

ORIGINAL RESEARCH ARTICLE

Histopathological and Oxidative Stress Responses of the African Catfish *Clarias gariepinus* in Heavy Metal Contaminated Water from the Hadejia-Nguru Wetlands of North Eastern Nigeria.

Musa Mohammed Ibrahim^{1*}  and Imam Tijjani Sabiu² ¹Department of Fisheries and Aquaculture, Federal University Dutse, Jigawa State²Department of Biological Sciences Bayero University Kano, Kano State.**ABSTRACT**

Hadejia-Nguru Wetland is a source of drinking water, agriculture, natural fertilization of fields, fishing and transportation. Effluents from agricultural activities, sewage, and chemical use enter this water body. This study investigated the levels of several heavy metals (Hg, Pb, Cd, Cr, and Al) in *Clarias gariepinus* tissues (gills, liver, muscle) collected from five sampling sites labeled A–E. Histopathological examination and the presence of antioxidant enzymes revealed the degree of tissue damage and stress in fish. The results for heavy metals show concentrations in the order Pb > Cr > Hg > Al > Cd, which are higher than the maximum residue limits recommended by FAO and WHO. Superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and reduced glutathione activities were observed at high concentrations in gills, liver, and muscle in comparison to the control fish. The highest SOD concentration was detected in the liver, with an average concentration of $32.43 \pm 2.05 \text{Ug}^{-1}$ tissue, followed by a concentration of $12.35 \pm 2.10 \text{Units g}^{-1}$ in the gills, and CAT concentration with a highest concentration of $14.92 \pm 2.81 \text{Ug}^{-1}$ in the liver. MDA was highest in gills at a concentration of $9.06 \pm 0.01 \mu\text{min}^{-1}\text{g}^{-1}$, and there was no significant difference between MDA concentrations in liver and other organs ($P < 0.05$). GSH levels were highest in gills at a concentration of $1016.64 \pm 0.54 \mu\text{min}^{-1}\text{g}^{-1}$ and there was a significant difference ($P < 0.05$) in GSH concentrations in gills compared with other organs. Histopathology showed different detrimental effects in gill filaments, hepatocytes, and Bowman's spaces in liver and muscle cells, respectively. The presence of metal toxicity, antioxidant enzymes, and tissue damage in fish is an indication of contamination and serves as a bio surveillance model for the safety of freshwater organisms.

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INTRODUCTION

The Hadejia-Nguru wetland is a vast land of Agricultural activities and fisheries known for its richness which allows for intensive farming and fishing activities, it covers a massive expanse of land covering an area of 3,500Km². This water receives varying level of waste which are discharges of agricultural, municipal, residential or industrial waste products and when contamination in water occurs in high concentration it can be a serious threat because of their toxicity, long persistence and bioaccumulation in fish. Heavy metals are commonly found in natural

waters and some are essential to living organisms as these metals gains entrance contamination and accumulation sets in. The continuous accumulation of pollutants affects the aquatic organisms (Maruf et al., 2021) Rate of bioaccumulation of heavy metals in aquatic organisms depends on their ability to digest and absorb the metals from their environment. Essential metals of the likes of Copper (Cu), Iron (Fe), Manganese (Mn), Nickel (Ni) and Zinc (Zn) are basic requirement for biochemical and physiological functions in the body of fish. The non-essential heavy

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metals in the likes of Mercury (Hg), Chromium (Cr), Lead (Pb), Cadmium (Cd) have no particular biological role in fish (Saleh et al., 2016) and classified among the metals of public health significance, they accumulate in the food chain and become carcinogenic with other adverse risks to health of humans as a result of bioaccumulation over time. Monitoring of contamination levels is necessary as it provides important information about contamination levels (Maurya et al., 2018). *Clarias gariepinus* is a tropical freshwater fish with great economic importance in Nigeria, its hardy nature and ability to survive under harsh environmental conditions has made it amongst the most successful fresh water fish in Nigeria. It can as well be used as a biomarker in assessing the quality of aquatic environments (Farombi et al., 2007) it is a known fact that heavy metals cannot be destroyed by biodegradation because they accumulate in the aquatic environment into harmful toxins that can adversely affect humans. When heavy metals accumulate in fish tissue, they catalyze reactions that lead to the production of reactive oxygen species (ROS). Oxidative stress biomarkers commonly used in monitoring aquatic ecosystems include superoxide dismutase (SOD), catalase, reduced glutathione, and the lipid peroxidation biomarker malondialdehyde (Gharred et al., 2015, Ochuwa et al., 2017). Oxidative stress causes cell damage, often associated with tissue damage and structural damage to organs. Histopathological examination provides useful information about changes in tissue architecture that occur due to external influences. The purpose of this work is to assess the effects of heavy metals on inducing body changes in fish. Metals such as mercury, lead, cadmium, chromium and aluminum are known to be harmful to health even at low concentrations. Heavy metal accumulation leads to histopathological changes in fish tissue (Ochuwa, 2017).

MATERIALS AND METHODS

Ethical Approval

The research methodology and its ethics have been fully reviewed and approved in the 2020/2021 academic session by the Department of Biological Science, Bayero University Kano, Post-Graduate Committee. The study also followed the World Medical Association Principles on the treatment of animals used in research (<https://www.wma.net/policies-post/wma-statement-on-animal-use-in-biomedical-research/>)

Study Area

Hadejia-Nguru Wetland (HNW) lies between latitudes 12°10'N and 13°N, and longitudes 10°15'E and 11°30'E. HNW is located in the semi-arid region of Nigeria. The area of wetlands is about 3,500 km². The terrain of the area is predominantly low-lying on the northeastern side, with limited local relief in the south and west. Precipitation patterns at the Nguru-Hadejia Wetlands (NHW) have not been stable over the years, but for the most part starts in June and last through September. The vegetation consists mainly of Sudanese savannah, including transitional savannah from northern Guinea and Sahelian savannah on the southern-northern border, respectively, Abubakar et al. (2015).

Site A is Hadejia barrage dam (Kalgwai)

Site B: Kirikasanma

Site C: Maikintari

Site D: Nguru lake

Site E: Dagona

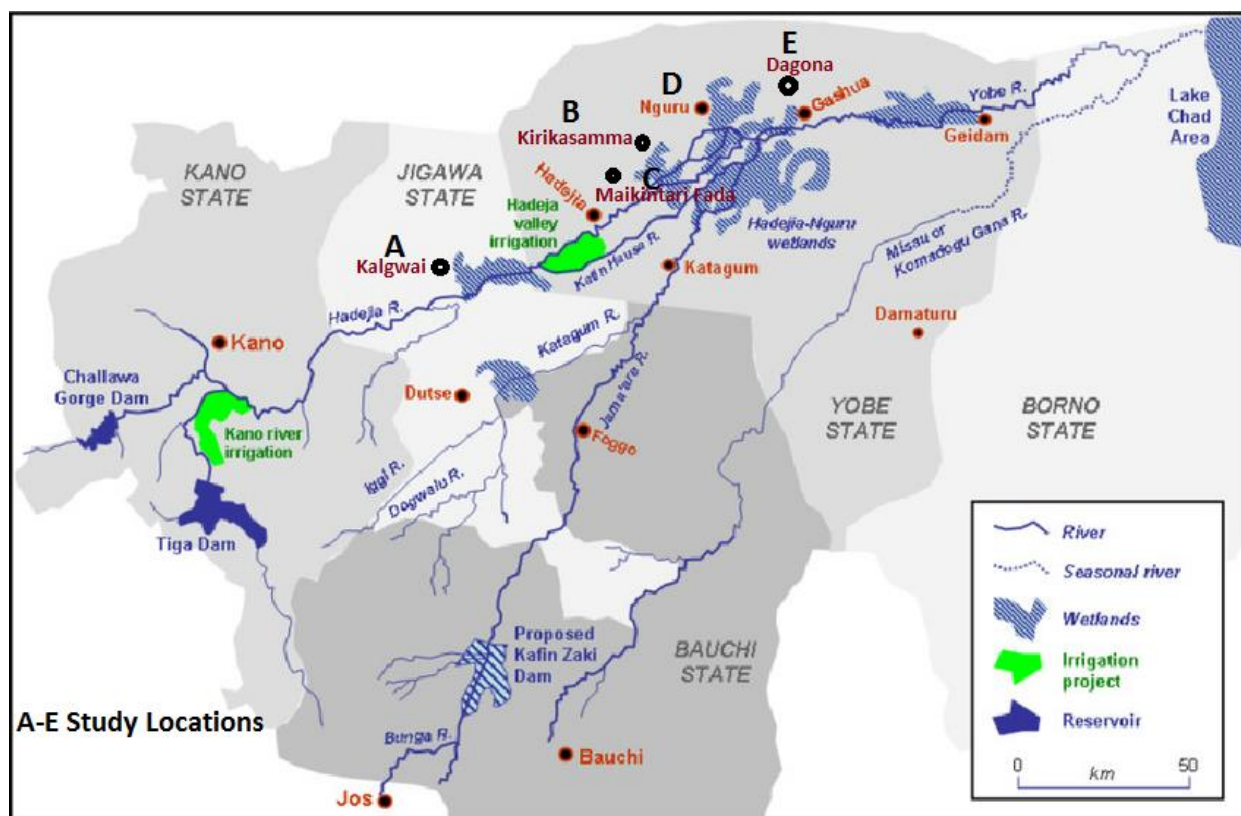


Figure 1: Map of Hadejia-Nguru wetland showing sampling sites (Source: Uploaded by Sulaiman, I. M., 2014)

Fish sampling and analysis:

Fish samples (*Clarias garrepinus*) were captured from five sampling sites through the services of hired fishermen and collected bimonthly for a period of six months. A total of 140 fish were used in the experiment (Twenty-eight fish per site). Fish samples were taken in the early morning at 06:00. They were then transported in ice-cold containers to the Laboratory at the Federal University Dutse for dissection and analysis. Control fish were obtained from Rumbun Kifi Fish Farm, Modobbi Road, Dutse, Jigawa. Fish were dissected to remove gills, liver and muscle and stored in a -40 °C refrigerator for further experiments.

Heavy metal analysis

Analysis was performed according to the wet method used in Tyokumbur (2016). Dissected gills, livers, and muscles were removed, oven-dried at a temperature of 105 °C to constant weight, and the dried samples were pulverized in a porcelain mortar before crushing. To digest the samples, powdered muscle, gills, and liver were homogenized and powdered samples of concentrated nitric acid and hydrogen peroxide (1: 1) v/v were placed in 250 mL round-bottom flasks and 10 mL each. of HNO₃ (65%) and H₂O₂ (30%) were

added to react the contents of the flask. The contents of the flask are heated in a hood on a heating mantle to a temperature of 130°C to reduce the volume to 3-4 mL, the digested sample is allowed to cool, filtered into an Erlenmeyer flask, and the filtered sample is added then transferred to a 50ml volumetric flask. The concentrations of Cd, Al, Cr, Pb, and Cd were determined using an atomic absorption spectrophotometer (Buck Scientific Model 230) at the Department of Soil Science, Ahmadu Bello University, Zaria.

Measurement of Markers of Oxidative Stress

Liver samples were analyzed for markers of oxidative stress: superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and lipid peroxidation (malondialdehyde MDA). These were determined according to the analytical method described by Achuba et al., (2014). Preparation of extracts for determination of lipid peroxidation (MDA) 0.5 g was isolated from excised gills, liver and muscle, ice-cold 0.05 M phosphate buffer pH 7.0 1% (w/v) 10 homogenized with ml. Triton X-100, excess butylated hydroxytoluene (BHT) and some crystals of protease inhibitors, phenylmethylsulfonyl fluoride using an ice-immersed MSE blender. Triton X-100 lyses membrane-bound.

Preparation and Extracts for determination of Lipid Peroxidation (MDA)

Of the isolated gills, liver and muscles, 0.5g were separated and homogenized with 10ml of ice-cold 0.05M phosphate buffer pH 7.0 containing 1% (w/v) Triton X-100, excess butylated hydroxyl toluene (BHT) and a few crystals of protease inhibitor, phenylmethylsulfonyl fluoride using an MSE blender immersed in ice. Triton X-100 solubilizes membrane-enclosed organelles while BHT prevents in vitro oxidation of lipid during homogenization. The extract was centrifuged at 7000g for 20 min (40°C). The supernatant (S1) was used for the determination of lipid peroxidation by the method of Hunter et al., (1963) as modified by Gutteridge and Wilkins (1982).

Catalase Extraction and Assay

(CAT) Catalase activity was determined according to Beers and Sizer (1952) by measuring the decrease in H₂O₂ concentration at 240 nm absorbance. An extinction coefficient of 40 M⁻¹ cm⁻¹ for H₂O₂ (Abel, 1974) was used for calculations.

Extraction and Assay of Superoxide Dismutase (SOD)

The resulting supernatant was used to assay superoxide dismutase (SOD) activity based on its ability to inhibit the oxidation of epinephrine by superoxide anions (Aksnes and Njaa, 1981) Enzyme activity was analyzed with an SP 1800 UV/VIS spectrophotometer.

Glutathione (GSH)

Glutathione (GSH) was determined by adopting the method described by Sedlak and Lindsay, 1968. Where the tissue sample was prepared by washing with PBS twice, 0.1g of the sample was added into homogenizer, 1mL reagent was added (the proportion of tissue and reagents are kept constant) and this was fully grinded on ice (using liquid nitrogen gave a better grinding effect) centrifuge was done at 8000x g for 10minutes at 4°C. Spectrophotometer was then preheated for 30minutes and adjustment was made to a wavelength of 412nm with distilled water before the values for GSH was measured.

Histopathological Examination

At the point of fish dissection, the gills, liver and muscle of the exposed and control fish were removed and stored in Bouin's fluid prior to examination. They were later dehydrated in ascending alcohols, and treated with toluene and infiltrated with molten paraffin wax. Microtome sections were stained with the

hematoxylin and eosin staining technique, examined with a Leica DM 750 microscope and photographed with a Leica ICC 50HD camera (Roberts, 2001; Auwioro, 2010)

Statistical Analysis

The generated data were analyzed using the Social Science Statistical Package (SPSS) version 25. All results are expressed as mean \pm standard deviation and data were analyzed using analysis of variance (ANOVA). Significant differences between contaminated sites and controls were determined at a 5% confidence level ($P < 0.05$) using Duncan's multiple test range

RESULT

Heavy Metals

The results for heavy metal concentrations in the gills of *Clarias gariepinus* are shown in Figure 3. The results show that the Al concentration was highest in the fish gill at site A with a concentration of 0.89 ± 0.16 mg/Kg with no significant difference ($P < 0.05$) with the gills concentration of 0.12mg/Kg in the control fish Figure 1. The concentration of cadmium (Cd) was 2.25 ± 0.33 mg/Kg at sites B and A and the average concentration of chromium (Cr) was the highest at site E at 75.75 ± 12.03 mg/kg, followed by site B at 59.25 ± 12.36 mg/Kg, values for Cr showed significant difference ($p < 0.05$) in comparison to the control fish. The highest concentration of lead (Pb) of 50.75 ± 8.90 mg/Kg was observed at Site E and all other values for Pb in the various organs of *C. gariepinus* were significantly different ($P < 0.05$) from the control fish. Concentration of mercury recorded in the gills was 120.75 ± 17.50 mg/kg in site E, indicating the highest overall value for heavy metal in the gills of the fish. Figure 4 showed the average concentration of heavy metals in fish liver. Aluminum was not detected at site A, but was highest at site B with a concentration value of 0.8 ± 0.16 mg/Kg, and Cd was highest at site B with a concentration of 2.25 ± 0.33 mg/Kg. Cr in liver had the highest mean concentration at site A. Concentration values of 81.25 ± 12.36 mg/Kg followed by 30.25 ± 12.03 mg/Kg at Site E. Sites B, C, and D had values of 18.50 ± 4.01 mg/Kg, 30.00 ± 6.09 mg/Kg, and 10.50 ± 2.44 mg/Kg, respectively, there was significant difference ($P < 0.05$) between heavy metal concentration in the liver to the control fish. Fish in Site D had the highest average concentration value of 20.05 ± 3.85 mg/Kg for Pb concentration, followed by

Site E with 18.95 ± 1.08 mg/Kg. Mercury in the liver had a value of 105.55 ± 17.50 mg/Kg at Site A, followed by a value of 100.05 ± 7.92 mg/Kg at Site D. Site B values were 35.50 ± 5.62 mg/Kg, site C was 86.50 ± 13.46 mg/kg, and site E was 20.01 ± 5.48 mg/Kg. Figure 5 showed the average concentration of heavy metals in muscle of fish. The highest Al concentration was detected at site A with a value of 1.20 ± 0.21 mg/Kg and no Al was detected at sample site B. Cd concentrations ranged from 0.25 ± 0.15

mg/Kg at sites C and D to 1.50 ± 0.26 mg/kg at site A. The highest Cr value of 94.25 ± 15.58 mg/Kg was observed at Site A. The Pb concentration was lowest at Site B with a value of 0.45 ± 1.38 mg/Kg and highest at Site E with a value of 25.75 ± 4.55 mg/Kg. The highest Hg value was measured at site D with a value of 98.05 ± 7.92 mg/Kg, followed by sites C and E with the same concentration of 95.15 mg/Kg, site A with a concentration value of $55.75 \pm 4.38 \pm 10.54$ mg/Kg and site B with a value of 80.30 ± 12.68 mg/Kg.

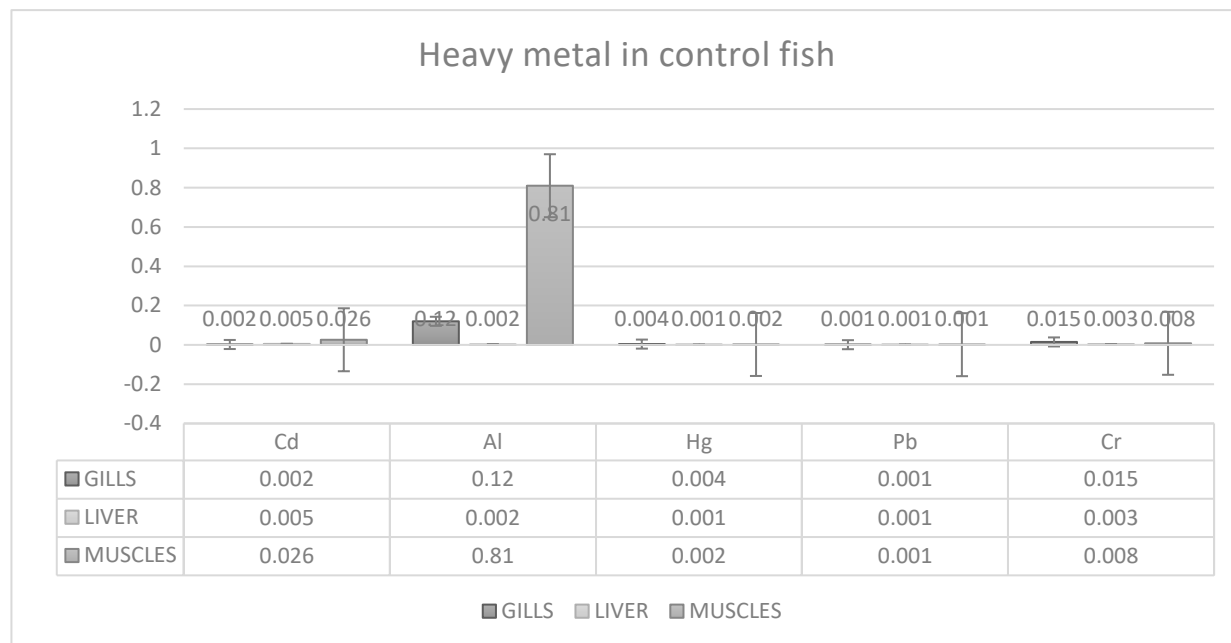


Figure 2: Control fish showing heavy metal concentration in gills, liver and muscle

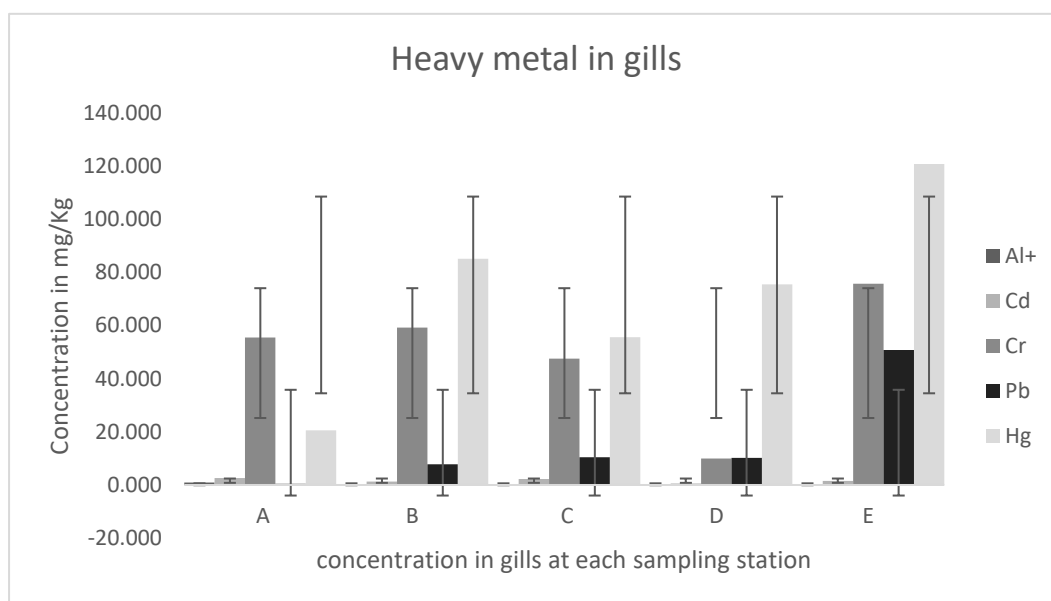


Figure 3: Mean concentration of heavy metal in fish gills in sampling

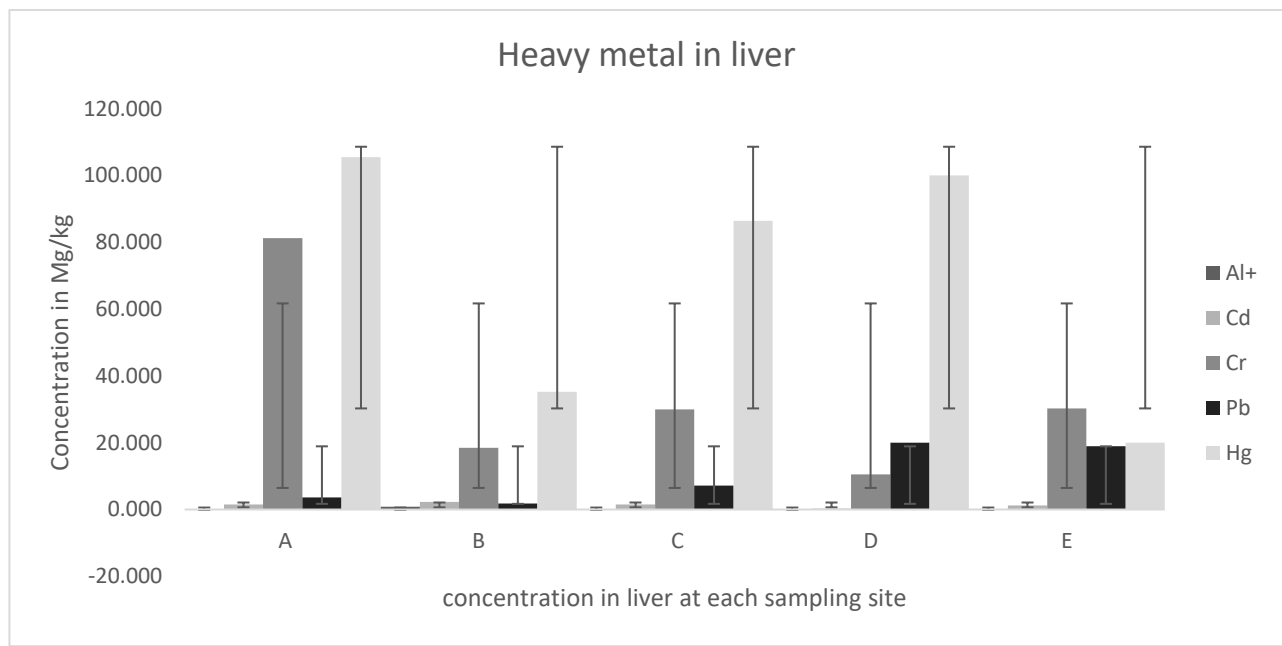


Figure 4: Mean concentration of heavy metal in fish liver in sampling site

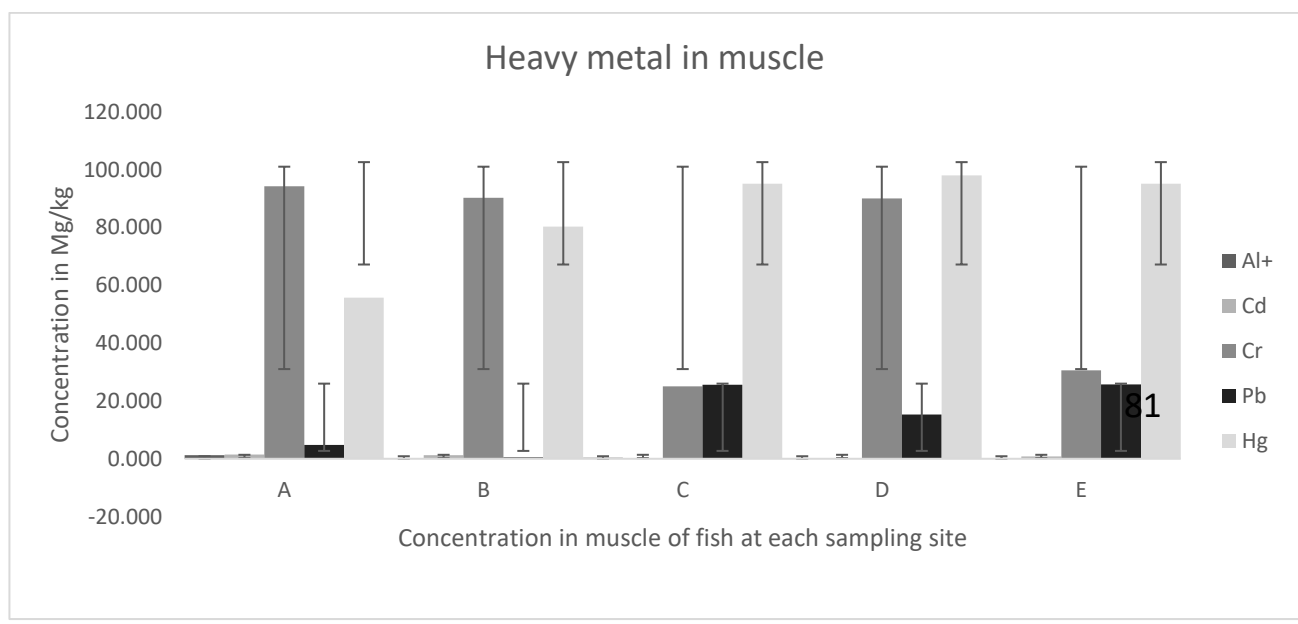


Figure 5: mean concentration of heavy metal in fish muscles in sampling site

Oxidative stress biomarkers

Oxidative stress enzymes were analyzed in gills, liver, and muscle of *C. gariepinus* from five sampling stations. Observed oxidative stress enzymes include superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), and lipid peroxidation (malondialdehyde). Table 1 shows activities of superoxide dismutase in the fish, the gill SOD

concentration was highest at site C, the liver concentration was $32.43 \pm 2.05 \text{Ug}^{-1}$ from site B, and the muscle SOD concentration was highest at site C at $11.02 \pm 1.20 \text{Ug}^{-1}$. The SOD activities were found to be significantly different ($P < 0.05$) in comparison to the control.

Table 2 shows the levels of GSH activity in the organs of *C. gariepinus*, and GSH levels in various organs of

fish liver, gills, and muscle were considered to be significantly higher ($P < 0.05$) in comparison to the control. Fish samples from site A showed the highest GSH levels in the gills, GSH levels in the liver at site D were 263.17 ± 3.04 U/mg and the concentration at site E was 262.44 ± 6.34 U/mg. Concentrations in muscle showed a highest value of 454.62 ± 4.47 U/mg at site B and a lowest value of 57.65 ± 7.10 U/mg at site A.

Level of CAT formation in *C. gariepinus* are shown in Table 3. High levels of CAT are present in the gills of fish. The highest CAT values for gills are from site B, followed by site E with values of 14.92 ± 2.81 $\mu\text{molmin}^{-1}\text{g}^{-1}$ and 10.17 ± 0.20 $\mu\text{molmin}^{-1}\text{g}^{-1}$. Liver

samples had the highest CAT content at Site B with a value of 0.86 ± 0.38 $\mu\text{molmin}^{-1}\text{g}^{-1}$, which is significantly ($P < 0.05$) different from other gills in Site B fish.

MDA production levels in *C. gariepinus* are shown in Table 4. An MDA value of 9.06 ± 0.01 μmolml^{-1} was found at site B and a value of 3.41 μmolml^{-1} was found at site C. The concentration of MDA in liver was highest at sites B and C with a value of 7.28 ± 1.71 μmolml^{-1} . And 7.28 ± 0.16 μmolml^{-1} . The highest MDA level observed in muscle was 3.62 ± 0.68 μmolml^{-1} at site C, with no significant difference between sites A, D and E ($P < 0.05$).

Table 1: SOD levels in *C. gariepinus* organs from HNW (in Units g^{-1} wet wt)

<i>C. gariepinus</i>		SITE					
Organ/Tissue	Control	A	B	C	D	E	
Gills	ND	9.40 ± 4.50^b	2.33 ± 1.03^a	12.35 ± 2.10^c	10.11 ± 0.11^b	8.33 ± 0.17^b	
Liver	1.83 ± 0.01^a	32.43 ± 2.05^d	30.83 ± 2.82^d	29.32 ± 7.02^d	13.42 ± 6.82^b	24.23 ± 4.01^c	
Muscle	0.41 ± 0.01^a	1.55 ± 0.50^a	ND	11.02 ± 1.20^c	4.66 ± 0.60^b	6.99 ± 0.99^b	

Values are mean \pm SD of single fish species determinations from 5 points in HNW. ND = Unrecognized

Table 2: GSH levels in *C. gariepinus* from HNW (in U/mg)

<i>C. gariepinus</i>		SITE					
Organ/Tissue	Control	A	B	C	D	E	
Gills	0.54 ± 0.39^a	1016.64 ± 0.54^f	95.36 ± 5.11^b	547.64 ± 10.44^d	331.41 ± 1.7^c	707.71 ± 6.39^e	
Liver	0.34 ± 0.04^a	102.51 ± 2.06^c	21.88 ± 2.81^b	11.20 ± 2.10^b	263.17 ± 3.05^c	262.44 ± 6.34^c	
Muscle	4.97 ± 0.07^a	57.65 ± 7.10^b	454.62 ± 4.47^d	208.70 ± 10.05^c	245.28 ± 30.17^c	247.80 ± 7.40^c	

Values are mean \pm SD of single fish species determinations from 5 points in HNW. ND = Unrecognized

Table 3: Catalase levels in *C. gariepinus* organs from HNW (in $\mu\text{molmin}^{-1}\text{g}^{-1}$)

<i>C. gariepinus</i>		SITE					
Organ/Tissue	Control	A	B	C	D	E	
Gills	ND	0.68 ± 0.00^a	ND	3.39 ± 1.00^b	6.78 ± 0.81^c	10.17 ± 0.20^d	
Liver	ND	0.86 ± 0.38^a	14.92 ± 2.81^b	ND	0.65 ± 0.04^a	0.68 ± 0.01^a	
Muscle	ND	4.75 ± 1.23^b	0.68 ± 0.01^a	2.03 ± 0.05^b	ND	0.68 ± 0.01^a	

Values are mean ± SD of single fish species determinations from 5 points in HNW. ND = Unrecognized
Table 4: Malondialdehyde levels in *C. gariepinus* organs from HNW (in μmolml^{-1})

<i>C. gariepinus</i>	SITE					
	Control	A	B	C	D	E
Gills	0.67±0.23 ^a	3.22±0.11 ^b	9.06±0.01 ^c	3.41±0.40 ^b	2.60±0.10 ^b	3.36±1.90 ^b
Liver	0.47±0.22 ^a	6.09±0.08 ^c	7.28±1.71 ^c	7.28±0.16 ^c	3.42±1.13 ^b	4.64±0.01 ^b
Muscle	ND	0.86±0.03 ^a	2.64±0.08 ^b	3.62±0.68 ^b	1.74±0.84 ^a	1.44±0.44 ^a

Values are mean ± SD of single fish species determinations from 5 points in HNW. 0.005. ND = Unrecognized

Histopathological Examination

Microscopic histopathological examination of various organs of catfish shows some damage/changes in gills, liver and muscle affected by the presence of heavy metals in the Hadejia Nguru wetlands. Plates 1 and 2 showed the changes seen in the organization of *C. gariepinus*. Gill filaments show more detrimental effects such as cell proliferation, clubbing, hyperplasia and fusion of lamellar cells, loss of secondary lamellae

and inflammatory cells (Plate 2; D and E). There was hepatocyte degeneration and erythrocyte distortion, and hepatocyte vacuolization was noted. Hepatocyte clogging and degeneration were observed in the tissues observed (Plate 2, A and C), with no evidence of tissue damage, color change, odor and texture changes observed prior to examination, and all tissues was consistent. Muscle tissue degeneration and vacuolated blood vessels have been observed.

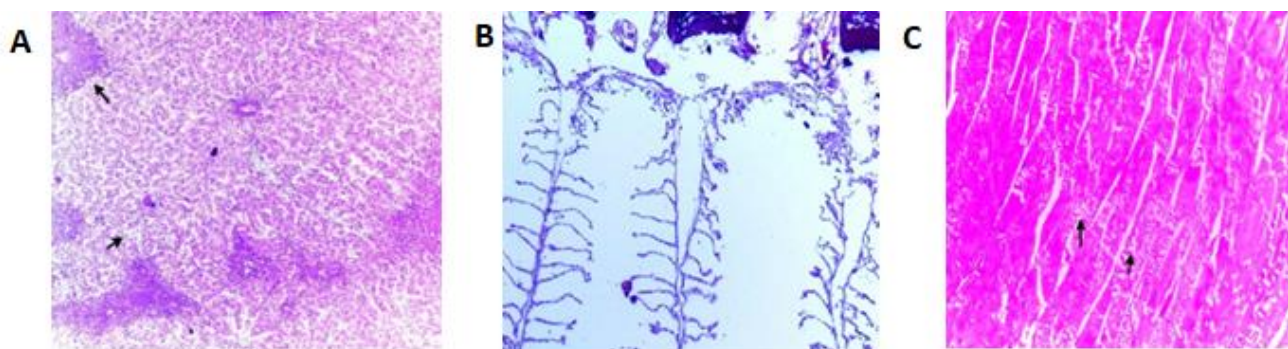


Plate 1: Histology of control fish *Clarias gariepinus* Showing no distinct alteration in liver of the fish, revealing normal hepatocyte (A) gills with normal primary and secondary lamellae (B) and muscle with normal position of blood vessels (C).

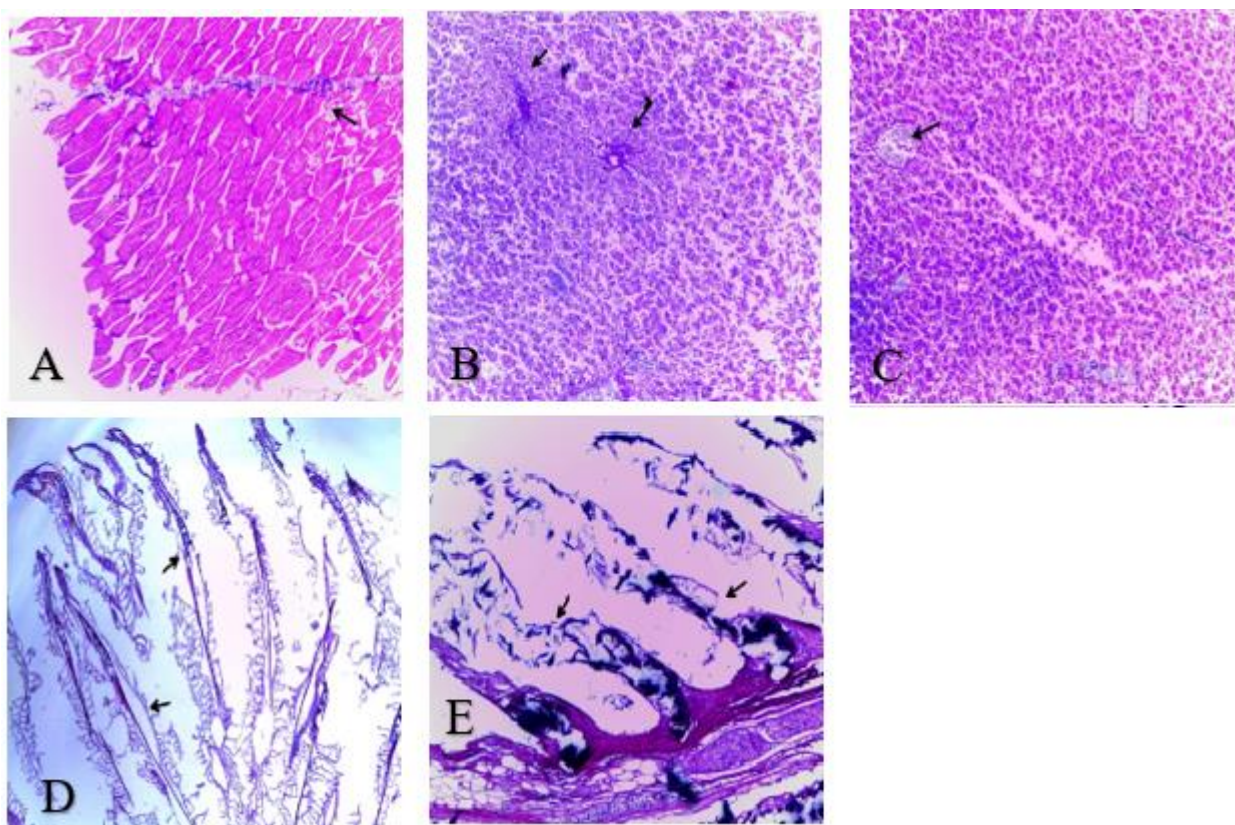


Plate 2: Tissue histology of *Clarias gariepinus* Showing (A) areas of degeneration of hepatocytes and distortion of red-blood cells, (B) shows degeneration of muscle tissue and vacuolated blood vessels {C} shows liver tissue with vascular congestion and degeneration of hepatocytes (D) gills showing areas of lamellae clubbing with hypertrophy and hyperplasia of the epithelial cells of gills (E) degeneration of primary and secondary lamellae, hemorrhage prominent in primary and secondary lamellae (Stain uptake H&E X100).

Table 5: Maximum Permissible Limit (MPL) of Heavy metals according to some International Standards

Organization	Heavy Metals (mg/Kg)					
	Aluminum	Chromium	Lead	Cadmium	Mercury	References
WHO/FAO	1.0	0.05	0.5	0.05	0.002	FAO(2004)
EU	0.2	0.05	0.5	0.1	0.05	EU (2002)
EC	0.3		0.025	0.007	0.0016	EC (2006)
FEPA	0.0016	0.15-1.0	0.2	0.2	0.003-0.007	FEPA(2003)

EU – European Union EC- European Communities FEPA- Federal Environmental Protection Agency.

DISCUSSION

The contamination levels observed in this study exceeded acceptable levels for most heavy metals (as presented in Table 5). High concentrations of mercury and chromium can result from human activities such as mining, fertilization, and chemicals rich in these heavy metals, even though water contains trace amounts of mercury (Authman et al., 2015). Mercury in the gills of fish were higher than in other organs. Low mercury concentrations reduce sperm viability in fish, reduce egg production, and impair viability of developing eggs (Raldua et al., 2007). Cr concentration

was highest at sample location A, exceeding the recommended tolerance limit for food which is set as 0.5mg/Kg by WHO/FAO, 2004. Cr enters aquatic ecosystems through effluent emissions from mining, leather tanning, textiles and dyeing. Lead is a toxic metal that occurs naturally in aquatic ecosystems through anthropogenic activities such as metal-based mining, lead paint, and gasoline. The limits for lead residues recommended by WHO (1999) and FAO (2004) are between 0.3 mg/kg and 0.01 mg/kg in food. Lead poisoning can cause high blood pressure, kidney dysfunction, fatigue, insomnia, loss of appetite, headache, and numbness. Farombi et al., (2007)

observed similar trends in the liver and kidney *C. gariepinus* from the Ogun River. Cd concentrations in fish were higher than acceptable limits, and Cd concentrations in this study were similar to those reported by Hashim et al. (2014) who observed concentrations in fish higher than recommended permissible limits in a study conducted in the Keratan River, Malaysia. Cadmium can be introduced into water bodies using cadmium-rich fertilizers, septic agents and pesticides, which can affect the kidneys and cause chronic toxicities such as kidney damage, reduced fertility and liver dysfunction. Waakes, (2000).

High activities of SOD, redox-sensitive thiol compounds GSH, CAT, and MDA were observed in organs at increased proportions. A possible explanation for this increase detected in fish organs is the result of the presence of heavy metals in the water, and it is possible that the accumulation of heavy metal residues causes the production of superoxide anions, leading to the induction of transforming SOD. It converts superoxide radicals to H₂O₂ and catalytically scavenges the SOD superoxide radical, which appears to be a key factor in oxygen toxicity (Musa and Imam, 2021). GSH showed increased levels in all samples and GSH is known to be a substrate for the activity of GST (glutathione peroxidase). The highly detected increase in GSH formation suggests an adaptive and protective mechanism of this biomolecule against oxidative stress caused by heavy metal residues, consistent with the results of Farombi et al. (2007). Fish and their environment and such contaminants (heavy metals) can penetrate the thin epithelium of fish. Catalase activity was present in lower concentrations in some of the sampling sites but showed appreciable amount in some of the sites. As reported by Dautremepuits et al., (2004) increases in CAT and SOD activity are usually observed in the face of environmental contaminants. The amount of CAT reduction observed in this study is comparable to that reported by Stanic et al., (2005) who claimed that low amount of CAT activity can be attributed to superoxide radical shading ability. Significantly higher levels of lipid peroxidation in all organs observed indicated an accumulation of heavy metals in the organs, with increasing metal concentrations leading to higher levels of antioxidants, as can be seen from the results and in some cases damage in DNA, proteins and lipids. Pandey et al., (2003).

One of the organs more susceptible to toxic chemicals/pollutants due to direct contact with the environment is the gills, the absorption of toxic substance through the gills has been one of the effective means of measuring the effect of aquatic

pollutants into water bodies (Pandey et al., 2008, Khan et al., 2011) From results obtained there was a structural organization in the control group of fish however, the exposed fish to the metal pollutants showed hypertrophy and the clubbing of the epithelial cells. Khan et al. (2011) observed similar trend in the histology of gills of the African catfish *Clarias batrachus* exposed to lead and other metallic pollutants. Toxicity in metals interferes with vital functioning's and physiology which includes respiratory (Doaa and Hanan, 2013) Lifting and hyperplasia of lamellar epithelium can be interpreted as defense responses in fish as these changes in the gills increase the distance across which waterborne irritants must diffuse to reach the blood stream (Pandey et al., 2008) Oxygen deficiency as a result of gill toxicity has been described as the most common cause of cellular degeneration in gill filaments. Among abnormalities noticed in the liver are the degeneration of hepatocytes, the high alterations in the liver can be attributed to its functions of detoxification and accumulation of toxic elements in its cells, this is consistent with the findings of Ekeanyanwu et al. (2015) where concentrated levels of heavy metals were found in the liver of fish from Oguta lake. The concentration level of metals in the liver can cause a lot of alterations in the liver histology (Doaa and Hanan, 2013). Fish muscle showed many deleterious changes due to heavy metal toxicity, but the changes were more pronounced in gills and liver than in muscle, indicating that heavy metal concentrations were higher in gills and liver.

CONCLUSION

In this study, various organs of *Clarias gariepinus* in the Hadejia-Nguru Wetlands showed very high contamination with the heavy metals investigated. We can therefore conclude that polluted environment results to increase in the presence of superoxide dismutase, lipid peroxidation, catalase and glutathione activities in tissues of *C. gariepinus* and also brings about change in the histology of the fish organs. However, fish tested from sampling points are not suitable for human consumption due to high contamination level observed in them.

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