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Analysis of Heavy Metals Concentration and Its Impact on Hepatic Indexes in Clarias gariepinus from Ibi, Gidin-Dorowa, and Donga Rivers in Taraba State, Nigeria

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Abstract

Exposure to heavy metals, which are particularly detrimental to human health, has increased as a result of anthropogenic activities and modern industry. Increased levels of heavy metals in African catfish (*Clarias gariepinus*) can be a good sign that human activity has contaminated an aquatic ecosystem. The study aimed to evaluate the levels of heavy metals and their potential effects on hepatic indexes in *Clarias gariepinus* collected from three different rivers (Ibi, Donga, and Gindin Dorowa) in Taraba State, Nigeria. The fish samples were collected from the three different rivers during the dry season. The gills were removed from the fish and subjected to oven drying and grinding before being analyzed for heavy metal concentrations. Blood samples were also collected from the fish for liver biomarkers analysis. The heavy metal concentrations (specifically Pb, Cd, Zn, As, and Hg) were determined using Micro Plasma Atomic Emission Spectroscopy (MP-AES) and compared to the maximum levels specified by the World Health Organization (WHO). The results showed that zinc had the highest mean concentration in all three river samples. However, all heavy metal concentrations in the gills of the fish were found to be below the maximum allowed limits advised by standard bodies, indicating

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that the gills were safe for consumption. Also, the liver biomarker analysis showed higher levels of ALP, total bilirubin, and albumin activity in the fish from Gindin-Dorowa compared to Donga and Ibi. However, all liver biomarker values fell within the reference standards, indicating that the fish from the three rivers were free from heavy metal toxicity. In conclusion, the study suggests that the *Clarias gariepinus* samples from the three rivers in Taraba State, Nigeria, had concentrations of heavy metals within safe limits, as determined by the WHO. The liver biomarker analysis further supported the absence of heavy metal toxicity in the fish.

Keywords: Heavy Metals, Hepatic indexes, *Clarias gariepinus*, Rivers**,** Taraba State, Nigeria

INTRODUCTION

Human exposure to heavy metals has significantly increased over the course of several centuries due to the rates of hazardous material emission from various sectors. The most frequent heavy metals that cause human poisoning have been mercury, lead, chromium, cadmium, and arsenic. Poisonings that are either acute or chronic may accompany exposure to water, air, or food. These heavy metals bioaccumulate in the body and have a variety of harmful effects on various tissues and organs. The cellular processes of growth, proliferation, differentiation, damage repair, and apoptosis can all be impacted by heavy metals. Similar pathways for these metals to cause toxicity, including ROS production, weakened antioxidant defense, enzyme inactivation, and oxidative stress, are revealed by comparing the mechanisms of action. However, some of them bind to particular macromolecules in a selective manner. Within this framework, ferrochelatase and aminolevulinic acid dehydratase interact with lead. Genomic instability is a result of some hazardous metals, including chromium, cadmium, and arsenic. Trace metals that are heavy or poisonous are at least five times denser than water. They often don't degrade further into less dangerous components; instead, they build up in the areas where they are discharged (Kennicutt *et al.,* 1998). They are bioaccumulative and stable (cannot be digested by the body). On sometimes, they reach people higher up the food chain (Fergosson, 1990). While some metals are necessary for biota, they can be exceedingly dangerous when present in high concentrations. At a certain amount of exposure, heavy metals are non-biodegradable and extremely damaging to aquatic life, plants, and people's health (Mustafa

et al., 2006). Some heavy metals, like copper or cobalt, are necessary for enzymatic function at low concentrations, but at greater quantities, they act as enzyme inhibitors. Other metals, like cadmium and lead, are poisonous even at low doses and serve no biological purpose (Bryan, 1976).

According to Kheradmand *et al.* (2006), heavy metals can be divided into three categories: possibly hazardous (arsenic, cadmium, lead, mercury, etc.); probably essential (nickel, vanadium, cobalt); and necessary (copper, zinc, iron, manganese). Because they inhabit diverse tropic levels and range in size and age, fish have been recognized as useful indicators of heavy metal contamination in aquatic systems (Burger *et al.,* 2002). One of the most important indicators for determining the amounts of metal pollution in freshwater systems is fish (Rashed, 2001). Additionally, fish consumption by humans is common in many regions of the world, and contaminated fish may be harmful to human health. Studies on heavy metals in rivers, lakes, fish, and sediments have received significant attention in the environmental field, particularly over the past ten years (Zmen *et al*., 2004; Begüm *et al*., 2005; Ztürk *et al.,* 2008; Praveena *et al.,* 2008). Approximately 70 well-known trace elements, including heavy metals, are needed by the human body. However, there are 12 hazardous heavy metals that operate as toxic interference with the body's enzyme systems and metabolism, including lead, mercury, aluminum, arsenic, cadmium, nickel, and chromium. The presence of metal contaminants in freshwater upsets the delicate balance of the aquatic ecosystem, making it imperative to regularly monitor the amounts of heavy metals in aquatic habitats.

MATERIALS AND METHODS

Sampling Locations

The study samples were collected in three separate places: **Ibi River, Donga River, and Gidin Dorowa River**

Sample Collection

During the dry season in February, this investigation was conducted. To assess the levels of lead, cadmium, chromium, zinc, and arsenic, samples of *Clarias gariepinus* were taken from the three Rivers.

Experimental procedure

From the Ibi, Donga, and Gidindorora Rivers, mature catfish samples weighing between 1-1.5 kg were collected. Gills were removed from fish samples using stainless steel dissection kits after the fish were slaughtered and brought to the lab. Prior to digestion and analysis, fish organ was oven-dried at 80^0C for 48 hours, ground separately in a porcelain mortar and pestle, packed in labeled plastic bags, and stored at -10^0C. Using the organic extraction technique described by (sreedivi *et al.*, 1992), fish organ was treated and digested. A 50ml kjedhal flask was filled with 1g of the milled sample. Nitric acid (10 ml), perochloric acid (2 ml), and sulfuric acid (2 ml) were added in a 5:1:1 ratio to the sample in the flask. The flask's contents were heated gently within a hood.

Ashing

Each organ sample weighed 2 grams and was placed in a platinum dish before being placed in a muffle furnace. The temperature was raised to roughly 55^0C for 4-5 hours, and once the sample had entirely turned to ash, it was removed and allowed to cool in a desiccator.

Digestion of Samples

25ml of digesting acid (Aqua regia HCl: HNO_3, 3:1) was applied to 2.00g of the ash-prepared samples (muscles) in a kjeldal flask. Allow to cool, then transfer the digest into a 100ml volumetric flask. Make up to mark with distilled water, and filter using Whatman No. 1 filter paper. Swirl and heat gently at first until frothing stops, then more forcefully until a clear light yellow solution results. After that, the filtrate was transferred to the AAS (Bulk Scientific, VPG 20, La Vegas, USA). For the measurements, the wavelength characteristics of each heavy metal were established using the air acetylene integrated flame mode (all heavy metals) and a hollow cathode lamp of each desired metal was put into the instrument. Standard for each metal was obtained through extrapolation from the calibration curve of the standard. The aforementioned steps in this section were performed in accordance with the recommendations from the analytical methods for atomic absorption spectrometry (Perkin Elmer, 1996).

Liver Function Test (LFT)

The liver function enzymes (ALT, ALP, AST, total bilirubin, total protein, and albumin) were measured in the blood samples. In Taraba State, samples were taken from the Rivers

Ibi, Donga, and Gidin Dorowa and processed using a semi-automatic analyzer and clinical chemistry reagent kits.

Heavy Metals Analysis

Atomic absorption spectrometer (Shimazu- AA- t300) analysis was done on the concentrations of the heavy metals (Cd, Zn, As, Hg, and Pb) after digestion. The AAS values were given as µg/g, which were then converted to mg/kg in the final results. Analytical-grade reagents were utilized throughout.

RESULTS

Heavy Metals Composition in Fish Gills Samples from River Ibi, Donga and Gidin-Dorowa

Table 1 shows the concentrations of heavy metals in the gills of Clarias gariepinus from river Donga, Ibi and Gindin-Dorowa. Zn and Cd were present in the gills from the three rivers, Pb was only present in the gills of the fish sample from Gidin Dorowa at a concentration of (0.014mg/kg), which is significantly different from Ibi and Donga. Hg and As were both found to be absent in all the gills samples from the three locations. There was a significant ($p < 0.05$) difference among the Zn with the highest found in Gidin Dorowa (12.65mg/kg) followed by Ibi (9.140 mg/kg) and the least Donga (7.52 mg/kg). However, there is a significant ($p < 0.05$) difference between the Cd content of sample from river Ibi (0.165 mg/kg) when compared with that of Gidin Dorowa (0.063 mk/kg) and Donga (0.03 mg/kg).

Table 1: The Concentrations of Heavy Metals in the Gills of *Clarias gariepinus* from Donga, Ibi and Gidin Dorowa Rivers in Taraba State, Nigeria.

Heavy metals	LOCATIONS		
	Donga (mg/kg)	Ibi (mg/kg)	Gindin Dorowa (mg/kg)
Lead	$0.000^{a}\pm0.000$	$0.000^{a}\pm0.000$	$0.014^{b}\pm0.021$
Zinc	$7.750^{a}\pm0.077$	$9.140^{b}\pm0.014$	$12.65^{c}\pm0.000$
Arsenic	$0.000^{b}\pm0.000$	$0.000^{b}\pm0.000$	$0.000^{b}\pm0.000$
Cadmium	$0.037^{b}\pm0.070$	$0.165^{b}\pm0.007$	$0.063^{a}\pm0.045$
Mercury	$0.000^{b}\pm0.000$	$0.000^{b}\pm0.000$	$0.000^{b}\pm0.000$

N=3; Results are expressed as Mean± Standard deviation(SD). Means having the same letters in a column are not significantly different ($p<0.05$)

Liver Biomarkers Activities

As displayed in Table 2, the result of liver function test shows that blood sample from river Gidin-Dorowa has the highest concentration of ALP, Total bilirubin and albumin (7.12 U/L, 0.06 mg/dL and 1.90 g/dL respectively) when compared with Donga and Ibi. The highest concentration of ALT was recorded in blood sample from Ibi (1.19 U/L) when compared with Gidin-Dorowa (0.47 UL) and Donga (0.43 U/L), while Donga has the highest concentration of AST (7.60 U/L) and direct bilirubin (0.70 mg/dL), followed by Ibi (1.19 U/L and 0.20 mg/dL respectively). However, there is no significant ($p < 0.05$) difference between direct and indirect bilirubin concentrations in the blood samples from the three rivers.

Table 2: Effect of Heavy Metals on Hepatic indexes in Clarias gariepinus from Ibi, Donga and Gidin Dorowa Rivers in Taraba State, Nigeria.

Parameters/Locations	Donga	Ibi	Gidin Dorowa
ALT (U/L)	$0.43^{a}\pm0.06$	$1.19^{b}\pm1.27$	$0.47^{a}\pm0.52$
ALP (U/L)	$6.35^{b}\pm5.30$	$2.10^{a}\pm1.41$	$7.12^{b}\pm1.44$
AST (U/L)	$7.60^{b}\pm2.12$	$1.19^{b}\pm1.27$	$0.47^{a}\pm0.52$
Total bilirubin (mg/dL)	$0.02^{a}\pm0.02$	$0.05^{a}\pm0.07$	$0.06^{a}\pm0.07$
Direct bilirubin(mg/dL)	$0.70^{a}\pm0.14$	$0.20^{a}\pm0.07$	$0.19^{a}\pm0.12$
Total protein(g/dL)	$0.10^{a}\pm0.13$	$0.05^{a}\pm0.07$	$0.08^{a}\pm0.02$
Indirect bilirubin(mg/dL)	$0.05^{a}\pm0.07$	$0.05^{a}\pm0.04$	$0.01^{a}\pm0.01$
Albumin (g/dL)	$1.10^{a}\pm1.41$	$0.14^{a}\pm0.10$	$1.90^{b}\pm1.35$

N=3; Results are expressed as Mean± Standard deviation(SD). Means having the same letters in a column are not significantly different ($p<0.05$)

DISCUSSION

Given that they occupy many food chain levels, fish are effective indicators of the extent of heavy metal contamination in aquatic systems (Karadede-Akin et al., 2007). The aquatic ecology is significantly impacted by heavy metals. Table 1

illustrates the findings of a study on the concentrations of heavy metals in the gills of Clarias gariepinus from Donga, Ibi, and Gindin-Dorowa rivers. Pb was present in the gills of fish samples from Gindin-Dorowa at a concentration of (0.0145mg/kg), whereas, it was absent from Ibi and Donga at a concentration of (0.00mg/kg), which was significantly different ($p < 0.05$). The Food and Agricultural Organization (FAO) and World Health Organization (WHO) reported that the results from the three rivers were all within the acceptable levels (0.5 mg/kg, respectively) (FAO, 2005; WHO, 2005). Every organ and system in the body is susceptible to lead, which is a possible human carcinogen. According to the Center for Hazardous Substance Research (CHSR), 2009, exposure to high levels of lead can seriously harm the kidney and brain and finally result in death. Pb is a hazardous environmental pollutant that has drawn attention because of the serious threats it poses to human health (Afshan et al., 2014). Gidin-Dorowa (12.65 mk/kg)>Ibi (9.140 mk/kg)>Donga (7.52 mk/kg) but the values in Donga are lower than the permissible limits, the values in Ibi was slightly above the permissible limits, and the values in Gidin-Dorowa was above the permissible limits (8.0 mg/kg respectively), according to data from (WHO, 2005). Particularly when given orally, zinc has been reported to be generally harmless. Zinc is a necessary element, but higher concentration may be toxic to fish (Buthelezi et al., 2000). However, high level of zinc intake will result in over toxicity symptoms such as fatigue, nausea, and epigastric pain. As was not discovered in the gills of fish samples from the three separate rivers at a concentration of (0.00mg/kg), which is not statistically ($p<0.05$) different. This amount is below the permitted limits (2.0 mg/kg, respectively), according to data from FAO/WHO (2004). Both lung and other malignancies may be brought on by arsenic (NRC 2000; IARC 2004). The king of poisons and poison of kings are two well-known nicknames for it (Gupta et al., 2017). Cd was discovered in the gills of fish samples from Gidin Dorowa at concentrations of (0.0636 mg/kg), Donga (0.037 mg/kg), and Ibi (0.165 mg/kg), with no appreciable difference ($p < 0.05$) between the three. According to data from (WHO, 2011), the values in Donga and Ibi are below the acceptable limits whereas Gidin Dorowa is only slightly above it (0.05 mg/kg, respectively). Gidin Dorowa and Ibi's findings, however, are greater than the mean value of 0.065 mg/kg reported by Akaninyene et al. (2016). The presence of

Cd in contaminated water may interfere with vital bodily functions, leading to short or long-term illnesses (Jiang et al., 2015; Richter et al., 2017; Cao et al., 2018). Muscle soreness, fever, and lung damage can all be brought on by acute inhalation exposure (high amounts over a short time). The fish samples from the three rivers did not have Hg in their gills. The acceptable limit of mercury is reported by (WHO/FAO, 2004) to be 0.108 mg/kg. Mercury vapour inhalation may be lethal and has negative effects on the lungs, kidneys, neurological, digestive, and immune systems. According to Olsen et al. (1997), the skin, eyes, and digestive system are corrosive to the inorganic salts of mercury.

Gidin Dorowa had higher levels of ALP, total bilirubin, and albumin activity (7.12 U/L, 0.06 mg/dL, and 1.90 g/dL, respectively) when compared with Donga and Ibi. Blood samples from Ibi (1.19 U/L) had the highest level of ALT when compared with Gidin-Dorowa (0.47 U/L) and Donga (0.43 U/L), while Donga has the highest concentration of AST (7.60 U/L) and total protein (0.70 mg/dL), followed by Ibi (1.19 U/L and 0.20 mg/dL respectively). However, the ranges of all liver biomarkers are within the reference standards, indicating that the fishes from the three rivers are free from heavy metal toxicity

CONCLUSION

The study's findings showed that Pb was only found in the gills of the fish sample from Gidin Dorowa, Zn and Cd were present in the gills from all three rivers. The variations in all heavy metals concentrations in the fish gills and liver parameters from the three locations are below the permitted limits and the reference standard.

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