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HEAVY METAL BIOACCUMULATION IN LIVER AND BLOOD TISSUES OF *Clarias gariepinus* (AFRICAN SHARP TOOTH CATFISH), FOUND IN RIVER BENUE, BENUE STATE, NIGERIA

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***Abstract*: Heavy metal refers to any such metallic chemical element that has a relatively high density and is toxic or deleterious at low concentrations. Heavy metal concentration in liver and blood tissues form *Clarias gariepinus* from selected sites along River Benue was assayed for using atomic absorption spectrophotometer (AAS Buck Scientific 205). This research is geared towards ascertaining the level of heavy metals in the liver and blood tissues of the cat fish from River Benue, considering the fact that they are a widely consumed fish species. Concentration of the metals in the Liver are in the following decreasing order Zn> Mg > Fe > Cd > Mn > Cr > Pb. Concentration of the metals in the Blood are in the following decreasing order Fe> Zn > Mg > Mn > Cd > Pb > Cr. Also, evidences from the photomicrograph of the liver tissues showed hydroscopic swellings and slight morphological changes to the structure of the liver. In conclusion, findings from this research showed that the liver and blood of the sample fishes (*Clarias gariepinus*) bioaccumulated considerable concentrations of heavy metals. Despite the bioaccumulation of metals in the liver, there were no visible lesions on the liver cells except for the morphological changes in some and hydroscopic swellings in some of the liver tissues.**

***Keywords*: Heavy Metal; Bioaccumulation; River Benue; *Clarias gariepinus*.**

I. INTRODUCTION

Heavy metal refers to any such metallic chemical element that has a relatively high density and is toxic or deleterious at low concentrations [13]. Examples of heavy metals include mercury (Hg), cadmium (Cd), zinc (Zn), arsenic (As), chromium (Cr), thallium (Tl), lead (Pb), silver (Ag), selenium (Se) and iron (Fe).

Heavy metals are natural components of the earth's crust. They cannot be degraded or destroyed. As trace elements, some heavy metals (e.g. copper, selenium, zinc) are vital to maintain the metabolism in the human body. Heavy metals are hazardous due to their tendency to bioaccumulate. Bioaccumulation refers to an increase in the concentration of a chemical in a biological organism over time, compared to the chemicals concentration in the environment [9]. These compounds (heavy metals) accumulate in living things any time they are ingested and are stored faster than they are metabolized or excreted.

Human mediated industrial activities have increased in recent times, leading to the introduction of various potentially hazardous inorganic compounds (e.g. heavy metals) into the environment [18]. Our water bodies (streams, rivers and oceans) receives a large influx of industrial waste emanating from mining, agricultural activities, manufacturing, metal fishing plants, domestic waste water and atmospheric precipitation [19]. Increasing environmental contamination by hazardous substances is of growing concern in Nigeria and the world at large [6].

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Clarias gariepinus of the family *claridae* is the most common Nigerian fresh water fish species and is prominent in aquaculture practice. They are easy to grow and have large economic gains because of their air-breathing and hardy nature, suitable reproductive strategy, nutritional efficiency and ability to gain size within a short space of time [7]. This study is aimed at determining the level of heavy metal bioaccumulation in the blood and liver tissue of *Clarias gariepinus* from River Benue and also investigating the effects of this bioaccumulation on the liver through a histopathology analysis.

II. MATERIALS AND METHODS

Materials, reagents and equipment's used include the following African sharp tooth catfish (Clarias gariepinus), Non EDTA bottles, Heparinised 2ml syringes, 22G needles, Analytic grade Nitric acid (HNO_3), Analytic grade Perchloric acid, Distilled water, Ethanol, 10% Formol-saline (fixative), Xylene, Molten paraffin wax, Haematoxylin and Eosin dyes, Atomic absorption spectrophotometer (Buck scientific 205), Complex light microscope (Leica DM 750).

Twenty (20) Test Fish samples were bought from fishermen at landing sites along the banks of River Benue. Five (5) control fish samples were bought from commercial fresh water pond at North Bank area of Makurdi Town. Fish samples were identified as *Clarias gariepinus* by the department of Fisheries of the University of Agriculture, Makurdi.

A. Study design

Twenty (20) fishes (*Clarias gariepinus*) were collected with the aid of local fishermen, across four sites along the River Benue (5 per site) within Makurdi metropolis as test samples, for the purpose of this research. The sites along the river for sample collection include:

- **Site 1**: Area around where effluent from Nigeria Breweries enters the river
- **Site 2**: Area around the new bridge where the abattoir is located, and there is also presence of a rice mill which burns old motor tires to make fire for processing.
- **Site 3**: Along the old bridge, close to where the Benue state water treatment plant is located.
- **Site 4**: Behind Wadata market; at this site is a very large dump site, where all the waste from the market is disposed and there is usually indiscriminate burning of these refuse.

Five (5) fishes (*Clarias gariepinus*) were bought from a commercial fresh water pond at North Bank area in Makurdi metropolis and are referred to as **Site 5** samples. These fishes were used as control samples for this research.

Blood sample was collected from the central vein of the fish, located just below the spinal cord, caudal to the cloaca and using the lateral line as reference point. The blood was collected with heparinised 2ml syringes and 22G needles into the non EDTA container. The blood sample was stored in a deep freezer for further analysis.

B. Determination of heavy metal concentration liver and blood tissues of the fish

Preparation of liver samples for heavy metal analysis was as described by POPs kits, (2012). Each fish was dissected using stainless steel instruments. The liver was taken out and composite sample of 2-5 g was used for subsequent analysis.

The sample was digested with ultra-pure nitric acid and Perchloric acid at 95^{o}c until the solution became clear. The solution was made up to known volume with deionized distilled water and analysed for Cu, Zn, Pb, Cd, Fe and Mn using the Atomic Absorption Spectrophotometer (Buck Scientific 205) as described by Ang and Lee (2005).

The non-haemolysed plasma stored in the deep freezer was also digested with ultra-pure nitric acid and Perchloric acid at 95^{o}c until the solution became clear. The solution was made up to known volume with deionized distilled water. The digested blood was then analysed for heavy metals using an Atomic Absorption Spectrophotometer (AAS Buck Scientific 205) as described by Ang and Lee (2005).

C. Determination of Histopathology of the Liver tissues

Clarias gariepinus liver tissues were prepared for histopathology analysis as described by Winsor, 1994. The sample fish was dissected, and the liver removed and preserved with a fixative (10% Formol saline). The fixative helps to keep the liver tissue in a near life like form, by preventing the activity of microbes and enzyme action, therefore stopping decay. The fixative forms cross linkages with the amino acids in the tissue, thereby preventing the degeneration of the tissue. Parts of the liver tissue was then cut off, dehydrated, cut into thin sections by a microtome and stained with Haematoxylin and Eosin dye, before being viewed under the microscope.

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D. Statistical Analysis

Results were analysed using the SPSS software (Version 18.SPSS). Results were reported as mean ± standard deviation. Duncan multiple test was used for analysis to test for significant difference at $p<0.05$

III. RESULTS

Table 1: Concentration in (PPM) of heavy metals in blood samples of *Clarias gariepinus* fish species from selected sites along River Benue

Sample	Concentration (PPM)						
	Pb	Zn	Cd	Cr	Mn	Fe	Mg
Site 1	0.12 ± 0.02^{c}	8.03 ± 0.61^{c}	0.31 ± 0.19^{ab}	0.05 ± 0.01^{b}	0.05 ± 0.02^{a}	1141.60 ± 35.15^{a}	1.15 ± 0.05^{c}
Site 2	0.03 ± 0.01^{ab}	8.53 ± 0.23^{c}	0.67 ± 0.17^{b}	0.01 ± 0.01^{a}	0.10 ± 0.01^{a}	1042.06 ± 50.34	1.01 ± 0.03^{bc}
Site 3	0.07 ± 0.02^{bc}	5.40 ± 0.29^{a}	0.36 ± 0.09^{ab}	0.04 ± 0.01^{ab}	0.03 ± 0.02^{a}	1174.90 ± 87.20^{a}	0.92 ± 0.03^{b}
Site 4	0.07 ± 0.02^{bc}	6.72 ± 0.15^{b}	0.37 ± 0.17^{ab}	0.03 ± 0.01^{ab}	0.43 ± 0.25^{ab}	1106.40 ± 30.85^{a}	0.86 ± 0.03^{b}
Site 5	0.01 ± 0.00^{a}	5.12 ± 0.27^{a}	0.02 ± 0.01^{a}	0.01 ± 0.01^{a}	0.61 ± 0.24^{b}	1096.00 ± 39.54^{a}	0.64 ± 0.14^{a}

Values are expressed as Mean ± SME; n=5, values with different alphabet as superscripts are considered significant at $p<0.05$ down the column

In Table 1, metal bioaccumulation in the blood of Clarias gariepinus fishes from River Benue was found to be in the following ascending order Pb< Cr< Cd< Mn< Mg< Zn< Fe. Pb levels in blood of fishes from all the sites (1-4), showed a significant increase at $p<0.05$ when compared to the control. There was no significant difference in Pb levels in blood of samples from site 3 and those of site 4. Zn levels in all the sites except from site 3 showed significant increase when compared with the control (site 5). Zn levels in site 1 showed no significant difference when compared to Zn levels in site 2. Cd in test groups showed a significant increase in comparison to the control. Cd levels at site 1, 3 and 4 are not significantly different at $p<0.05$. Cr showed a significant increase when all test sites where compared to the control (site 5) except for site 2. Also site 3 and 4 are not statistically significant at $p<0.05$. Mn levels in the blood showed a significant decrease when all groups where compared with the control (site 5). Mn levels in site 1, 2 and 3 are not statistically significant at $p<0.05$. Fe levels where the highest in the blood of fishes used for this work. There was no statistical difference between the control and test samples at $p<0.05$ as well as between test groups (site 1-4). Mg showed a significant increase between samples from all test sites when compare to the control. Samples from site 3 and site 4 where not statistically significant at $p<0.05$

Table 2: Concentration in (PPM) of heavy metals in liver tissues of *Clarias gariepinus* fish species from selected sites along the River Benue

Sample	Concentration (PPM)						
	Pb	Zn	Cd	Cr	Mn	Fe	Mg
Site 1	0.39 ± 0.03^{b}	$7.38\pm0.1.15^{a}$	1.75 ± 0.11^{b}	0.78 ± 0.01^{b}	2.57 ± 0.57^{a}	6.78 ± 1.73^{a}	10.98 ± 0.57^{b}
Site 2	0.84 ± 0.01^{c}	15.13 ± 0.07^{c}	3.20 ± 0.57^{b}	0.99 ± 0.57^{b}	1.93 ± 0.01^{a}	8.42 ± 1.15^{b}	12.50 ± 1.15^{bc}
Site 3	0.47 ± 0.05^{b}	12.24 ± 0.05^{b}	2.39 ± 1.15^{b}	0.78 ± 0.11^{b}	2.24 ± 1.14^{a}	7.28 ± 1.76^{b}	10.53 ± 0.11^{b}
Site 4	0.79 ± 0.11^{c}	15.11 ± 0.02^{c}	2.75 ± 1.15^{b}	0.62 ± 0.12^{b}	1.63 ± 0.17^{a}	8.47 ± 0.57^{b}	14.41 ± 1.15^{c}
Site 5	0.08 ± 0.04^{a}	7.91 ± 0.58^{a}	0.22 ± 0.06^{a}	0.03 ± 0.01^{a}	1.01 ± 0.01^{a}	6.24 ± 0.02^{a}	9.14 ± 1.15^{a}

Values are expressed as Mean ± SME; n=5, values with different alphabet as superscripts are considered significant at $p<0.05$ down the column

In table 2, Pb showed a significant increase when samples from test sites where compared with the control at $p<0.05$. There was no significant difference in Pb levels of site 1 compared to that of site 3, and that of site 2 compared to site 4. Zinc is the metal with the highest level of accumulation in the liver with a value of 15.11±0.02 PPM in site 4. Zn showed a significant increase in test samples when compared to that of control except in site 1. There was also no significant difference in Zn levels of site 2 compared to that of site 4. Cd levels are significantly increased when test samples from (site 1-4) where compared to the control, but there was no significant difference in Cd levels between the test groups at

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$p<0.05$. Cr levels are significantly increased when test samples from (site 1-4) where compared to the control, but there was no significant difference in Cr levels between the test groups at $p<0.05$. Mn showed no significant difference between test samples and control. Also, there was no significant difference at $p<0.05$ between all test groups (site 1-4). Fe levels showed a significant increase when control was compared to samples from all test sites except for those from site 1. There was also no significant difference between site 2, 3 and 4. Mg showed a statistically significant increase when control was compared to other test sites at $p<0.05$. Samples from site 1 and 3 showed no significant difference between them.

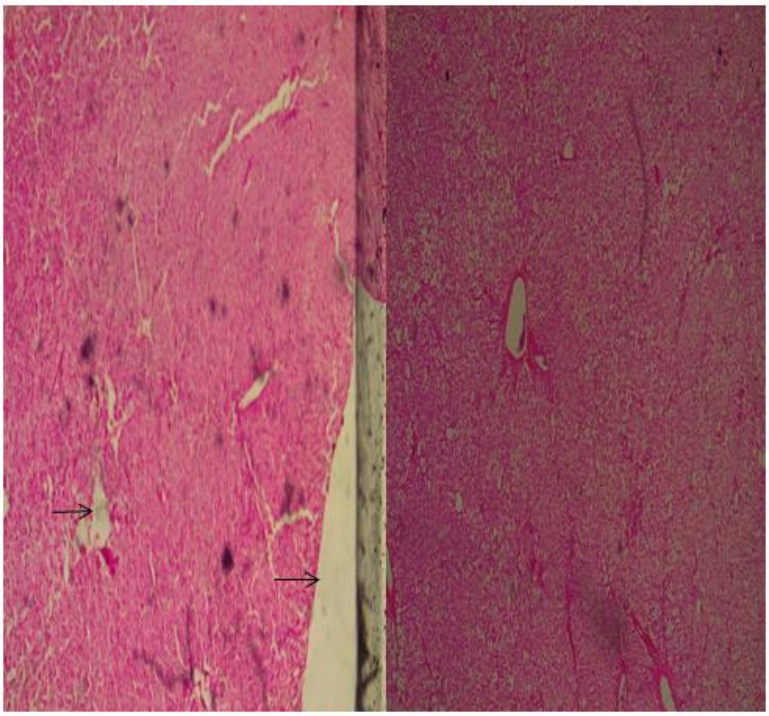

Fig 1: Micrograph of liver tissue of *Clarias gariepinus* from site 1 compared with that from site 5 (control). Magnification= (x40). There was a slight folding and hydroscopic swelling in the liver tissue from site 1.

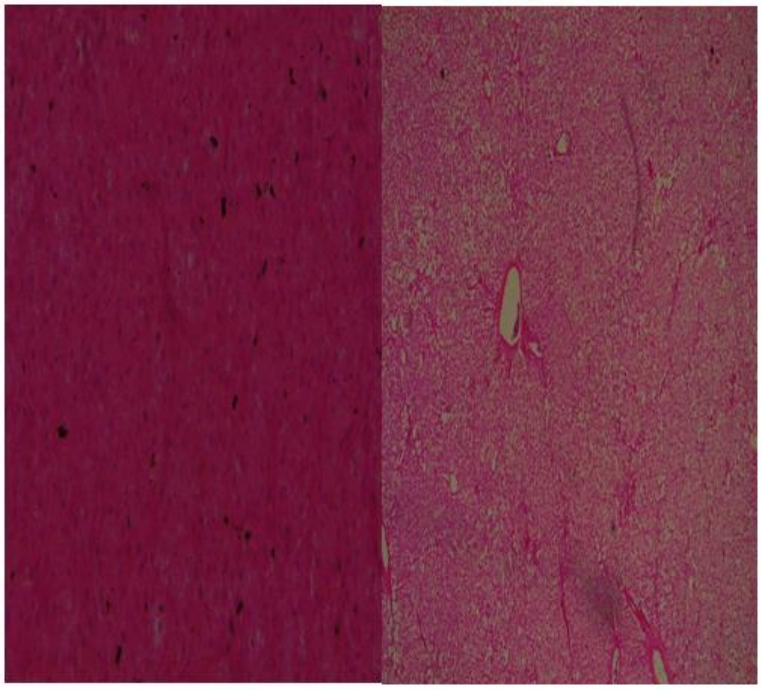

Fig 2: Micrograph of liver tissue of *Clarias gariepinus* from site 2 compared with that from site 5 (control). Magnification= (x40). Liver tissue from site 2 showed no swelling or lesions as is the case in the control (site 5).

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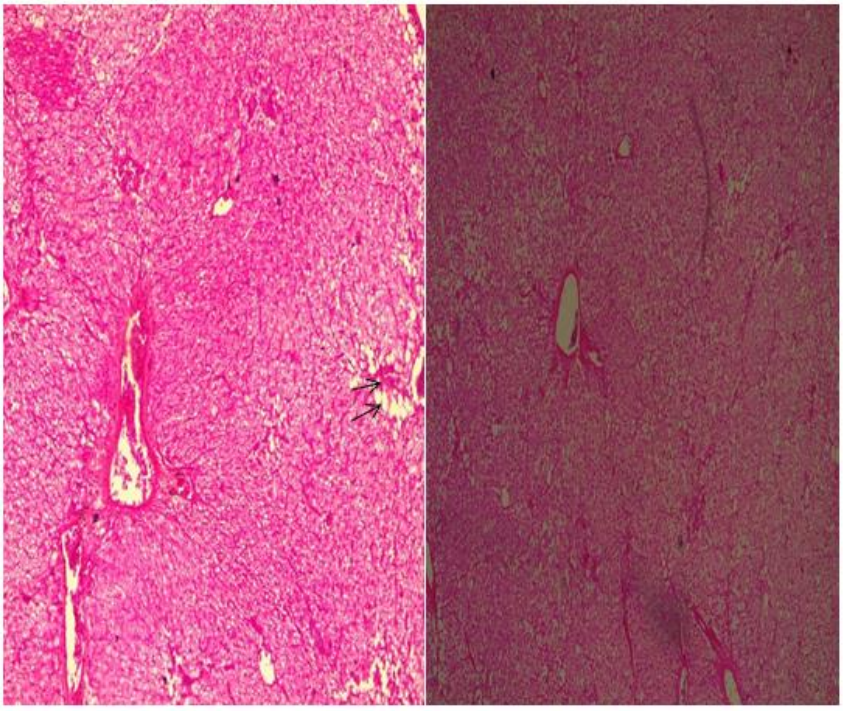

Fig 3: Micrograph of liver tissue from *Clarias gariepinus* from site 3 compared with that from site 5 (control). Magnification= (x40). There is a very mild diffuse vacuolation of hepatocytes in liver tissues from site 3

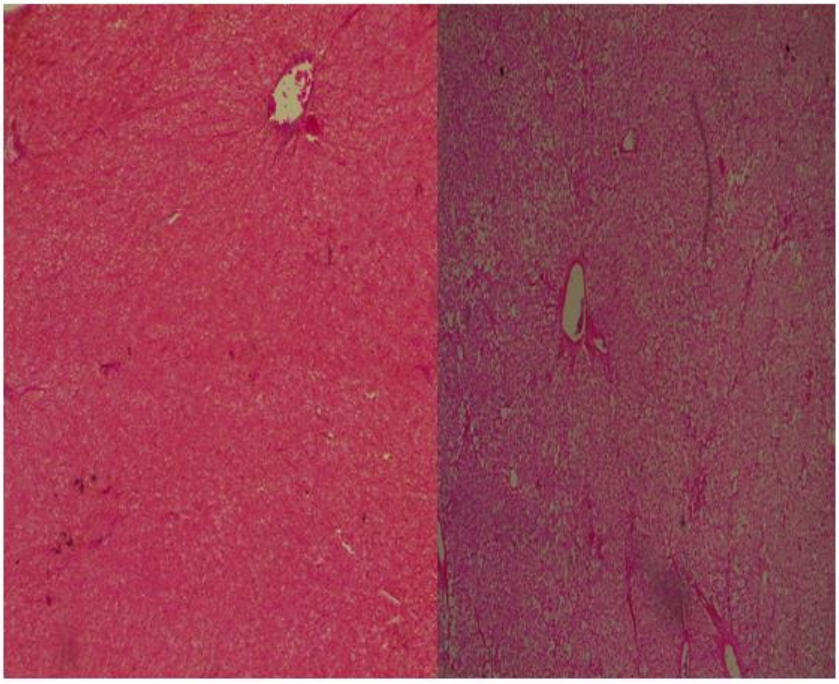

Fig 4: Micrograph of liver tissue from *Clarias gariepinus* from site 4 compared with that from site 5 (control). Magnification= (x40). Liver tissue from site 2 showed no swelling or lesions as is the case in the control (site 5).

Images of histopathology slides of liver tissues, as shown in figure 1 to figure 4 shows liver with normal parenchymal cells and normal distribution of hepatocytes in all cases. Presence of black pigments in figure 2 and 3 was as a result of the reaction of formalin used for preservation of the liver, with some of the hepatocytes. In figure 3 and 4 the hepatic portal vein can be clearly seen, which is responsible for carrying blood away from the liver. No abnormal growth or lesions was found on the liver from the fishes used for this research.

IV. DISCUSSION

Fish for this present study (Clarias gariepinus) showed high levels of accumulation of certain essential metals such as Fe and Zn in its blood, while Zn, Mg and Fe where highly accumulated in the liver. Mn, Cd and Pb accumulated more in the liver than in the blood. This agrees with the work of El-moselhy, et al; 2014, on Bioaccumulation of heavy metals in some tissues of fish in the Red Sea, Egypt.

In this study metals with highest concentrations in the liver are Mg and Fe respectively. These metals are regarded as essential, and the accumulation of essential metals in the liver are likely linked to its role in metabolism [21]; elevated levels of Zn and Mg in hepatic tissues are usually related to a natural binding protein like metallothioneins (MT) [8], which serves as a vital metal store (i.e. Zn and Mg) to fulfil enzymatic and other metabolic demands [2]. The high accumulation of Fe in the blood is likely related to the fact that Fe is a central metal in haemoglobin, an essential constituent of the blood.

In this present study, the concentration of the selected heavy metals (Pb, Zn, Cd, Cr, Mn, Fe and Mg) where significantly higher in the blood of *Clarias gariepinus* from River Benue than that from the fresh water pond, with the exception of Fe in site 2 (10.4206 ± 1.127) which is less than Fe in site 5 (10.9600 ± 0.884) and also Mn in all the sites from the river being less than that of the control from fresh water pond. In both blood and liver of *Clarias gariepinus* analysed for heavy metals, Pb and Cr where metals with the lowest concentrations.

The high levels of Mn in control samples could be attributed to the presence of this essential metal in the feed administered to these fishes in the fresh water pond were they are grown.

The rate of bioaccumulation of metals in organisms depends on the ability of the organs to digest the metals and the concentration of such metals in the river. It also has to do with the concentration of the heavy metal in the surrounding soil as well as the feeding habits of the fish species. The tendency for Fe to accumulate in the liver is due to the physiological role of the liver in blood cells and haemoglobin synthesis [8].

On the other hand, the liver of the test fishes showed considerably high levels of Cd, this agrees with the work of El-Moselhy *et al*., 2014, on Bioaccumulation of heavy metals in some tissues of fish in the Red sea, Egypt; this finding could be explained by the ability of Cd to displace the normally MT-associated essential metals in hepatic tissues [2].

Values of bioaccumulation of non-essential metals (Pb, Cr and Cd) where highest in site 2 (i.e. area around the new bridge at Wurukum), this is not unconnected to the level of human and small scale industrial activities within the area that could possibly generate heavy metal waste discharge that flows into the surrounding water.

There is a lot of irrigational farming going on around this area, and lots of herbicides, pesticides and fertilizers are used on these farms. Run offs and leaching, could lead to these heavy metals containing chemicals, getting into the surrounding water body and the hence the fishes in this area of the river are exposed to increased concentration of these toxicants [1].

Also, there is an abattoir and a rice mill along the river bank. Motor tires and plastic materials are used to make fire by the rice millers and butchers alike, and this generates a lot of heavy metal pollution that is dumped into the surrounding water body and consequently the aquatic life therein.

Macroscopic description of the liver showed all specimens to have a similar gross appearance and measured between 2.8*2.7*2cm on the average. They all were tan to brown and cut sections of the liver also had a tan to brown colour.

Although it has been reported that changes in liver enzymes activities in the serum shows major pathologic changes or liver damage [5], photomicrographs of liver cells of *Clarias gariepinus* used for this research when viewed under the microscope as shown in fig 1-4, showed sections of all specimens to be similar. There where hydroscopic swellings within the liver tissues of the test samples, although this could be reversible. The cells were observed to have a preserved parenchymal architecture. There were no lesions in the liver but the liver tissues from the test sites showed certain morphological changes in their structures.

V. CONCLUSION AND RECOMMENDATION

In this work it was noticed that concentration of Heavy metals was higher in fishes from the river than in the control fishes from fresh water pond. There was also a higher bioaccumulation of essential metals such as Fe, Zn and Mg in both blood and liver tissues of both test and control fish samples. Evidence from the microscopy evaluation of the liver tissue

showed little or no visible damage to the liver. Given its wide distribution, the African Sharptooth catfish is a suitable bio-indicator of pollution in a variety of freshwater aquatic ecosystems. More research should be carried out on *Clarias gariepinus* fish species from the River Benue to ascertain if any damage will be done on the liver over a prolonged exposure to heavy metal contamination. Regular monitoring of heavy metal pollution on the river Benue should be encouraged.

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