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Effects of sex and season on levels of heavy metals in muscles tissue of catfish (*Heterobranchus bidorsalis*) from River-Rima, Sokoto, Sokoto Nigeria

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Abstract
Geochemical processes and anthropogenic activities are the measure processes that help in the exposure of aquatic organisms to heavy metals. This study was conducted for the purpose of determining the level of heavy metals in gills and muscles tissue of male and female *Hetrobranchus bidorsalis*. Samples were collected in two Seasons; rainy and dry seasons from three different landing sites (kwalkwalawa, Hayi and Kagara-Rima) taken as stations and marked as A, B, and C along River Rima in Sokoto State, Nigeria. One gram of gill and muscle tissues from each of the stations was collected for the determination of heavy metals concentration, using AAS method. Data obtained was subjected to statistical analysis at $P<0.05$. The highest concentration of Pb in muscle tissue when comparing between the stations was recorded at station C (2.874 ± 0.611), the concentration of Pb in gills tissue of the sample was also recorded at station C (3.101 ± 0.618). Chromium was recorded to be the highest concentration in gills tissue in station A (1.429 ±0.271) and its concentration in muscles tissue was highest in station A (1.210 ± 0.313) While Cd and Cu were observed to have highest concentration in both gills and muscle tissues at station C, when considering the variation of concentration of heavy metals at seasons. Rainy season has the highest concentration. It was observed that there was higher level of heavy metals in males than females. The results for the concentration of heavy metals in gills and muscles tissues were found to be within the permissible limits when compared with the WHO and EPA guidelines.

Keywords: *Heterobranchius,* stations, session, heavy metals, River Rima

Introduction

Heavy metals are part of the natural chemical properties present in aquatic environment, which are also increasing due to high anthropogenic activities, that in turns affect the physical and chemical properties of the water and also the flora and fauna such as phytoplankton, zooplankton, vertebrate and invertebrates presents in the water. This directly affect the wellbeing and quantity of fish stocks [1]. The African Catfish *Heterobranchus bidorsalis* is one of the four identified species of *Heterobranchus*; *H. bidorsalis, H. longiflis, H. isopterus,* and *H. boulengaris*. The specie of concerned gained more attraction of farmers and consumers due to its economics importance, high protein content, palatability, hardness and ability to resist stress [2]. *H. bidorsalis* has a depressed head, with granulated upper surfaced, the unique feature which differentiate it with other members of the family, is the position of the length of their adipose and dorsal fin. The rayed dorsal fin, and caudal fin is usually longer than the adipose fin, and caudal fin relatively long and slightly pointed. The dorsal has 38-45 rays with the anal fin having the greater rays 50-45 [3]. *H. bidorsalis* is one the popular fishes that dominate River- Rima that are always found in all seasons [4].

The existence of trace metals in water bodies comes from the natural sources between water, sediment and atmosphere which contained the water. The bioaccumulation of metals in the body of aquatic organisms fluctuate as a result of natural hydrodynamic chemical biological forces [5]. Humans, through industrialization and technology, has influenced the capacity to alter these natural interactions to the level that the water and aquatic fauna therein have been threatened to a devastating point [6]. Human activities (e.g. mining excessive use of chemicals, industrial waste from refineries) have negative effect on many biological process and as time goes on these will continue to affect the wellbeing of highly productive coastal ecosystem [7]. However, fish are present at the top of aquatic food chain, this give them chances to accumulate heavy metals from food, water and sediments [8, 9].

The negative effect of heavy metals in fish can counteract there beneficial effect to humans, to an extent that the effect it will cause to humans will multiply the benefits they gets from the fish consumed [10], this may include serious threats like heart diseases, liver damage and renal failure. Therefore, many international monitoring programs, Federal Environmental Protection Agencies (FEPA) have been established in order to monitor the health of the aquatic environment [11]. This study was therefore designed to investigate the effects of sexual dimorphism and season on the level of bioaccumulation of heavy metals in gills and muscle tissue of *H. bidorsalis.*

Materials and Methods

Study Area.

The study area is River-Rima in Sokoto State located in the North-Western region of Nigeria, the area is found between Latitude 12˚ N and 13˚58´ N and Longitude 4˚ 8`E and 6˚ 5`E. Sokoto. The River covers a land area of approximately 131,600km^2 and shares its borders with Niger Republic to the North, and covers Sokoto, Kebbi, Zamfara and large part of Katsina State to the East. It also borders Niger State to the South-east, and Benin Republic to the west. The whole basin can be described as Sudan and Sahel Savanna, and it extends beyond the border to Niger Republic and the Northern part of Benin Republic. The basin topography consists of a vast floodplain (fadama land) and rich alluvial soils that is suitable for the cultivation of different variety of crops. There are also isolated hills (inselberg) and hill ranges scattered all over the area (Ekpoh and Ekpenyong, 2011). Temperature is generally extreme, with average daily minimum of 16°C during cool months of January and December, and in the hottest months of April to June, an average maximum of 38°C and minimum of 24°C. Throughout the year the average maximum is 36°C and the average daily minimum is 21°C. Rainfall is generally low. River Rima flows in a south western direction over 100km and joins the major River Sokoto to form the Sokoto Rima River system. The average annual rainfall for 35 years is about 470mm. much of the rain falls between the month of May to September, while the rainless months are October to April. Evaporation is high ranging from 80mm in July to About 210 mm in April to May [4].

Sample Collection

This study was carried during the peak period of two season's rainy season (July, August, and September) dry season (November December and January 2014). Three (3) males, 3 female fish samples were collected from three identified landing sites (kwalkwalawa, Hayi and Kagara-Rima) monthly, along River –Rima. Samples when collected were immediately taken to fisheries laboratory of Usmanu Danfodiyo University for the removal of selected tissues. Sterilized scissors was used to cut up gill and muscle tissues and placed on a weighing balance to measure 1g each.

Determination of Heavy Metals

Analysis of heavy metals was conducted using Atomic Absorption spectrophotometer. The use of AAS for determining the extent of metal concentration in fish tissues and other materials has been used by various workers in their studies [12].

One gram of the gills, and muscles of each sample was introduced into 100 ml digestion flask; 10 ml of concentrated HNO_3 and 2 ml $HClO_4$ was added. It was then allowed to stand for 24 hours. Samples were digested until clear, cooled and addition of 10 – 20 ml of deionized water was prepared and heated for 10min, removed and chilled, it was finally filtered with Whatman filter paper No. 4. Sample was prepared and analyzed in accordance with the testing of heavy metals (Pb, Cr,Cd, and Cu) for digested sample analysis.

Results

The average bioaccumulation of heavy metals in gills and muscles tissue of the samples with effect on station revealed that concentration of Lead (Pb), Chromium (Cr) in both gills tissue (GT) and muscles tissue (MT), concentration of Cadmium (Cd) in GT and Copper (Cu) in MT shows no significant difference between the three stations (A, B and C). The concentration of Cadmium in MT and Cupper in (GT) show significant difference (P<0.05), the mean and standard error of Cadmium in (MT) of station A (0.147 ± 0.044) was significantly different from station C (0.314 ± 0.074). The concentration of Copper in (GT) of station B (0.189 ± 0.076) was also significantly different at station C with 0.580 ± 0.176 (Table 1).

Table 1: Bioaccumulation of heavy Metals in gills and muscles tissue in *Hetrobranchus bidorsalis*

Heavy Metals	Stations		
	A	B	C
Lead (GT)	2.372 ±0.503	2.244 ±0.581	3.101 ±0.618
Lead (MT)	2.634 ±0.624	2.335 ±0.634	2.874 ±0.611
Chromium (GT)	1.429 ±0.271	1.157 ±0.222	1.265 ±0.189
Chromium (MT)	1.210 ±0.313	0.954 ±0.255	1.076 ±0.249
Cadmium (GT)	0.187 ±0.055	0.120 ±0.037	0.198 ±0.047
Cadmium (MT)	0.147 ±0.044^a	0.171 ±0.052^{ab}	0.314 ±0.074^{bc}
Copper (GT)	0.278 ±0.121^a	0.189 ±0.076^{ab}	0.580 ±0.176^{ac}
Copper (MT)	0.193 ±0.088	0.265 ±0.123	0.427 ±0.162

Foot note: Means followed with different superscript across the same row are statistically significance, at 5% level of significance. A= Kwalkwalawa, B= Hayi, C= Kagara-Rima, GT= gills tissue, MT= muscles tissue.

Table 2: Seasonal level of bioaccumulation of heavy metals in gill and muscle tissues of *Heterobranchus bidorsalis*

Heavy metals	Rainy season	Dry season
Lead (GT)	3.134± 0.621^a	2.011 ±0.190^a
Lead (MT)	3.760 ±0.669^a	1.469 ±0.131^b
Chromium (GT)	1.330 ±0.231^a	1.237 ±0.130^a
Chromium (MT)	1.500 ±0.292^a	0.661 ±0.086^b
Cadmium (GT)	0.153 ±0.043^a	0.183 ±0.033^a
Cadmium (MT)	0.231 ±0.047^a	0.190 ±0.049^a
Copper (GT)	0.333 ±0.135^a	0.364 ±0.074^a
Copper (MT)	0.304 ±0.126^a	0.286 ±0.078^a

Means followed with different superscript across the rows are statistically significance, at 5% level of significance. Keys: GT= gills tissue, MT= muscles tissue.

However the seasonal level of bioaccumulation of heavy metals in gills and muscles tissue of *Heterobranchus bidorsalis* shows that concentration of Pb, Cr, Cd and Cu in gills tissue (GT) and concentration of Cd and Cu in muscles tissue (MT) do not show significant difference between the two seasons (rainy and dry). While concentration of Pb and Cr in muscles tissue show significant difference between the two seasons. Between the two seasons, rainy season was recorded with the highest concentration of all the heavy metals in both gills and muscles tissue. Lead has the highest bioaccumulation level 3.760mg/kg and 3.134mg/kg, followed by chromium 1.500mg/kg and 1.330mg/kg in muscles and gills tissue respectively, then copper 0.333mg/kg in gills and 0.304mg/kg in muscles tissue, and lastly cadmium has the least concentration in both muscles and gills tissue 0.231 and 0.153 respectively (Table 2).

The level of bioaccumulation of heavy metals in gills and muscles tissue with respect to their sexes also revealed that concentration of Lead (Pb) in both gills and muscles tissue and concentration of Chromium in only muscle tissue, and that of Cadmium in only gills tissue show no significant difference between the sexes. The concentration of Copper in both gills and muscle tissue, and concentration of Chromium in gills tissue and that of Cadmium in muscles tissue show significant difference (*P*<0.05) between the sexes. Male at station "A" gill tissue has the highest mean value or concentration of Chromium (2.215 ± 0.399 mg/kg) than female at station "A" (0.644 ± 0.267 mg/kg), with significant difference between the two at *P*<0.05% level of significance. Cadmium present in muscles tissue of male at station "C" (0.421 ± 0.124 mg/kg) shows significant difference from all the muscle tissue of the remaining sexes. For the concentration of Copper in gills tissue, only male at station "C" (0.932 ± 0.322 mg/kg) differ significantly from the remaining sexes. There is no significant difference in the concentration of Copper in muscles tissues of both sexes except in female at station "A" (0.041 ± 0.035 mg/kg) and male at station C with 0.660 ± 0.293 mg/kg (Table 3).

Table 3: Bioaccumulation of heavy metals base on sex in gill and muscle tissues for *Heterobranchus bidorsalis*

Heavy metals (mg/kg)	Station A		Station B		Station C	
	Males A	Females A	Males B	Females B	Males C	Females C
Lead (GT)	2.814 ±0.798^{a}	1.930 ±0.618^{a}	2.254 ±0.830^{a}	2.235 ±0.837^{a}	3.941 ±0.880^{a}	2.261 ±0.846^{a}
Lead (MT)	3.130 ±0.958^{a}	2.137 ±0.810^{a}	2.665 ±0.978^{a}	2.005 ±0.830^{a}	3.228 ±0.724^{a}	2.520 ±1.000^{a}
Chromium(GT)	2.215 0.399^{b}	0.644 ±0.267^{a}	1.514 ±0.324^{b}	0.799±0.288^{ab}	1.543 ±0.245^{b}	0.987 ±0.280^{ab}
Chromium(MT)	1.674 ±0.534^{a}	0.747 ±0.302^{a}	1.282 ±0.389^{a}	0.627 ±0.323^{a}	1.360 ±0.392^{a}	0.791 ±0.306^{a}
Cadmium (GT)	0.172 ±0.060^{a}	0.202 ±0.093^{a}	0.209 ±0.068^{a}	0.030 ±0.013^{a}	0.194 ±0.061^{a}	0.203 ±0.074^{a}
Cadmium (MT)	0.140 ±0.065^{a}	0.154 ±0.060^{a}	0.268 ±0.090^{a}	0.074 ±0.043^{a}	0.421 ±0.124^{b}	0.207 ±0.078^{a}
Copper (GT)	0.320 ±0.124^{a}	0.236 ±0.211^{a}	0.114 ±0.068^{a}	0.264 ±0.137^{a}	0.932 ±0.322^{b}	0.227 ±0.098^{a}
Copper (MT)	0.345±0.167^{ab}	0.041 ±0.035^{a}	0.253±0.175^{ab}	0.276±0.179^{ab}	0.660 ±0.293^{b}	0.194 ±0.126^{ab}

Footnote: Means followed with different superscript across the rows are statistically significance, at 5% level of significance.
A= Kwalkwalawa, B= Hayi, C= Kagara-Rima, GT= gills tissue, MT= muscles tissue.

Discussion

Heavy metals contamination has been reported in aquatic organisms and considered to be serious pollutants inducing their effects on aquatic fauna [13]. In the present study average value of Pb in both gills and muscles was higher in station C than in A and B which may be due to domestic sewage and other human activities. The range of Pb (2.244 – 3.101) recorded in gills and muscles of the fish species were higher than the maximum recommended limits of 2.0ppm [14, 15]. (FAO, 1983, FEPA, 2003) in fish food. The increased level of industrialization is continuously increasing the risk and damages of Pb to human via different types of food chains. It is quite considered that industrial effluents and domestic sewage should approach the main flow after its proper treatment [16]. The higher concentration of Pb (3.134) recorded in gills tissue in rainy season was higher than the recommended limits of 2.0ppm, which may be due to drainage of large amounts of metal to our water bodies during rainy season.

Chromium (Cr) is an essential trace element and plays an important role in fish metabolism [16]. The level of Cr in both the muscles and gills were higher in station A than station B and C. The mean concentration of Cr in the gills and muscles tissues were found higher than the permissible limit (1.0µg/g) of [14] in station A. The muscles has the highest concentration of the heavy metals than gills. This is contrary to the previous study of [17], which showed that the muscle is not an active organ in the accumulation of heavy metals.

Cupper (Cu) The mean value of Cupper ranged from 0.580 – 0.189. Samples from station C were higher in concentration of Cu in both gills and muscles, due to the pollution of water caused by human activities. The level of Cu (0.580 – 0.189) recorded in fish samples were higher than the maximum recorded limits of 0.15 – 1ppm [15], but was lower than the values recorded by [18] 3.979 ± 0.013, which can induced respiratory stress in fish and its striking that the most hypoxia sensitive species are also the most Copper sensitive.

Cadmium (Cd) is a non-sensitive and toxic element; it can easily cause chronic toxicity even when present in low amount below 1.00µg/g. The mean value of Cd in both the gills and muscles in Table 7, were below the acceptable limit of 2.00 µg/g [14]. The values of Cd in respect to effect on station were higher in station C, while highest value was recorded in gills tissue (0.364) in dry season.

Regarding the Seasonal variation of metals, indicated consistent increase of metals in gills and muscles tissue between the seasons, except for Pb and Cr in muscles tissues that showed significant decrease from rainy season to dry season. The mean value of Pb in muscle tissue for rainy and dry season were 3.760 – 1.469 respectively. While the values of Cr in muscle tissue for rainy and dry season were 1.500 – 0.661, which may be due to high flood of water experienced in months of August and September 2015, during sampling period on River-Rima. The highest concentration of metals in the seasons fluctuated between the tissues. Pb, Cr, Cd and Cu were accumulated high in muscle tissues during rainy season. Reason for this variation was that, during the rainy season the prevailing environmental conditions such as excess run-off water from various locations, sewage, emission etc. bring to the river huge amounts of nutrients through which the aquatic animal by way of accumulation and bio-accumulation ingest in these heavy metals. The accumulation pattern of metals differed between the sexes, where the highest concentration in both the gills and muscles were found in males. These may be due to sex differences or males are more active and aggressive than the females.

Conclusion

Geochemical processes and anthropogenic activities are the measure processes that help in the exposure of aquatic organisms to heavy metals. It was observed during this study, that there was higher level of heavy metals in males than females. The level of heavy metals in gills and muscles tissues was also found to be within the permissible limits when compared with WHO and EPA guidelines.

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