gills of catfish were carried out using standard procedure. Copper was the most abundant metal in the studied tissues of the fishes. Copper revealed its maximum accumulation in the kidney and gills of the three species. The pattern of occurrence of these heavy metals are 92.92>58.08>23.23 as observed in the kidney of Clarias gariepinus (Burchell, 1822), Heterobranchus bivorsalis (É. Geoffroy Saint-Hilaire, 1809) and Clarias anglarius (Linnaeus, 1758) (Siluriformes; Clariidae). In the gills of C. gariepinus, H. bivorsalis and C. anglarius the pattern of occurrence of these heavy metals are 4.85>2.23. As observed in the kidney of C. gariepinus, H. bivorsalis and C. anglarius the level of accumulation of cadmium are 0.985>0.520>0.500. 0.439>0.435>0.345 were the level of accumulation of cadmium in the gills of H. bivorsalis, C. anglarius and C. gariepinus. The accumulation of iron in kidney of C. gariepinus, H. bivorsalis and C. anglarius were in the following order 1.044>0.696 while 0.696>0.522 were its occurrence in the gills of C. gariepinus, H. bivorsalis and C. anglarius. In relation to zinc accumulation in the kidney of C. anglarius, H. bivorsalis and C. gariepinus the level of accumulation are 318.95>293.75>256.25 while 318.75>287.50>250.00 were the level of accumulation of these heavy metals in the gills of C. anglarius, H. bivorsalis and C. gariepinus.

1. Introduction
Fish accumulate toxic chemicals such as heavy metals directly from water and diet, and contaminant residues may ultimately reach concentrations hundreds or thousands of times above those measured in the water, sediment and food (Osman et al., 2007). Heavy metals are normal constituents of marine environment that occur as a result of pollution principally due to the discharge of untreated wastes into rivers by many industries. Bioaccumulation of heavy metals in tissues of marine organisms has been identified as an indirect measure of the abundance and availability of metals in the marine environment. For this reason, monitoring fish tissue contamination serves an important function as an early warning indicator of sediment contamination or related water quality problems and enables us to take appropriate action to protect public health and the environment (Mansour and Sidky, 2002). Gill tissue is an organ having a large surface and separates blood from water in fish and is very susceptible to changes in concentrations of the variables (heavy metals, temperature, pH etc.) in the environment. These variables affect the structural integrity of the gill and cause morphological changes.
For this reason gills are good indicators of water pollution (Bhagwant and Elahee, 2008). It is estimated that fish can act as front-line indicators of suspected aquatic pollutants such as metals (Fatma et al., 2005). Fish may absorb dissolved elements and heavy metals from surrounding water and food, which may accumulate in various tissues in significant amounts (Fatma et al., 2005) and are eliciting toxicological effects at critical targets. However, fish may accumulate significant concentrations of metals even in waters in which those metals are below the limit of detection in routine water samples (Fatma et al., 2005). Fish might, therefore, prove a better material for detecting heavy metals contaminating the freshwater ecosystems (Fatma et al., 2005). Accumulation of metals may lead to high mortality rate or cause many biochemical and histological alterations in the survived members. Several investigations had concerned with the effect of metals on the levels of the tissue protein and lipid. (Fatma et al., 2005). Lead and other trace metals have high affinity for animal tissues where they are concentrated to varying levels (Martinez et al., 2004). The uptake from the medium continues passively against a concentration gradient since backflow is limited by the excess high affinity binding sites within the body of the fish (Martinez et al., 2004). The positive correlation between the concentration of metals in the aquatic environment and the tissue of fish often pose serious health problems to fish consumers especially man. Consumption of fish with high amount of lead is a major route of human exposure to lead when contaminated fish are consumed (Nnaji et al., 2007). Lead is known to be accumulated in different organs of fish including the bone, gills, kidneys, liver and scales (Javid et al., 2007). Fish constitutes an important and cheap source of animal protein to human beings and a large number of people depend on fish and fishing activities for their livelihood. Increasing human influences through heavy metal pollution have however led to the depletion of our fish resources and substantial reduction in the nutritive values. The danger of these heavy metals is their persistent nature as they remain in the biota for long period of time when they are released into the environment. As a result of these heavy metals pollution several endemic fish species have become threatened. Realizing this, concern for assessment of heavy metals in the tissues of fish species in most of our water bodies have increasingly been gaining ground throughout the world. Predator fish like European catfish (Silurus glanis) are at the top of food chain in the ecosystem, and can reflect the ambient metal concentrations. Determination of trace metal levels in this fish is reported in few papers: Mendil and Uluzlu (2007) studied different lakes in Turkey, while Matasin et al. (2011) considered a fish pond in the continental part of the Republic of Croatia. The levels of contamination by cadmium in fish are of considerable interest because fish consumption is an important source of intake cadmium for the general population. Most of the cadmium content in fish or other seafood is highly absorbable in CdCl2 form; in humans cadmium is particularly accumulated in kidney; in muscles the concentrations are low. Once absorbed, lead accumulates in high concentrations in bone, teeth, liver, lung, kidney, brain, and spleen, and it goes through the blood–brain barrier and the placenta. Thus this paper seeks to determine the concentration of heavy metals (Zn, Cu, Cd, Fe and Pb) in kidney and gills of catfish, to show the correlation between heavy metal intake in catfish and potential human health hazard and to understand the ecological role catfish plays in heavy metal uptake as well as its impact in the food web/trophic level.

2. Materials and Methods

The study was carried out in Ebonyi River an area covering about 6,421.2 square kilometer of the crystalline complex in South Eastern Nigeria. It lies at latitude 6°15’ North and 8°05’ East.

2.1. Sample Collection

The fish samples were collected from Ebonyi River. The samples were collected using cast net and were transported immediately to the laboratory for analysis using standard procedure. The samples were pre-treated for analysis as follows; Total length (TL) was measured to the nearest 0.1cm with a meter rule measuring board. Weight measurements were made with a FEJ-1500A electronic compact weighing balance to the nearest 0.1g. A clean stainless knife was used to dissect the fish sample and the gills and kidneys of the dissected fish sample were collected and placed inside labeled coracles and weighed.

2.2. Procedure for Preparation of Solution for Analysis

2.2.1. Lead Determination

The samples were ashen in a muffle furnace at the temperature of 600°C for 2hours. It was allowed to cool. After cooling the sample was dissolved with 5ml 30% HCl and made up to 50ml with distilled water. 5ml of the filtrate was pipette into a test tube; 5ml of sodium citrate (buffer) was added after which 0.1ml of dithzone solution was also added. It was allowed to stands for 20 minutes for colour development and the absorbance was read in a calorimeter.

2.2.2. Zinc Determination

To a 10.0ml of the digested sample was added 5ml acetate buffer and 10.0ml sodium thiosulfate solution and mixed. A 10.0ml dithizone solution (ii) was added and shaken vigorously for 5 minutes. The lower CHCl3 layer was transferred to a beaker. The standard and blank were prepared also and the absorbance read at 535nm.
2.2.3. Iron Determination

The samples were dissolved with 5ml 30% HCl and made filled into the 50ml mark with distilled water. 5ml of the filtrate sample was pipette into a test tube an about 2.5ml sodium citrate was added and to this was added 2.5ml phenoptroline.

2.2.4. Copper Determination

The samples were dissolved with 5ml 30% HCl and made filled into the 50ml mark with distilled water. 5ml of the filtrate sample was pipette into a test tube an about 2.5ml sodium citrate was added and to this was added 2.5ml phenoptroline. 2.5ml of hydroquenole was added and was allowed to stand for 20 minutes and the absorbance was read in a calorimeter.

2.2.5. Cadmium Determination

The sample was dissolved with 5ml 30% HCl and distilled water was added to the 50ml mark. 5ml of the filtrate sample was pipette into attest tube and 5ml of carbon nitrate was added. 5ml of acetic acid was also added. It was allowed to stand for 20 minutes and the absorbance was read with the calorimeter.

3. Results

The results of the heavy metal analysis in the catfishes are shown in Table 1-3. Copper was the most abundant metal in the studied tissues of the fishes. Copper revealed its maximum accumulation in the kidney and gills of the three species. The pattern of occurrence of these heavy metals are 92.92>58.08>23.23 as observed in the kidney of C. gariepinus, H. bivorsalis and C. anglarius. In the gills of C. gariepirus and C. anglarius/H. bivorsalis the pattern of occurrence of these heavy metals are 4.85>2.23. As observed in the kidney of C. gariepinus, H. bivorsalis and C. anglarius in the accumulation of cadmium, the pattern of occurrence are 0.985>0.520b>0.500b. 0.439b>0.435b>0.345b were the level of accumulation of cadmium in the gills of H. bivorsalis, C. anglarius and C. gariepinus. The accumulation
of iron in kidney of C. gariepinus and C. angliarius/H. bivorsalis were in the following order 1.044>0.696 while 0.696>0.522 were its occurrence in the gills of C. gariepinus and C. angliarius/H. bivorsalis. In relation to zinc accumulation in the kidney of C. angliarius, H. bivorsalis and C. gariepinus the level of accumulation are 318.95>293.75>256.25 while 318.75>287.50>250.00 were the level of accumulation of these heavy metals in the gills of C. angliarius, H. bivorsalis and C. gariepinus.

Table 1. Heavy metal assay for C. gariepinus

<table>
<thead>
<tr>
<th>Sample/Heavy metals</th>
<th>Cu (mg)</th>
<th>Cd</th>
<th>Fe</th>
<th>Pb</th>
<th>Zn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>92.92\textsuperscript{a}</td>
<td>0.985\textsuperscript{b}</td>
<td>1.044\textsuperscript{b}</td>
<td>-</td>
<td>265.25\textsuperscript{b}</td>
</tr>
<tr>
<td>Gill</td>
<td>34.85a</td>
<td>0.345b</td>
<td>0.696</td>
<td>-</td>
<td>250.00</td>
</tr>
<tr>
<td>WHO limit</td>
<td>30.00</td>
<td>2.00</td>
<td>42.625</td>
<td>2.00</td>
<td>1000.00</td>
</tr>
</tbody>
</table>

\textsuperscript{a} values above WHO limit hence consumption of such food poses a very serious health risk
\textsuperscript{b} values below WHO limit, hence consumption of such food does not pose any health risk since they are insignificant

Table 2. Heavy metal assay for C. angliarius

<table>
<thead>
<tr>
<th>Sample/Heavy metals</th>
<th>Cu (mg)</th>
<th>Cd</th>
<th>Fe</th>
<th>Pb</th>
<th>Zn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>23.23\textsuperscript{b}</td>
<td>0.500\textsuperscript{a}</td>
<td>0.696</td>
<td>-</td>
<td>318.95\textsuperscript{a}</td>
</tr>
<tr>
<td>Gill</td>
<td>23.23\textsuperscript{b}</td>
<td>0.435\textsuperscript{b}</td>
<td>0.522\textsuperscript{b}</td>
<td>-</td>
<td>318.75\textsuperscript{b}</td>
</tr>
<tr>
<td>WHO limit</td>
<td>30.00</td>
<td>2.00</td>
<td>42.625</td>
<td>2.00</td>
<td>1000.00</td>
</tr>
</tbody>
</table>

\textsuperscript{a} values above WHO limit hence consumption of such food poses a very serious health risk
\textsuperscript{b} values below WHO limit, hence consumption of such food does not pose any health risk since they are insignificant

Table 3. Heavy metal assay for H. bivorsalis

<table>
<thead>
<tr>
<th>Sample/Heavy metals</th>
<th>Cu (mg)</th>
<th>Cd</th>
<th>Fe</th>
<th>Pb</th>
<th>Zn (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>58.08\textsuperscript{b}</td>
<td>0.520\textsuperscript{b}</td>
<td>0.696\textsuperscript{b}</td>
<td>-</td>
<td>293.75\textsuperscript{b}</td>
</tr>
<tr>
<td>Gill</td>
<td>23.23\textsuperscript{b}</td>
<td>0.439\textsuperscript{b}</td>
<td>0.522\textsuperscript{b}</td>
<td>-</td>
<td>287.50\textsuperscript{b}</td>
</tr>
<tr>
<td>WHO limit</td>
<td>30.00</td>
<td>2.00</td>
<td>42.625</td>
<td>2.00</td>
<td>1000.00</td>
</tr>
</tbody>
</table>

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\textsuperscript{b} values below WHO limit, hence consumption of such food does not pose any health risk since they are insignificant

4. Discussion

Copper was the most abundant metal in the studied tissues of the fishes. Copper revealed its maximum accumulation in the kidney and gills of the three species; this agrees with the findings in Carassius auratus (De Boeck et al., 2004). Cu accumulation exhibited in gills of C. gariepinus, C. angliarius and H. bivorsalis in the present study is comparable to O. mykiss (De Boeck et al., 2004), Oreochromis niloticus and L. niloticus, Cu accumulation in gills of C. gariepinus corroborates with O. mossambicus (Jenny and Avenant-Oldewage, 2006). The present data also indicated that accumulation levels for Cu in liver and kidney of C. gariepinus, C. angliarius and H. bivorsalis corroborates to the O. mossambicus and Onchorynchus mykiss (De Boeck et al., 2004) respectively. Cu can induce respiratory distress in fish, and it is striking that the most hypoxia sensitive species is also the most Cu sensitive. Copper accumulation was followed by Zn in the present study where the values were observed to be highest in kidney and gills of C. angliarius followed by the kidney and gills of H. bivorsalis and least in the gills and kidney of C. gariepinus respectively. Accumulation reported in the present study for gills of C. gariepinus are comparable to O. niloticus (Mohammed, 2008) and C. punctatus respectively (Murugan et al., 2008). In the fishes analyzed, Fe accumulation was observed in all the organs but its highest value was observed in kidney of C. gariepinus. Studies reported in C. gariepinus showed highest accumulation to be seen in organs such as gills in the fishes O. niloticus, L. niloticus (Mohammed, 2008), O. mossambicus (Jenny and Avenant-Oldewage, 2006), Liza aurata, Mugil cephalus, Liza ramada. Kalay and Canli (2000) reported that metal accumulation in the tissues of fish varied according to the rates of uptake, storage and elimination. Accumulation of each metal varied between the species of fishes used in this research of the same river as well as between the rivers. For Cu in Ebonyi River, copper level were in the following order C. gariepinus: kidney> gills followed by H. bivorsalis: kidney> gills with equal occurrence in C. angliarius. Tissues mean levels of Cu seemed to have conformed to the distribution in water. Similar findings were reported on bio-accumulation by fish of Ogba River (Wangboje and Oronsaye, 2001). Variations in metal bioaccumulation in fish tissues depend on a number of factors such as food habits and foraging behavior of the fish; trophic status, source of a particular metal, distance of the organism from the contamination source and the presence of other ions in the milieu; food availability; bio-magnification and/or bio-diminishing of a particular metal; metallothioneins and other metal detoxifying proteins in the body of the fish; temperature, transport of the metals across the membrane and the metabolic rate of the animal; species, age, size of fish and exposure time (Idodo-Umeh, 2002) and the position and...
function of the organ in the fish. Cu and Zn are essential elements for the growth and well-being of living organisms. They show toxic effects when organisms are exposed to levels higher than normally required. However, Cd, and Pb are not essential for metabolic activities and exhibit toxic properties. Metal contamination of the aquatic environment may lead to deleterious effects from localized inputs which may be acutely or chronically toxic. The essential elements such as zinc are regulated to maintain certain homeostatic status in fish. On the contrary, the non-essential elements, such as cadmium and lead, have no biological function or requirement. The amount of a metal bioaccumulated is influenced by various environmental and biological factors, leading to differences in metal bioaccumulation between different individuals, species, seasons and sites. Also, different degrees of the metals accumulated in various tissues depend on the biochemical characteristics of the metal (Farkas et al., 2000) Generally in fishes, the gill is the tissue that is more often found to have high heavy metal concentrations (Farkas et al., 2000). However, feeding habits and the metal concentrations in the environment could be responsible for metals accumulation in various tissues in fish (Farkas et al., 2000). Thus, the concentrations of metals in gills reflected the concentrations of metals in the water, where the fish species lives.

Furthermore, metal accumulations in tissues are generally found to be species specific. The observed differences between the metal concentrations in fishes may be related to their feeding habits and the bio-concentration capacity of each species (Farkas et al., 2000), WHO (1992) and Environmental Protection Administration (1991), reported that Municipal refuse contains cadmium derived from discarded nickel-cadmium batteries and plastics containing cadmium pigments and stabilizers. The incineration of refuse is a major source of atmospheric cadmium release at country, regional, and worldwide level. Also, cadmium upon getting to the environment as reported by WHO (1992) may interact with the calcium metabolism of animals and in fish may cause hypocalcaemia, probably by inhibiting calcium uptake from the water.

Copper is readily accumulated by plants and animals; bioconcentration factors ranging from 100 to 26 000 have been recorded for various species of phytoplankton, zooplankton, macrophytes, macro invertebrates and fish. Toxic effects of metals occur when the rate of uptake exceeds the rates of physiological or biochemical detoxification and excretion. The sources of copper into the environment could be traced the use of the metal compounds in fish culture and fisheries as algacides and in the prevention and therapy of some fish diseases. Copper compounds including cuprous oxide, cupric sulphate and cupric acetate are used as fungicides, in the manufacture of wood preserving agents, rayon and paint pigments. In products such as wire, piping and plated metal copper is generally immobilized, although some release can occur, for example, from water heating systems. Copper from fungicide products may also find its way into the aquatic environment. Using copper sulphate as an algacide can result in its direct addition to water supply reservoirs. Copper is also an essential nutrient and is therefore present in human and animal wastes. The animals used in this research showed a trend of increasing iron concentration in all the organs studied. It was observed that the concentration of iron was consistent.

Domestic sewage, combustion emission, mining operations, metallurgical activities and industrial effluents are among the sources of anthropogenic metal inputs and heavy metals such as Pb, Cd, Cu and Zn are released as a result of these processes. Data obtained in this study indicate that Zn, Cd, Fe, Pb and Cu were accumulated in certain concentrations in the kidney and gills of C. gariepinus, C. angliarius and H. bivorsalis from the Ebonyi River. High accumulation of Zn in the kidney could be based on specific metabolism process and co-enzyme catalyzed reactions involving Zn taking place in the kidney as reported by Jaffar and Pervaiz (1989). Zn also acts as a catalyst in metal biomolecules bound to amino acid side chains containing N, O and (or sulphur legends) to form tetrahedral zinc metalloproteins and metalloenzymes in kidney tissues. The highest copper concentration in the kidney may be due to the fact that fish kidney contains a cystine rich copper binding protein which is thought to have either a detoxifying or storage function as reported by Luckey and Venugopal (1977). Copper though essential in the diet can be harmful when large intake occurs. The harmful toxicity is largely attributed to its cupric (Cu2+) forms which, is the species commonly found. The cadmium concentration reported in this study is consistent and within the range reported in other studies that evaluated metal contamination of fishes. Kalay et al. (1999) reported cadmium level of 1.98 ppm in the liver of Mullus barbatus from the coastal region of Mediterranean Sea. Lower concentration of Cd in kidney has been reported in previous studies and may possibly be due to low tendency of Cd species towards the available active sites (N and/or O donor atoms) in kidney tissues to form tetrahedral or square planer Cd (II) complex species. In aquatic ecosystems cadmium can bio-accumulate in fish; the susceptibility to cadmium can vary greatly between aquatic organisms: salt waters organisms are more resistant to cadmium poisoning than freshwaters organisms. Cadmium is first transported to the liver through the blood, where it is bound to proteins to form complexes that are transported to the kidneys. The metal then accumulates in kidneys, damaging filtering mechanisms.

In conclusion, this study shows that the concentration of Zn, Fe, Cd and Pb in the kidney and gills of all the fish species were within the WHO limits. Cu in the gills and kidneys of C. gariepinus was beyond the limits. These metals could pass to humans through the food chain and thus predispose the consumers to possible health hazards. Periodic monitoring of these and other heavy metals in both the fishes and river system to ensure continuous safety of people in the area is recommended. Safe disposal of domestic wastes and industrial effluents should be practiced and where possible recycled to avoid these metals and other contaminants from going into the environment.
References


[10] De Boeck G., Meeus W., De Coen W., Blust R. (2004), Tissue specific Cu bioaccumulation patterns and differences in sensitivity to water borne Cu in three fresh water fish: rainbow trout (Oncorhyncus mykiss), common carp (Cyprinus carpio), and gibel carp (Carassius auratus gibelio). Aquatic Toxicology 70: 179-188.


