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Assessment of some heavy metals in the tissues (gills, liver and muscle) of *Clarias gariepinus* from Calabar River, Cross River State, South-eastern Nigeria

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ABSTRACT

Objective: To assess the pollution status of Calabar River in relation to the levels of heavy metal in the tissue of the African catfish [*Clarias gariepinus* (*C. gariepinus*)].
Methods: A total of 45 samples of *C. gariepinus* were purchased from fishermen on landing at Nsidung beach along Calabar River within three months (15 samples monthly) from June to August, 2014. The samples were then put into a cold box containing ice blocks immediately after buying from the fishermen. The fish samples were transported immediately to the Chemistry Laboratory, University of Calabar for digestion and heavy metal analysis. Portions of the muscle, gills and liver were removed from the fresh samples and oven dried at temperature of 120 °C to constant weight and digested using standard methods. The digested tissue portions were analyzed for lead, iron, manganese, cobalt, chromium and cadmium concentrations using atomic absorption spectrophotometer.
Results: Mercury was not detected at all in the muscle, liver and gills of *C. gariepinus*. The mean ± SD of metals in liver of *C. gariepinus* were: (0.080 ± 0.014), (0.110 ± 0.014), (6.480 ± 1.279) and (0.295 ± 0.021) mg/kg for Cd, Cr, Fe and Mn, respectively. In the gills, heavy metals values were: (0.065 ± 0.021), (0.115 ± 0.035), (5.843 ± 0.558), and (0.345 ± 0.007) mg/kg for Cd, Cr, Fe and Mn, respectively. In the muscles, heavy metals values were (0.045 ± 0.021), (0.115 ± 0.353), (5.150 ± 1.075), and (0.187 ± 0.045) mg/kg for Cd, Cr, Fe and Mn, respectively. The general trend of metals accumulation in tissues of *C. gariepinus* showed a decreasing trend of Fe > Mn > Cr > Cd. Also, the metal accumulation in the three tissues showed a decreasing trend of liver > gills > muscle.
Conclusions: The metal concentrations in the muscle, gills and liver of *C. gariepinus* were all below the World Health Organization acceptable range, and as such fishery resources from Calabar River are safe for consumption.

1. Introduction

Heavy metals from natural and anthropogenic sources are continually released into rivers, and they are serious threats

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because of their toxicity, long persistence, bio-accumulation and bio-magnification in the food chain[1-3]. Heavy metal contamination in water and its uptake by fishes is a direct consequence of urban and industrial pollution[4,5]. Fish is always at the top of aquatic food chain and may concentrate large amounts of these metals, which increase with increments of the metal levels in fish food organisms[6]. Fish accumulate contaminants from the environment and therefore have been extensively used in pollution monitoring programs. Heavy metal

pollution of terrestrial and aquatic eco-systems has long been recognized as a serious environmental concern[7]. This is largely due to their non-biodegradability and tendency to accumulate in plants and animals tissues. Fishes are excellent indicators for heavy metal contamination level in aquatic systems because it occupies different food chain levels[8].

Jobling[9] reported that heavy metals concentrate more in the gills and liver of fishes than in muscles. High accumulation of heavy metals in the liver and gill tissues is the result of the synthesis of metallothionein proteins within the gills and liver tissues when fishes are exposed to heavy metals, in order to detoxify them. Gills are the site directly exposed to the ambient conditions and are known for their excretory function even for some metals like zinc[10].

The rate of bioaccumulation of metals in aquatic organisms depends on the ability of the organisms to digest the metals. Also, it has to do with the concentration of the heavy metal in the surrounding waters and sediment as well as the feeding habits of the organism. Species differences in heavy metals bioaccumulation could be linked to differences in feeding habits and behavior of the species. The degree of contamination depends on pollutant type, fish species, sampling location, trophic level and their mode of feeding[3]. Heavy metals are accumulated through different organs of the fish because of the affinity between them. In this process, all heavy metals are concentrated at different levels in different organs of the fish body parts[11]. Different concentrations of heavy metals in different fish species might be a result of different ecological needs, metabolism and feeding patterns[12]. Several studies have shown that the order of heavy metal concentration in fishes is liver > gills > muscles[13-15]. The consumption of fishes polluted with metals can lead to serious health problems in humans and livestock. Excess cadmium causes lung and kidney damage, chromium causes cancer, and manganese causes kidney and lung failure. Excess iron causes nausea, vomiting, brain haemorrhage, anxiety, tension, cardiac arrest and metabolic disorders.

The Calabar River is dominated by human settlements which expose the river to intense human activities such as ferry boat transport, open defecation, waste dumping, effluent discharges, bathing, washing and so on. These human activities have led to the introduction of contaminants along with heavy metals into the river, thereby creating a possibility of the river being polluted with some toxic heavy metals. Therefore, this study was carried out to assess the pollution status of Calabar River in relation to the levels of heavy metal in the tissue of the African catfish [*Clarias gariepinus* (*C. gariepinus*)].

2. Materials and methods

2.1. Study area

The study area is located at Nsidung beach in Calabar River (Figure 1), between latitude: 40°57'326" N and longitude: 80°18'557" E at 26 feet altitude[16]. The climate of the area is characterized by a long wet season from April to October and a dry season occurring between November and March[17]. The annual total rainfall is about 2 000 mm. There is always a short period of drought in the wet season around August/September which is called August drought. There is usually a cold, dry and dusty period between December and January referred to as the harmattan season[18-20]. Air temperature generally ranges from 22 °C in wet season to 35 °C in the dry season with relative humidity, generally above 60% at all seasons and up to 90% during the wet season[21].

Cross River has a mangrove dominated vegetation consisting of mangroves like *Rhizophora racemosa, Avicennia africana* and *Nypa fruticans*. The vegetation also contains palm trees (*Elaeis guineensis*) as well as African oak species[22].

2.2. Collection and preservation of samples

A total of 45 samples of *C. gariepinus* were purchased from fishermen on landing at Nsidung beach along Calabar River within three months (15 samples monthly) from June to August, 2014. The samples were then put into a cold box containing ice blocks immediately after buying from the fishermen, in order to keep the specimens as fresh as possible to prevent tissue decay. These fish samples were transported immediately to the Chemistry Laboratory, University of Calabar, where the samples were preserved in a freezer while awaiting dissection and preparation of tissues for analysis of metal.

2.3. Digestion and metal analysis

The frozen fish samples were allowed to thaw at room temperature (20–27 °C). Portions of the muscle, gills and liver were obtained from the fresh samples and oven dried at temperature of 120 °C to constant weight. The dried samples were ground to powder with laboratory mortar. One gram of the ground sample (for muscle, gills, and liver each) was digested with 40% nitric acid. Cooled digested samples were washed with deionized water, filtered and made to desired volume. The digested tissue portions were analyzed for lead, iron, manganese, cobalt, chromium and cadmium concentrations using atomic absorption spectrophotometer.
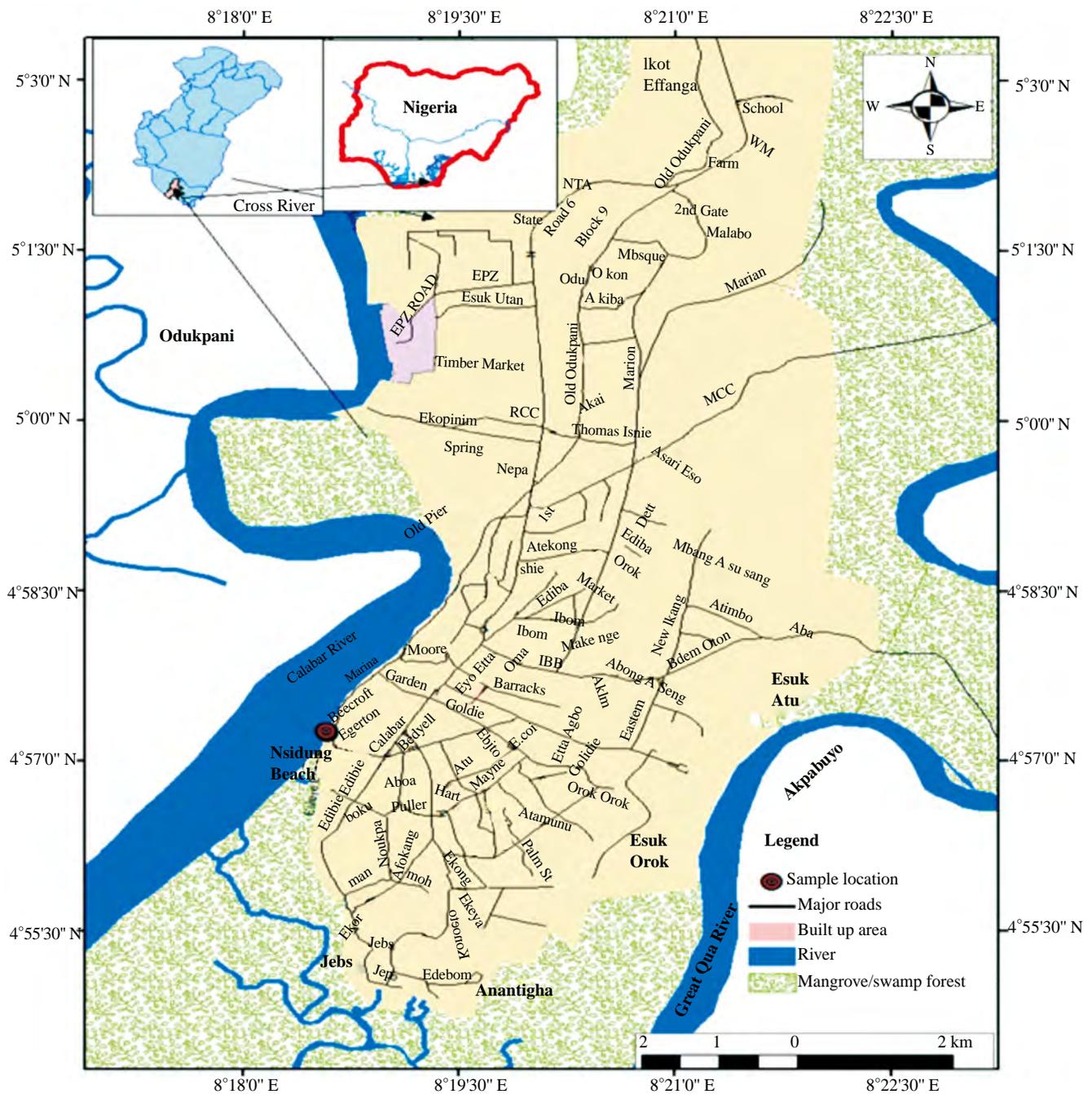
Figure 1. Map of Calabar River showing the sampling station at Nsidung beach.

3. Results

The mean ± SD of heavy metals in gills, liver and muscle of *C. gariepinus* from the study area was shown in Table 1.

The mean ± SD of metals in liver of *C. gariepinus* were: (0.080 ± 0.014), (0.110 ± 0.014), (6.480 ± 1.279) and (0.295 ± 0.021) mg/ kg for cadmium, chromium, iron and manganese, respectively. Mercury was not detected at all in the liver. The metal concentrations in the liver were all below the World Health Organization (WHO) acceptable range. Metal accumulation in the liver of *C. gariepinus* showed a decreasing trend of Fe > Mn > Cr > Cd.

The mean ± SD of metals in gills of *C. gariepinus* were: (0.065 ±

Table 1

Heavy metal accumulation in liver, gills and muscle of *C. gariepinus*.

Metals	Liver	Gills	Muscle	*F*-value	Significant value	*P*-test	Inference	WHO limits
Cd	0.080 ± 0.014[a]	0.065 ± 0.021[a]	0.045 ± 0.021[a]	1.682	0.324	$P > 0.05$	Not significant	2.00
Cr	0.110 ± 0.014[a]	0.115 ± 0.035[a]	0.115 ± 0.353[a]	0.019	0.982	$P > 0.05$	Not significant	0.15
Fe	6.480 ± 1.279[a]	5.843 ± 0.558[a]	5.150 ± 1.075[a]	1.282	0.344	$P > 0.05$	Not significant	-
Mn	0.295 ± 0.021[a]	0.345 ± 0.007[b]	0.187 ± 0.045[c]	14.442	0.015	$P < 0.05$	Significant	0.50
Hg	Below detectable level	Below detectable level	Below detectable level	-	-	-	-	-

Values with the same superscript are not significantly different ($P > 0.05$).

0.021), (0.115 ± 0.035), (5.843 ± 0.558), and (0.345 ± 0.007) mg/kg for cadmium, chromium, iron and manganese, respectively. Mercury was not detected at all in the gills. The metal concentrations in the gills were all below the WHO acceptable range. Metal accumulation in the gills of *C. gariepinus* showed a decreasing trend of Fe > Mn > Cr > Cd. The mean ± SD of metals in muscles of *C. gariepinus* were: (0.045 ± 0.021), (0.115 ± 0.353), (5.150 ± 1.075), and (0.187 ± 0.045) mg/kg for cadmium, chromium, iron and manganese, respectively. Mercury was not detected at all in the muscle. The metal concentrations in the muscle were all below the WHO acceptable range. Metal accumulation in the muscle of *C. gariepinus* showed a decreasing trend of Fe > Mn > Cr > Cd.

The distributions of the different heavy metals in gills, liver and muscles of *C. gariepinus* were varied, with the liver and gills accumulating more metals than muscles (Table 1) (Figure 2). However, ANOVA *F*-statistics showed that there were no significant differences in the metal accumulation of cadmium, chromium, and iron between the liver, gills and muscle of *C. gariepinus* at $P > 0.05$. Manganese metal accumulation differences were significant between the liver, gills and muscle of *C. gariepinus* at $P < 0.05$. The general trend of metals accumulation in *C. gariepinus* showed a decreasing trend of Fe > Mn > Cr > Cd. Also, the metal accumulation in the three tissues showed a decreasing trend of liver > gills > muscle.
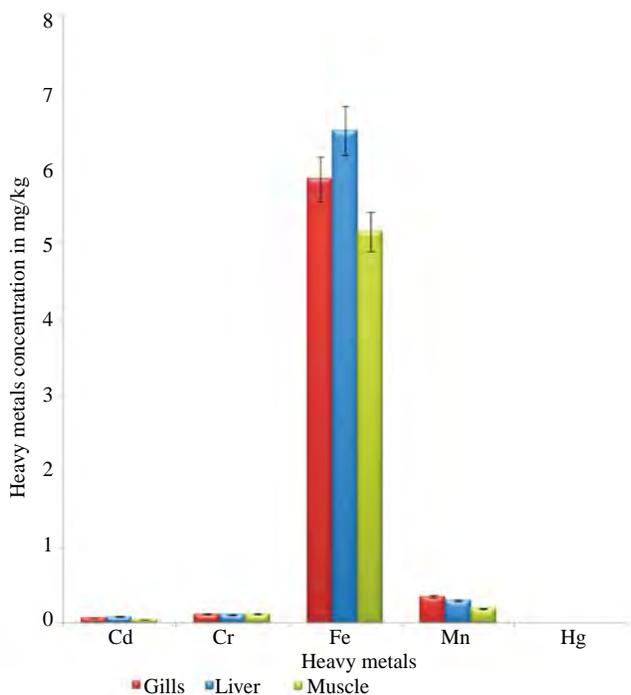

Figure 2. Heavy metal concentration in the gills, liver and muscle of *C. gariepinus.*

4. Discussion

Fishes are excellent indicators for heavy metal contamination level in aquatic systems because it occupies different food chain levels[8]. There were variations in the accumulation of heavy metals in the liver, gills and muscle of the fish species under study. This corroborated with the report of Rao and Padmaja[11], who reported that heavy metals are concentrated at different levels in different organs of the fish body parts. Indrajith *et al.*[23] reported lower values of cadmium and manganese in muscle and gills, lower values of cadmium and higher values in liver of *Etroplus suratensis* and *Ambassis commersoni* compared to the present study. Nwabueze[24] reported a higher mean accumulation for cadmium. These discrepancies could be due to the fact that the rate of bioaccumulation of metals in aquatic organisms depends on the ability of the organisms to digest the metals, concentration of the heavy metal in the surrounding waters and sediment, feeding habits of the organism, pollutant type, fish species, sampling location, and trophic level[3]. Metal accumulation in fish depends on pollution, and may differ for various fish species living in the same water body[25].

The general metal trend for this study was in the decreasing order of Fe > Mn > Cr > Cd. This trend did not corroborate with the trends reported by several authors. Eneji *et al.*[26] reported the trend of heavy metals for *Tilapia zillii* to be Cr > Zn > Cu > Fe > Mn > Cd > Pb, while the trend for *C. gariepinus* was Cr > Zn > Fe > Cu > Mn > Cd > Pb. Eneji[27] reported a trend of Cr > Zn > Cu > Fe > Mn > Cd > Pb. According to Yaduma and Maina Humphrey[28], the order of heavy metals accumulation in *Clarias anguillaris* was Cr > Mn > Pb > Zn > Cu and Mn > Zn > As > Cu for *Heterotis niloticus*. This variation could be due to the difference in species, sampling location, pollutant type, feeding habit and availability of the metals[26]. According to previous studies[9,26,29,30], a decreasing metal trend in tissues of fishes is as follows: liver > gills > muscles, showing that metals tend to accumulate more in liver and then gills, with muscles being the least preferred site for metal accumulation. This corroborated with the observation of the present study, as liver accumulated more metals, followed by gills and then muscles. The liver and gills accumulated more metals because of the synthesis of metallothionein proteins (metal binding protein) within the gills and liver tissues when *C. gariepinus* was exposed to heavy metals, in order to detoxify them[9]. The muscles of *C. gariepinus* accumulated the least heavy metals because fish muscles have low levels of binding proteins compared to the liver and gills.

In conclusion, the study confirmed that liver accumulates more heavy metals than the gills, and that the muscle accumulates heavy metals least. Also, there were variations in the accumulation of the different heavy metals in the different fish tissues. All the heavy metals studied in the gills, liver and muscles of *C. gariepinus* were all below the WHO acceptable limit, and as such, the fish is not polluted and so is the study area. This means that the fishes from Calabar River are safe for consumption. In order to maintain this safe status, the government should enforce policies against indiscriminate discharge of energy and waste by man into Calabar River, so as to prevent any health consequences associated with metal pollution.

Conflict of interest statement

We declare that we have no conflict of interest.

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