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±4.38±10.54 mg/Kg and site B with a value of 80.30±12.68 mg/Kg.

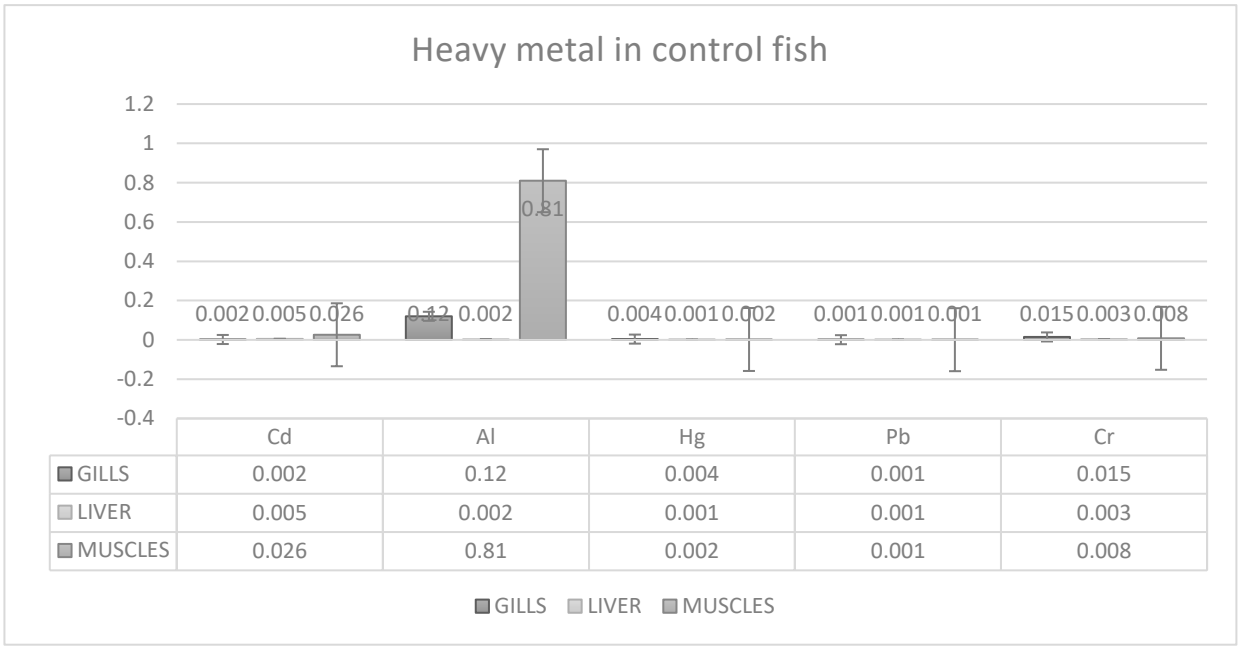

Figure 2: Control fish showing heavy metal concentration in gills, liver and muscle

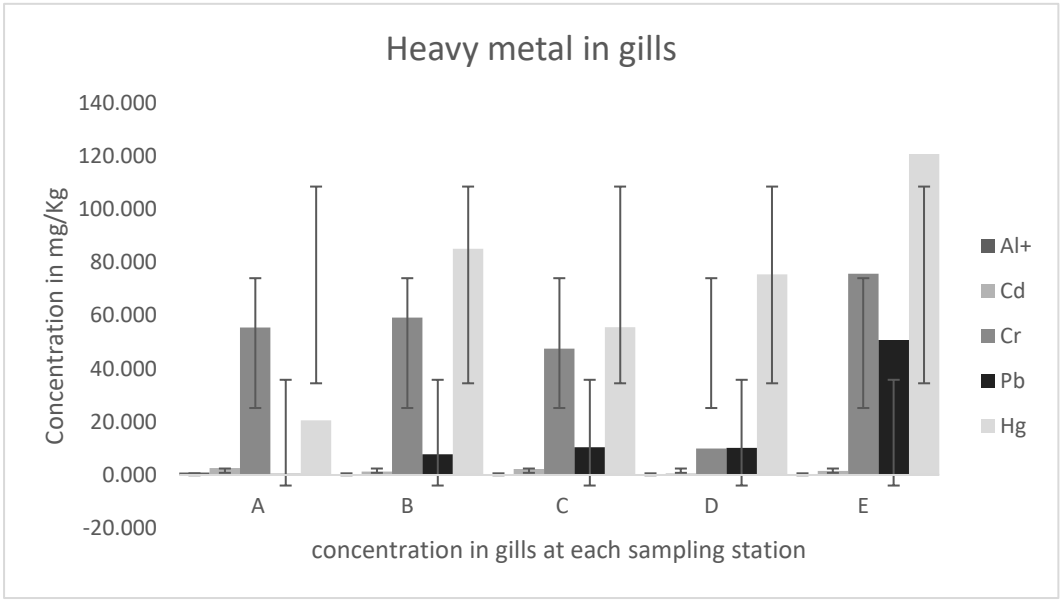

Figure 3: Mean concentration of heavy metal in fish gills in sampling

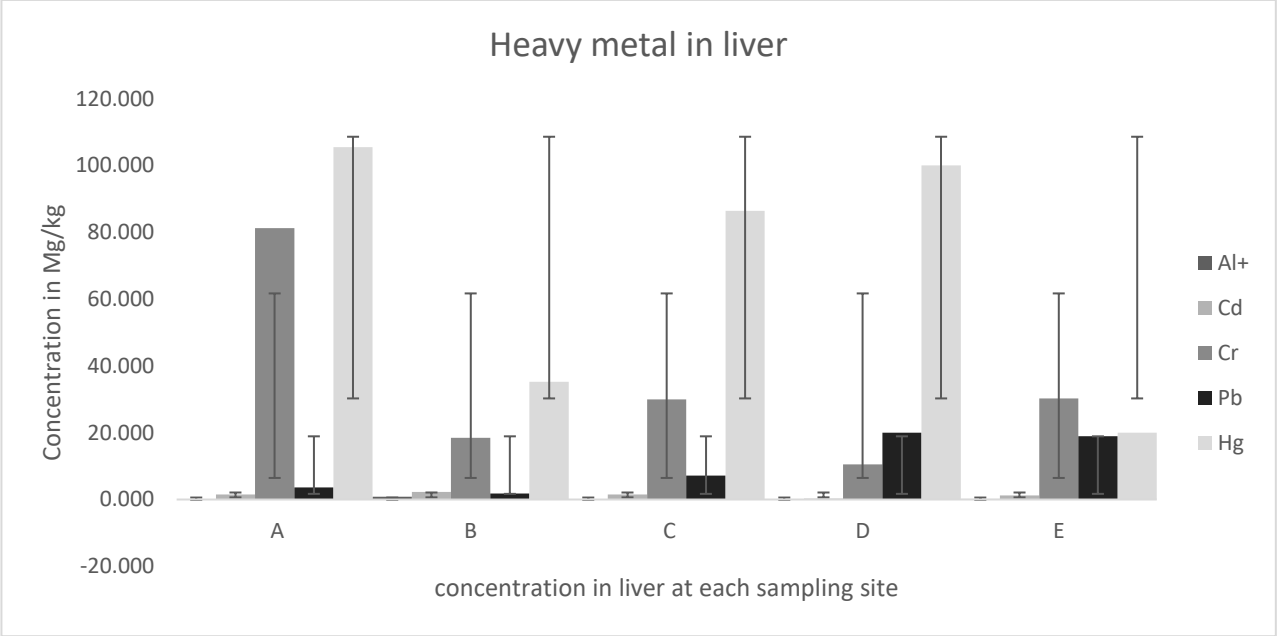

Figure 4: Mean concentration of heavy metal in fish liver in sampling site

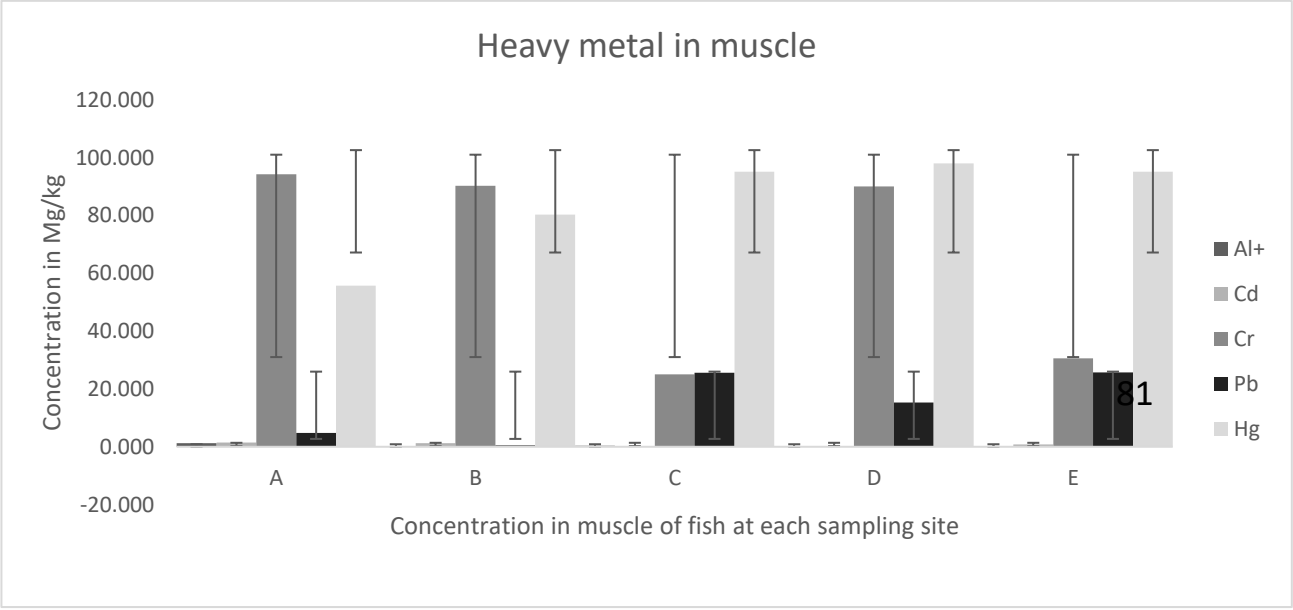

Figure 5: mean concentration of heavy metal in fish muscles in sampling site

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 ± 2.05Ug-1 from site B, and the muscle SOD concentration was highest at site C at 11.02 ± 1.20Ug-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±3.04U/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 μmolmin-1g-1 and 10.17±0.20 μmolmin-1g-1 .Liver samples had the highest CAT content at Site B with a value of 0.86±0.38 μmolmin-1g-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±0.01μmolml-1 was found at site B and a value of 3.41 μmolml-1was 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 Unitsg^{-1} wet wt)

C. gariepinus			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 ± 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)

C. gariepinus			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 ± SD of single fish species determinations from 5 points in HNW. ND = Unrecognized

Table 3: Catalase levels in *C. gariepinus* organs from HNW (in μmolmin^{-1}g^{-1})

C. gariepinus			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})

C. gariepinus			SITE			
Organ/Tissue	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.

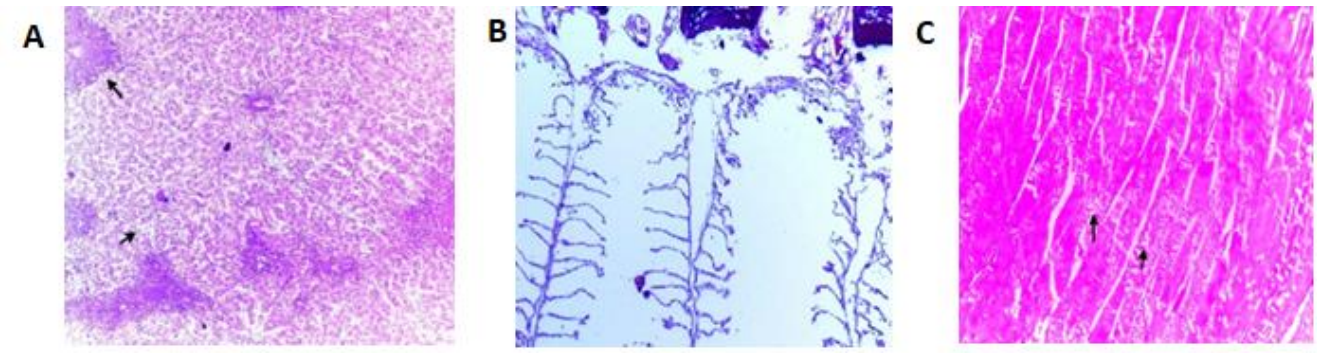

Plate 1: Histology of control fish *Clarias garipinus* Showing no distinct alteration in liver of the fish, revealing normal hepatocyte (A) gills with normal primary and secondary lamellae (B) and muscle with normal position of blood vessels (C).

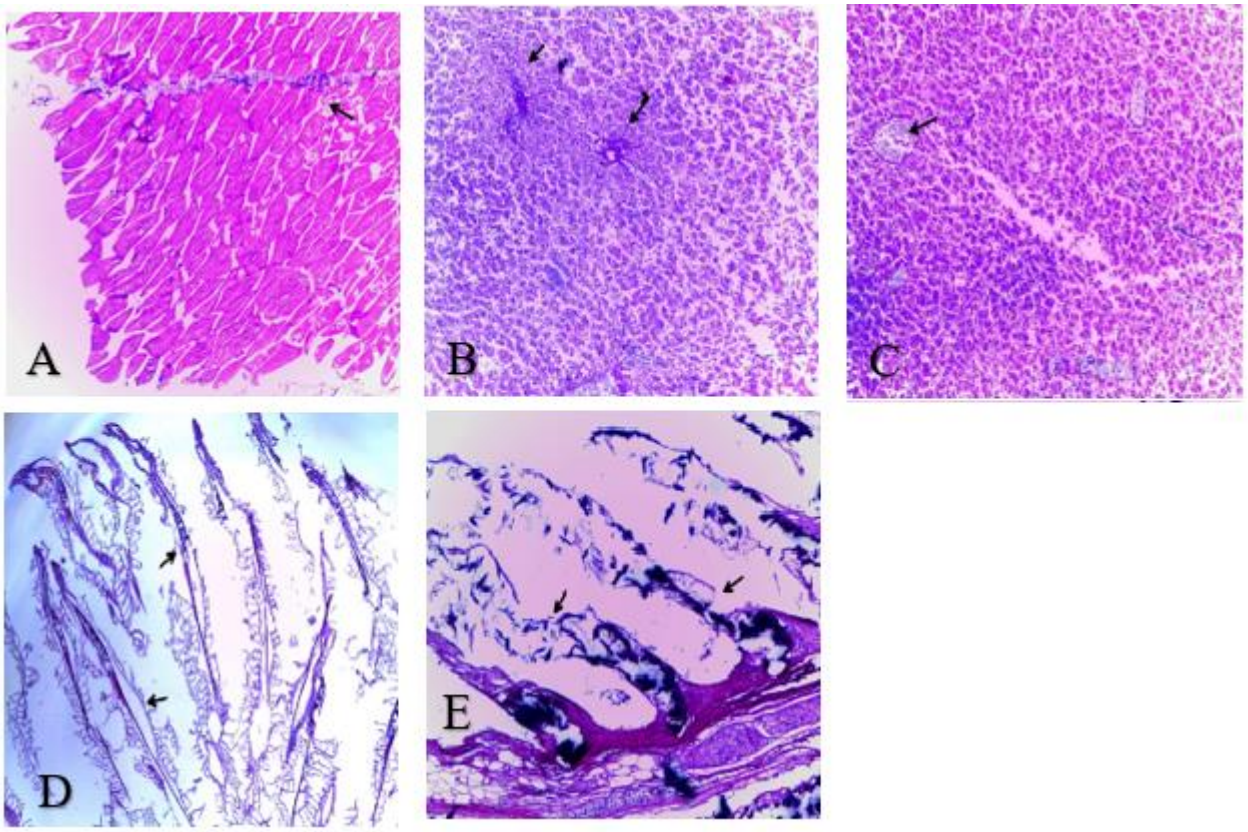

Plate 2: Tissue histology of *Clarias gariepinus* Showing (A) areas of degeneration of hepatocytes and distortion of red-blood cells, (B) shows degeneration of muscle tissue and vacuolated blood vessels {C) shows liver tissue with vascular congestion and degeneration of hepatocytes (D) gills showing 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

			Heavy	Metals (mg/Kg)		
Organization	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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