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Multiple biomarker response (Organ Histology, Serum Biochemistry and Oxidative Status) in Catfish Captured from Ureje River, Nigeria

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ABSTRACT

With rapid urbanisation coupled with the influx of more people to Ado-Ekiti, it is pertinent to assess the effect of human activities on living organisms in Ureje River, the main water body that cuts across Ado-Ekiti. This study examined the effects of environmental pollution on the Ureje River in Ado Ekiti, Nigeria with focus on water quality, heavy metal contamination, and the health of female African catfish. Samples of water were fetched from various fishing spots along the river, and an earthen pond, which served as a control. Blood samples were collected from fish samples farmed from different fishing points, and thereafter slaughtered and organ characteristics and histology assessed.

Fish from Emirin and Erinfun showed lower ($p<0.05$) weights, indicating impaired growth. Fish had significantly ($p<0.05$) elevated glucose at Ajebamidele, high total protein at Aduloju, Erinfun and Ajebamidele, and varied cholesterol levels, reflecting metabolic stress. Fish from polluted points had notably higher liver enzymes and alkaline phosphatase coupled with highest oxidative stress, indicating liver damage and stress. Fish from heavily polluted areas had necrosis in gills, liver steatosis, and kidney infiltration. Pollution from industrial, agricultural, and domestic sources significantly degrades the Ureje River ecosystem with negatives impacts on water quality and aquatic organism health

Keywords: Catfish, Environmental pollution, Heavy Metal, Histology, Oxidative stress

INTRODUCTION

Water and its resources play important roles in sustaining unhindered supply of food, as well as an environment conducive for all organisms, including humans. Water supports healthy ecosystems, drives economic growth, energy production, agricultural activities, sustainable waste management and sanitation (Onuh and Bassey, 2021).

Nigeria has vast water resources, which is evident in the volume of her rainfall, underground and surface water deposits, with the Niger Delta and tropical rainforest areas in the Southern part of Nigeria experiencing up to 3000 mm of rainfall yearly, which lasts up to eight months (Ezeabasili et al., 2014; Idu, 2015). However, the primary water sources accessible to local communities in Nigeria are impacted by alterations in their quality or composition as a result of natural and human-induced activities. This often renders the water unsuitable for domestic or industrial use, agricultural activities, fisheries, or other purposes. The gap between the need for water and water supply seems to be broadening yearly with growth in human population and increase in human activities of which indiscriminate dumping of untreated wastes into the water bodies' remains one of the most dominant problems (Chukwu, 2017). Currently in Nigeria, research indicates that significant numbers of available freshwater sources are polluted, leading to serious outbreaks of diseases (Galadima et al., 2011). Water pollution is the release of solid wastes or industrial and agricultural effluents into the water bodies, which in turn make the water bodies unfit for human use and uncomfortable to aquatic organisms or even lead to their death.

Numerous human activities such as industrialization, agricultural practices, and urban development have led to environmental degradation and pollution. These impacts adversely affect both rivers and oceans, which are essential for life, human health and sustainable social development (Xu et al., 2022). Improper disposal of solid waste, sand, and gravel or industrial waste drained into the water bodies without proper treatment has been the major reasons for the decline in water quality (Ustaoğlua et al., 2020). Polluted water, as result of various toxic, industrial pollutants often emit unpleasant odours and diminish biodiversity, creating conditions that are unsafe for human consumption and unsuitable for irrigation activities.

Heavy metal pollution poses a threat to various aquatic species, including fish as the continuous exposure to these toxic metals often results in their uptake directly from the environment that in turn affects the organs and tissues of these aquatic species through blood contact (Goher et al., 2018). Fish absorb heavy metals through their gills or digestive systems, which then enter their bloodstream and accumulates in their organs (Javed and Usmani, 2012; Al Taee et al., 2020). According to Bristy et al. (2021), this bioaccumulation of heavy metals in fish can lead to physiological, morphological, and behavioral abnormalities, as well as reproductive impairments. Consequently, consuming contaminated fish increases the risk of poor human health associated with exposure to heavy metals. Persistent intake of heavy metals through food, such as fish, can lead to chronic accumulation in the human body, and over time causing serious health damage (Adegbola et al., 2021).

Fresh water bodies are useful for fisheries, domestic use, drinking and also for irrigation. The fisheries sector plays a crucial role in generating income and employment, fostering growth in numerous related industries, and providing affordable and nutritious food through fish consumption. Global demand for fish is increasing due to its recognized health benefits, including being a rich source of essential nutrients such as protein, vitamins, minerals, and low-cholesterol unsaturated fatty acids (Medeiros et al., 2012). Research from the American Heart Association underscores the importance of consuming fish at least twice weekly to meet daily omega-3 fatty acid requirements (El-Moselhy et al., 2014).

However, concerns about health risks associated with heavy metal contamination in fish have led to the establishment of international monitoring programmes to assess fish nutritional safety prior to consumption (Tacon et al., 2020). Fish, living in close proximity to their environments are susceptible to chemical and physical changes that can manifest in their blood and bodily components (Witeska et al., 2022). Consequently, in toxicological studies and environmental monitoring, fish blood is increasingly recognized as a valuable indicator for assessing physiological changes, investigating diseases within fish populations, and monitoring environmental health (Fazio, 2019; Lawrence et al., 2020).

The quality of a water body is determined by its physico-chemical factors, making it crucial to continuously monitor these characteristics over both short and long periods. These factors play a significant role in shaping the distribution and productivity of aquatic organisms. Understanding the physico-chemical characteristics of a water body is essential for assessing its productivity, as it forms the foundation for evaluating the biological richness and productivity of the aquatic environment. In countries around the world especially in developing counties like Nigeria, over 99% of rivers natural states have been disrupted by indiscriminate human activities.

Many researchers have carried out different research on the physico-chemical parameters of water bodies, which are analysed to assess the concentrations of heavy metals in various rivers and sediments across different water bodies in Ekiti state, Nigeria including Elemi, Ogbesse and Ureje River (Oluwafemi et al., 2015, Bolaji et al., 2017, Adebayo and Familusi, 2020). The bioaccumulation of heavy metals and other toxic substances in fish flesh are also extensively studied but only a few have included the effects of these pollutants on the health of fishes in Ureje River in Ekiti State of Nigeria.

To address this knowledge gap, the bioaccumulation factors resulting from human activities on organ histology, serum biochemistry and oxidative stress status of fish caught at the five different fishing points along the Ureje River were investigated in the present study. The health status of female fish is taken into consideration since this largely determines the viability of these fishes to have viable seeds that can bring about high production of fish in this water body.

MATERIALS AND METHODS

Study area

Ureje River is the main river that flows through Ado Ekiti and its environs. The topography of the town lies between 1,200m-2,200m above sea level. The area enjoys heavy rainfall during the rainy season. The river is received in a Dam at waterworks, and flows through numerous tributaries, as source of livelihood for fishermen, motor and industrial laundry services, irrigation for crop fields, while also receiving domestic wastes, industrial waste and run-off from agricultural fields. As a result of human activities, large quantities of toxic industrial and domestic wastes, as well as chemical (herbicide, pesticides) run-off from various crop fields are deposited into the river as it flows through. Significant amounts of toxic industrial and domestic wastes can degrade water quality by altering the physico-chemical properties of Ureje River, which potentially leads to stunted growth among aquatic organisms, and ultimately affects their quality of life. Fish points along the river were identified (Ajebamidele, Emirin, Erinfun, and Aduloju), including the Dam holding the river before it enters the communities where it inter-faces with human activities (as shown in Figure 1). An earthen pond was selected inland, whose source of water is a borehole, which has no direct or indirect connection to the river as a control.

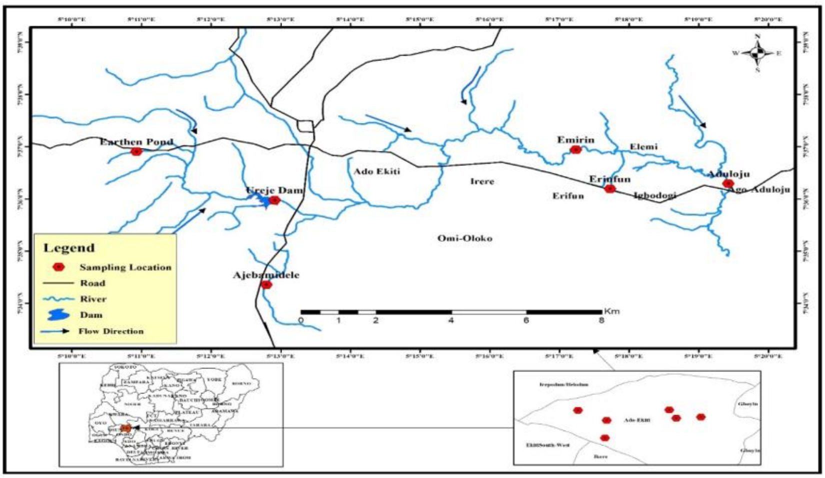

Figure 1. Map showing the location of the sampling points and the earthen pond

Sample Collection and Analysis

Water samples were gathered from various fishing locations along the Ureje River, these were carefully stored in sterile bottles and subsequently processed through digestion. Sediment samples were dried in the laboratory, pulverized, and sifted using a 2 mm mesh sieve to achieve uniformity before undergoing digestion.

Digestion of water sample

According to APHA/AWWA/WEF (2017), 2ml of concentrated HNO_3 and 5ml of concentrated HCl were added to 100ml of water sample. The sample was covered and heated on a hot plate for 2 hours at 900°C to 950°C until the volume was reduced to 15-20ml. It was cooled and the walls of the beaker was then washed down with distilled water and filtered (Whatman No. 1 filter paper) to remove silicates and other soluble materials. The volume was adjusted to 100ml and presented for metal/mineral analysis.

Metal/mineral analysis

The sediment samples were analysed for selected heavy metals: Sodium, Calcium, Magnesium, Potassium, Lead, Cadmium, Nickel, Chromium, Arsenic, Copper, Manganese and Zinc, while water samples were analysed for water quality parameters. Dissolved oxygen (DO), total dissolved solids (TDS), total suspended solids (TSS), total solids (TS), biochemical oxygen demand (BOD), and chemical oxygen demand (COD) were analyzed according to APHA methods (2005). Metal concentrations were determined using heavy metal kits (Merck) and atomic absorption spectrophotometry (AAS) on a Buck Scientific model 211 VGP, following the calibration plot method (APHA, 2005). The process included standard preparation, equipment calibration, and sample analysis. For each metal, the instrument was zeroed using distilled water (blank), followed by aspiration of standards from lowest to highest concentrations into the flame. The corresponding absorbance values were recorded by the instrument and used to plot a graph of absorbance against concentration. Metal concentrations in the samples were extrapolated from the standard curve and reported in parts per million (ppm).

Sampling of fish species

A total of 120 adult female African Catfish samples (20 per fishing point) were sampled. The collection was performed using gill nets and drag nets (van der Sleen and Albert 2018) operated by fishermen at each fishing point and in the earthen pond before 08:00hrs. The fish were sorted, identified as females at the species level by a fish taxonomist and weighed with well calibrated scale as described by Idodo-Umeh, (2003). They were transported in plastic tanks separately from each fishing point to the Animal Production Laboratory, Department of Agricultural Technology, Federal Polytechnic Ado Ekiti and allowed to rest for 2 hours.

Serum analysis

Blood samples were collected from each specimen's caudal vein near the ventral fin into plain bottles, and promptly centrifuged according to standard procedures to obtain serum.

Serum biochemical assay: glucose, total protein, albumin, creatinine, urea, alanine amino transferase (ALT), Aspartate amino transferase (AST), Alkaline phosphatase (ALP), cholesterol, triglyceride, high density lipoprotein (HDL) and bilirubin were carried out using fortress diagnostics commercial assay kits and its procedures. Determination of serum total antioxidant capacity activities, superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase activities and lipid peroxidation were assayed as outlined in Jimoh et al., (2019).

Preparation of Fish Gills, Liver, and Kidney for Histological Analysis

The fish samples were dissected from the anal opening to the head region. Organs including the brain, gills, kidneys, heart, liver, gastrointestinal tract (GIT), and eggs required for the study were removed, weighed, and dressed (Farombi et al., 2007; Mohammed, 2009). They were rinsed with deionized water and preserved in separate bottles with 70% ethanol.

Histopathological examinations of the gills, liver and kidneys were conducted according to Garcia (2021) as follows: the organs were collected and fixed in a 4% formaldehyde solution to prevent decay. They were dehydrated through sequential alcohol solutions of 50%, 70%, 80% and 100% for 90 minutes each. After dehydration, the organs were cleared in 100% xylene and left for 2 hours to remove any residual alcohol, followed by embedding in liquid wax for 2 hours.

The embedded organs were sectioned using a microtome, stained with haematoxylin-eosin (Ojokoh, 2006), and excess stain was removed under tap water. After clearing in xylene, DPX, Castor oil/xylene mixture was added, and a coverslip was placed on each slide (De Lillo et al., 2010). The preparations were oven-dried at 40°C, and histological structures and any histopathological changes in the gills, liver, heart, and kidneys were observed under a microscope equipped with a digital camera connected to a computer system. The findings were recorded and interpreted (Ross and Pawlina 2020).

Statistical Analysis

The data collected underwent descriptive statistics, one-way ANOVA, and means were separated using the Duncan multiple range test at a significance level of $\alpha = 0.05$. All statistical analyses were performed using IBM Statistical Package for Social Science (SPSS, version 20.0).

RESULTS

Analysis of water samples from different fishing points of Ureje River, Ado Ekiti is shown in Table 1. Water samples from Erinfun fishing point have the highest (210.17 mg/L) total solid, which was the absolute reflection of the total dissolved solids and total suspended solids, the result for the Earthen Pond water sample was the least when compared with other fishing points. Dissolved oxygen in water samples of Aduloju fishing point (7.16 mg/L) had the highest value, while the least was from Ajebamidele fishing point sample (3.63 mg/L). On the contrary, water from Aduloju had the highest biochemical oxygen demand (17.51mg/L) and chemical oxygen demand (31.30 mg/L), which was followed by that of Ajebamidele water 13.14 mg/L and 27.18 mg/L respectively.

Table 1. Water analysis of samples from different fishing points of Ureje river, Ado Ekiti

	Ajebamidele	**Emirin**	**Erinfun**	**Aduloju**	**Waterworks**	**Earthen pond**
Total dissolved solids (mg/L)	175.65	152.20	208.11	175.65	96.82	77.46
Total suspended solids (mg/L)	0.52	0.74	2.06	0.52	1.22	0.67
Total solid (mg/L)	176.17	152.94	210.17	5.55	98.04	78.12
Dissolved oxygen (mg/L)	3.63	5.55	4.85	7.16	6.12	4.16
Biochemical oxygen demand (mg/L)	13.14	7.16	8.63	17.51	6.56	10.24
Chemical oxygen demand (mg/L)	27.18	16.25	17.51	31.30	13.89	21.76

The result of the analysis of heavy metal from water samples obtained at the different fishing points is presented in Table 2. Earthen pond water sample had the least Sodium (6.65ppm), Magnesium (0.89ppm) and Copper (0.05ppm) when compared with samples from the five fishing points. The highest concentration of Sodium is at Aduloju (60.50ppm), Calcium at Erinfun (60.50ppm), and Magnesium at Aduloju (13.60ppm). However, only Earthen Pond had traces of Cadmium (0.01ppm), while Aduloju had traces of nickel, highest arsenic across the different fishing points. Copper was highest in water samples from Ajebamidele and Aduloju, Zinc values ranged from 0.12ppm in waterworks to 0.31ppm in Erinfun fishing points. Manganese was highest at Ajebamidele, Erinfun and Aduloju water samples and least values were obtained in waterworks sample.

Table 2. Metal analysis of samples from different fishing points of Ureje river, Ado Ekiti

	Ajebami dele	**Emirin**	**Erinfun**	**Aduloju**	**Waterw orks**	**Earthen pond**
Sodium (ppm)	21.30	11.50	31.30	60.50	7.40	6.65
Calcium (ppm)	36.40	27.25	60.50	2.10	10.80	15.45
Magnesium (ppm)	1.76	1.87	2.10	13.60	1.21	0.89
Potassium (ppm)	18.35	6.55	13.60	18.35	5.20	9.65
Lead (ppm)	0.01	0.02	0.02	0.01	0.01	0.01
Cadmium (ppm)	0.00	0.00	0.00	0.00	0.00	0.01
Nickel (ppm)	0.00	0.00	0.00	0.13	0.00	0.00
Chromium (ppm)	0.25	0.13	0.22	0.00	0.09	0.31
Arsenic (ppm)	0.01	0.00	0.00	0.06	0.01	0.02
Copper (ppm)	0.21	0.06	0.11	0.21	0.08	0.05
Manganese (ppm)	0.20	0.15	0.21	0.20	0.09	0.12
Zinc (ppm)	0.25	0.28	0.31	0.25	0.12	0.20

The live weight of female catfish from the different fishing points is shown in Table 3. It suggested that fishes caught at Emirin (314.80g) and Erinfun (388.78g) had the significantly ($p<0.05$) least values, the significantly ($p<0.05$) least organ weights and dressed weight (253.52g) was observed in catfishes caught at Emirin. Fish samples from Aduloju had higher ($p<0.05$) organ characteristics than fish from other fishing points. Egg weights were least ($p<0.05$) in fish sampled from waterworks and earthen pond, compared to the highest ($p<0.05$) value recorded in fish samples from Aduloju. Gonadosomatic index of fish from Emirin was significantly ($p<0.05$) highest, while that of fish samples from Ajebamidele were significantly ($p<0.05$) higher than those from Erinfun, with the significantly ($p<0.05$) least values obtained from those in waterworks and earthen pond.

Table 3: Liveweight, Organ weight and Gonadosomatic index of female catfishes from different fishing point of Ureje river in Ado Ekiti

	Ajebam idele	Emirin	Erinfun	Aduloju	Water Works	Earthen Pond	SEM
Liveweight (g)	583.92^{b}	314.80^{c}	388.78^{c}	866.50^{a}	508.34^{b}	809.57^{a}	40.06
Liver (g)	5.62^{b}	3.04^{b}	4.18^{b}	9.26^{a}	4.60^{b}	8.27^{a}	0.52
Heart (g)	0.54^{ab}	0.27^{b}	0.54^{ab}	0.82^{a}	0.60^{ab}	0.87^{a}	0.06
dressed weight (g)	442.12^{b}	253.52^{d}	321.68^{c}	680.56^{a}	446.06^{b}	707.07^{a}	32.08
Kidney (g)	1.72^{bc}	0.82^{c}	1.04^{c}	3.16^{a}	2.16^{ab}	2.33^{ab}	0.20
mesenteric fat (g)	9.24^{c}	0.84^{d}	3.12^{d}	17.14^{b}	8.04^{c}	32.08^{a}	2.25
GIT	12.14^{bc}	8.12^{c}	11.08^{bc}	16.32^{b}	21.44^{a}	20.13^{a}	1.10
Gills (g)	13.68^{b}	9.60^{c}	2.60^{d}	18.94^{a}	14.82^{b}	20.77^{a}	1.20
Brain (g)	4.12^{ab}	2.64^{b}	2.60^{b}	5.22^{a}	4.02^{ab}	6.15^{a}	0.35
Egg (g)	37.48^{b}	27.02^{c}	21.50^{c}	51.66^{a}	1.32^{d}	2.80^{d}	5.86
Gonadosomatic index	7.88^{b}	10.84^{a}	6.61^{c}	8.04^{ab}	0.30^{d}	0.38^{d}	1.34

abcd Means with the same superscript on the same row do not differ significantly (P> 0.05), SEM: standard error of means

The serum biochemistry of female catfish caught from the different fishing points of Ureje River is recorded in Table 4. Catfish from Ajebamidele fishing point had the highest glucose (29.07mmol/L) level and least was in Erinfun (9.78mmol/L), the highest total protein is from fishes caught at Aduloju, Erinfun and Ajebamidele, which were not significantly different, and the least value was obtained in Emirin. Albumin levels of fish from Emirin, Erinfun and Aduloju were significantly ($p<0.05$) higher than those from earthen pond and the significantly ($p<0.05$) least values were obtained in fish from Ajebamidele and waterworks. Alkaline phosphate of fish from water works was higher than those from the other fishing points. AST of fish from Emirin was higher than those from earthen pond. ALT of fish from earthen pond and water works were ($p>0.05$) similar and significantly ($p<0.05$) higher than those from Erinfun. Peak values of bilirubin, uric acid and creatinine were recorded in fish from Aduloju, earthen pond and waterworks respectively. The cholesterol level was found to be highest in catfishes caught at Erinfun (2.38 mmol/L) and Emirin (2.33 mmol/L) while the least value obtained in those caught at Ajebamidele (1.54 mmol/L). Triglyceride of fish from Ajebamidele, Aduloju and earthen pond was significantly ($p<0.05$) higher than those from Emirin, Erinfun and waterworks. HDL was significantly ($p<0.05$) highest in fish from

Emirin and the significantly (p<0.05) least value was obtained in earthen pond.

Table 4. Serum biochemistry of female catfishes from different fishing point of Ureje river in Ado Ekiti

	Ajebam idele	Emirin	Erinfun	Aduloju	Water Works	Earthen Pond	SEM
Glucose (mmol/L)	29.07^{a}	17.44^{b}	9.78^{c}	16.91^{b}	14.01^{b}	14.27^{b}	3.19
Total Protein (g/dL)	52.09^{a}	10.99^{c}	47.94^{a}	54.79^{a}	32.81^{b}	37.75^{b}	4.58
Albumin (g/L)	79.17^{c}	201.25^{a}	200.83^{a}	202.50^{a}	68.33^{c}	137.50^{b}	18.83
Alkaline phosphatase(U/I)	52.44^{b}	51.06^{b}	55.20^{b}	53.36^{b}	198.72^{a}	19.32^{b}	16.59
Aspartate amino transferase(U/I)	15.20^{ab}	44.68^{a}	24.04^{ab}	22.34^{ab}	13.84^{ab}	4.15^{b}	4.56
Alanine amino transferase(U/I)	26.26^{ab}	29.30^{ab}	19.27^{b}	25.71^{ab}	20.69^{a}	41.56^{a}	2.42
Bilirubin (mg/dL)	2.05^{b}	1.74^{bc}	2.34^{ab}	2.97^{a}	1.24^{c}	2.10^{b}	0.15
Uric acid (mmol/L)	2.49^{b}	3.73^{b}	3.11^{b}	4.46^{b}	3.07^{b}	6.82^{a}	0.40
Creatinine (mg/dL)	44.00^{a}	1.33^{b}	2.00^{b}	3.56^{b}	5.11^{a}	1.33^{b}	5.37
Cholesterol (mmol/L)	1.54^{d}	2.33^{a}	2.38^{a}	1.94^{bc}	1.62^{cd}	2.17^{ab}	0.09
Triglyceride (mmol/L)	21.90^{a}	9.93^{b}	7.49^{b}	22.73^{a}	11.04^{b}	26.06^{a}	1.97
High density lipoprotein (mmol/L)	3.17^{bc}	4.55^{a}	2.72^{cd}	3.97^{ab}	3.34^{bc}	1.86^{d}	0.24

abcd Means with the same superscript on the same row do not differ significantly (P> 0.05), SEM: standard error of means

Oxidative stress markers of female catfish from different fishing points of Ureje river is shown in Table 5. Total antioxidant activity, superoxide dismutase and catalase of African catfish from different fishing points in Ureje River in Ado Ekiti were not statistically (p>0.05) different from one another. The lipid peroxidation and glutathione peroxidase of catfish from Emirin was statistically similar to those from waterworks, but was significantly higher than those from Ajebamidele, Erinfun, Aduloju and Earthen pond.

Table 5: Oxidative stress markers of female catfishes from different fishing point of Ureje river in Ado Ekiti

	Ajebam idele	Emirin	Erinfun	Aduloju	Water Works	Earthen Pond	SEM
Total Antioxidant activity(mmol/L)	0.73	0.60	1.06	0.55	0.57	0.85	0.12
Lipid peroxidation ($x10^{-5}$MDA/mg protein)	7.70^{b}	42.10^{a}	7.20^{b}	7.60^{b}	30.60^{ab}	11.50^{b}	0.04
SOD (U/min/mg protein)	2.46	2.32	0.14	0.70	0.68	0.16	0.40
GPx (mg GSH/min/mg protein)	0.74^{b}	8.22^{a}	0.39^{b}	0.66^{b}	3.89^{ab}	0.54^{b}	0.89
CAT (nm H_2O_2/min/mg protein)	1.30	7.07	7.10	2.26	16.96	1.57	2.45

ab Means with the same superscript on the same row do not differ significantly (P> 0.05), SEM: standard error of means

The histopathology analysis of female catfish caught from the different fishing points of Ureje River is shown in Table 6 and representative images are in Figure 2-9. Only catfish from Waterworks, Ajebamidele fishing points and the Earthen Pond had normal hearts without any visible damages. Likewise, those at the Earthen Pond, Emirin and Aduloju fishing points did not show any alterations on the gills. While the different alterations such as loss of secondary lamellae and necrosis of the epithelia cells in gills, severe steatosis in liver, infiltration of lymphocytes and macrophages of the kidney, infiltration of the interstitium with coagulative necrosis of the tubular epithelial cells of kidney were evident in the fishes caught at Erinfun fishing point.

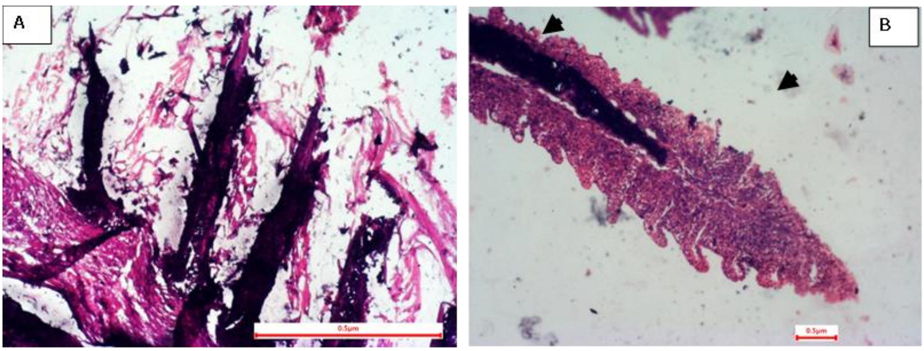

Figure 2. Photomicrograph of gill from A Ajebamidele: Severe necrosis of *lamillae*
B Waterworks : Blunting of secondary lamellae, necrosis of the epithelium (Arrow head)

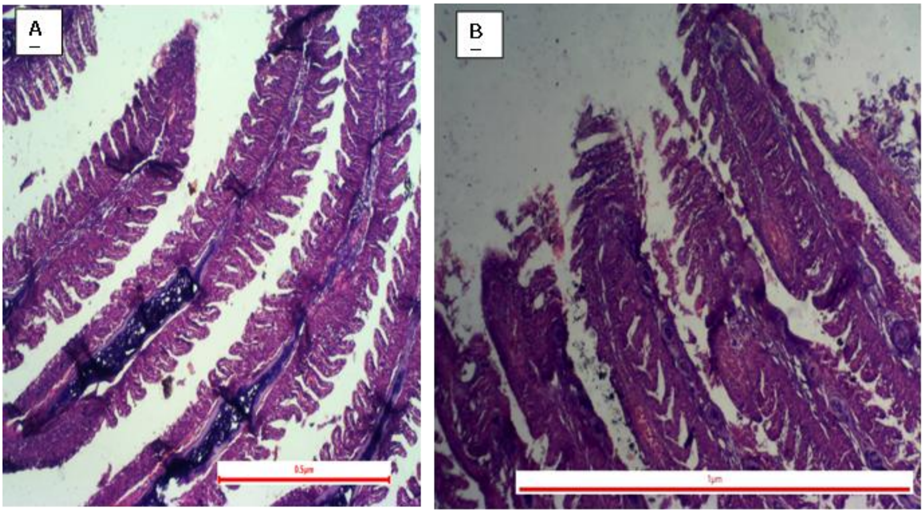

Figure 3. Photomicrograph of fish gills from **A Erinfun** : No pathology is seen in the gills **B Earthen pond**: mild sloughing of the lamilae and necrosis of the epithelial cells (arrow)

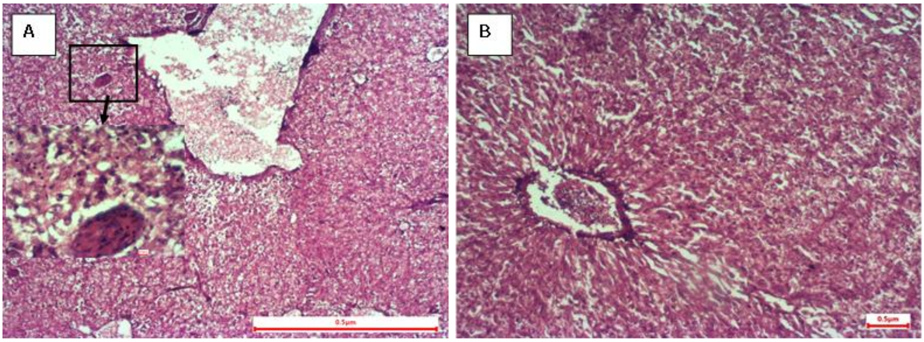

Figure 4. Photomicrograph of fish Liver from **A Waterworks**: Severe steatosis, cellular infiltrations, centrilobular Coagulative necrosis, loss of architecture, presence of giant cells**. B Ajebamidele**: Steatosis, congestion

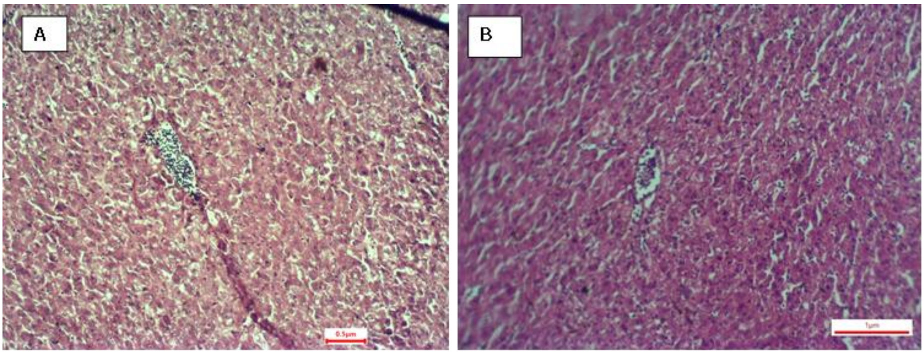

Figure 5. Photomicrograph of fish Liver from **A Erinfun**: Vacoulation of hepatocytes, coagulative necrosis and loss of architecture **B Earthen Pond**: mild mononuclear infiltration, Widespread vacuolation of hepatocytes

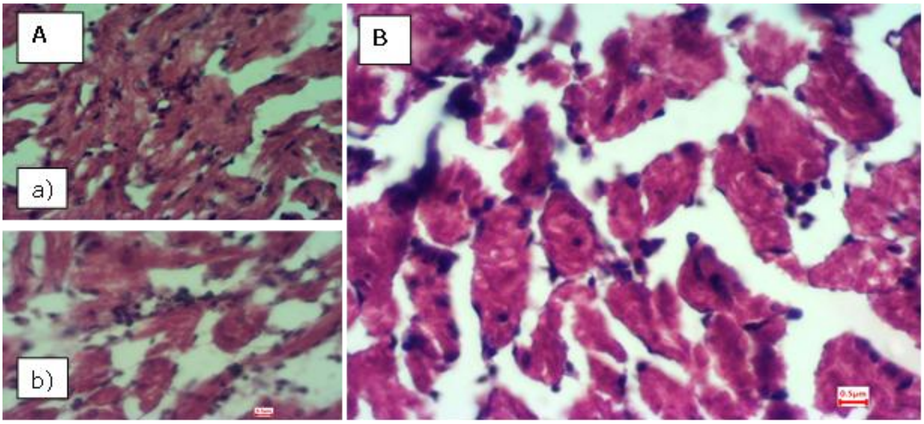

Figure 6. Photomicrograph of fish heart from **A Waterworks**: a) Female, No abnormal changes. B) Male, Widespread haemorrhages. **B Ajebamidele** :No changes

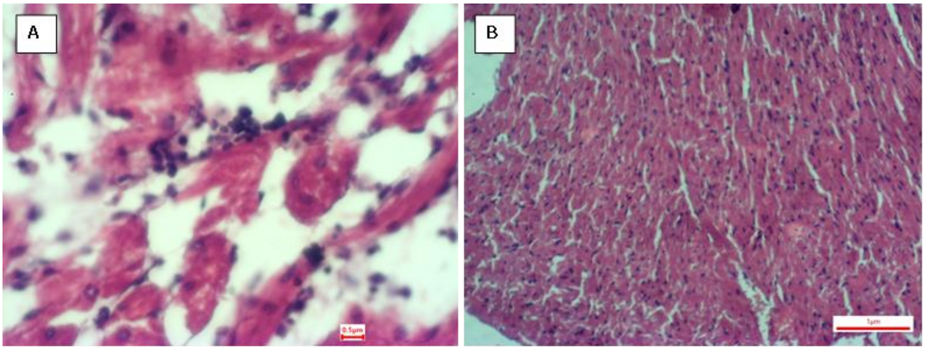

Figure 7. Photomicrograph of fish heart **A: Erinfun** Female: Mild lymphocytic infiltrations **B Earthen Pond**: no visible changes

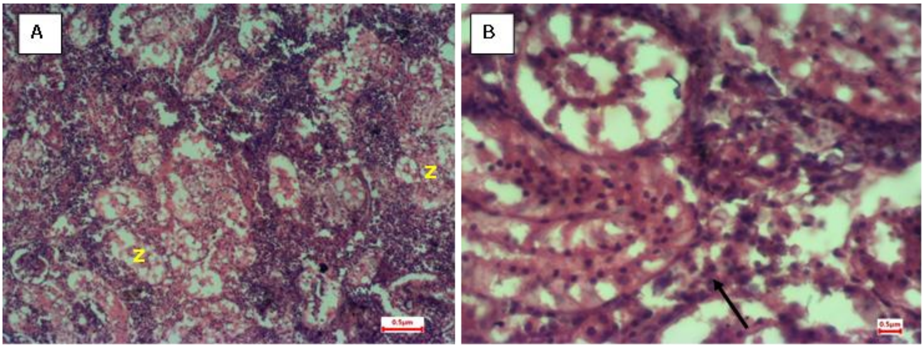

Figure 8. Photomicrograph of fish kidney from **A. Waterworks**: Severe necrosis (z) of tubular epithelial cells, Presence of necrotic debris within the tubular lumen, and edema **B. Ajebamidele**: Coagulative necrosis of the tubular epithilia cells and infiltration of the interstitium with lymphocytes and macrophages (arrow).

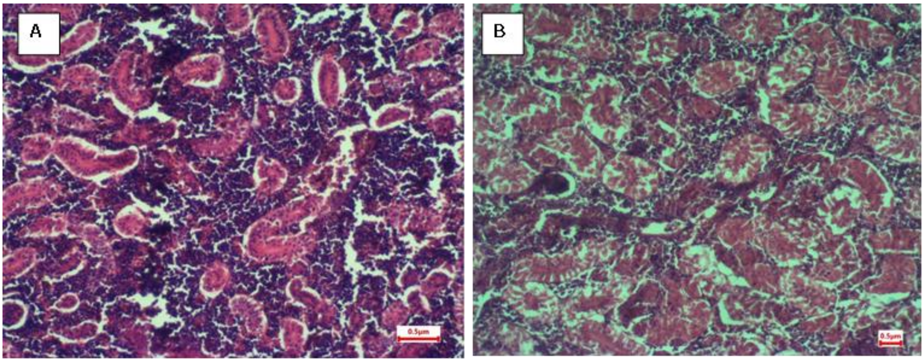

Figure 9. Photomicrograph of the fish kidney from **A Erinfun**: severe infiltration of the interstitium with inflammatory cells**. B. Female** Severe necrosis of tubular epithelial cells and moderate cellular infiltration in the interstitium Mild infiltration of the interstitium

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Table 6. Histopathology of fish organs from different fishing sites of Ureje river, Ado Ekiti

Location	Organs			
	Gills	Liver	Heart	Kidney
Ajebamidele	-Loss of secondary lamellae	-Steatosis Congestion	-No changes	-Coagulative necrosis of the tubular epithilia cells -Infiltration of the interstitial with lymphocytes and macrophages
Emirin	-No changes	-Presence of giant cells Vacoulation of hepatocytes -Coagulative necrosis -Loss of architecture -Presence of thrombus	-Mild lymphocytic infiltrations	-Severe infiltration of the interstitium with inflammatory cells
Erinfun	-Oedema -Loss of secondary lamellae -Necrosis of the epithelia	-Severe steatosis	-Mild lymphocytes and macrophages infiltration	-Massive infiltration of the interstitium with inflammary cells -Coagulative necrosis of the tubular epithelial cells
Aduloju	-There is no visible changes	-Congestion -Coagulative necrosis of the hepatocytes -Severe macrophage and lymphocytes infiltration	-Mild haemorrhages -Mild cellular infiltrations	-Loss of architecture -Mild cellular infiltration in the interstitium -Mild necrosis of the tubular epithelial cells
Water Works	-Blunting of secondary lamellae -Necrosis of the epithelium	-Severe steatosis -Cellular infiltrations -Centrilobular Coagulative necrosis -Loss of architecture Presence of giant cells	-Normal	-Severe necrosis of tubular epithelial cells -Flattening of the tubular epithelia -Presence of necrotic debris within the tubular lumen -Severe edema lymphocytes and macrophage infiltration in the interstitial -Haemosiderosis within the haemopoietic islands
Earthen Pond	-No changes	-Mild mononuclear infiltration Mild vacuolation of hepatocytes	-No visible changes	-Mild necrosis of tubular epithelial cells -Moderate infiltration of the interstitium

DISCUSSIONS

Ureje River is bound to increase as there is an increase in the number of commercial activities at Ado Ekiti. This river serves as source of water for laundry activities, construction works and a path for household water runaway, wash-away from farms and waste disposal, hence, the changes in water quality and evidence of some heavy metals (Zhang et al., 2023). The study shows elevated levels of total solids, BOD, and COD in various fishing points, particularly at Ajebamidele and the Earthen Pond. High BOD and COD indicate significant organic pollution, which can deplete oxygen levels in the water and harm aquatic life (Knapik, 2014; Ferreira et al., 2017). The presence of heavy metals like Sodium, Calcium, Magnesium, Potassium, Lead, Cadmium, Nickel, Chromium, Arsenic, Copper, Manganese, and Zinc in the water samples suggests contamination from industrial and agricultural runoff (Nouri et al., 2008; Sarkar et al., 2016).. These metals can be toxic to aquatic organisms, bioaccumulate in the food chain, and pose risks to human health if the fish are consumed (Malik and Maurya, 2014; Authman, 2015; Jamil et al., 2023).

The activities of humans around the Ureje River of Ado Ekiti no doubt have led to high level of contaminants in the water with adverse effects on fishes caught along its tributaries. The sources of contaminants along Ureje River include wastes from open dumpsites, agricultural wastes, and run-off from landfills, building and road construction works during erosion (Bolaji et al., 2017). The results of water analysis from the different sampling points considered in this study: Ajebamidele, Emirin, Erinfun, Waterworks and Aduloju as compared with water from Earthen Pond suggest that the physicochemical parameters of the Ureje River vary along its tributaries. Various particles are dissolved in rivers cutting across organic and inorganic sources. The concentrations of these particles are measured by total dissolved solids (Bolaji et al., 2017). Total solids are various dissolved solids together with suspended and settled- solids in water. For aquatic organisms like fish, constant mineral levels are required, as changes in the TDS concentrations can be detrimental as this controls the in-flow and out-flow of water through an organism's cells and may contribute to a rise in water temperature, decline in photosynthesis and decrease in water clarity. Although, some level of mineral content in water is necessary for healthy living, the lower the TDS, the better for quality of water (Zeshan and Saltanat, 2022).

The solid particles in the studied river are within the recommendation levels of World Health Organisation (WHO, 2009) of less than 80 mg/L for TSS and TDS within 500 mg/L in freshwater bodies according to Orabator et al. (2020). This revealed that the transparency level of this river is still normal and needs to be monitored to avoid negative shifts that could harm fish or other aquatic organisms in it. High suspended solid waste particles are associated with high turbidity, which will reduce light penetration and oxygen dissolution into the water, and this can eventually lead to decrease in fish production as a result of toxic effects on fish and fish eggs. Carballo et al. (2008) reported that dissolved suspended solids can clog filters and injure fish gills. Dissolved oxygen refers to the quantity of oxygen gas present in a water body (Ostroumov, 2017). For fish and other living organisms to perform optimally, the DO should not be less than 5mg/L and any value between 2-4mg/L is a sign of distress in fish (Bulba Ali and Abha 2022). The DO of the river, except at Ajebamidele fishing point (3.63mg/L) is within the permissible limit suggesting that the water is more polluted in this area. This calls for more attention, because a low DO is detrimental to the health of fishes. This is similar to the report of the research of physic-chemical analysis carried out by Bilewu et al. (2022).

Biochemical oxygen demand (BOD) refers to the quantity of dissolved oxygen required for the stabilization of biodegradable organic matter by aerobic microorganisms, along with the oxidation of specific inorganic substances (Tikariha and Sahu, 2014). BOD gave the qualitative index of degraded organic materials (Zeshan and Saltanat, 2022). Reports from this study revealed that the BOD of Ureje River is above the WHO prescribed BOD which is 5 mg/L. This is evidence that this river might be polluted (Edori and Nna, 2018). Chemical oxygen demand (COD) is a crucial parameter in assessing water quality, indicating the amount of oxygen required for the chemical oxidation of both organic and inorganic substances (Jeyaraj et al., 2014). The highest COD value among the fishing points is 31.30 mg/L at Aduloju, while the lowest value is 13.89 mg/L at Waterworks fishing point. All examined fishing points recorded COD values exceeding the WHO (2009) standard of 10 mg/L. This elevated level could pose risks to the survival of aquatic life in these areas.

With regards to calcium content, fishing points like Ajebamidele (36.40 ppm), Emirin (27.25 ppm), and Erinfun (60.50 ppm) fall within the WHO (2009) standard range of 25-200 mg/L, and this is essential for bone and scale formation in aquatic organisms. Other fishing points examined showed calcium levels below this range; lower Calcium is an indication that there is a high level of aquatic plants as a result of eutrophication that will easily use up the Calcium in the river. Because of its buffering properties, calcium improves the taste of water and is a significant factor in determining the hardness of the water. It also serves as a pH stabilizer (Bolaji et al., 2017). The Cadmium concentration reported in this study corroborate the work of Okoro et al. (2015) who reported that the presence of Cd was not found in Ureje river both at dry and wet season. The presence of traces of Cadmium in the Earthen Pond with a concentration of 0.01 ppm which exceeded the permissible limit for WHO (0.003 mg/L) is a proof that the possible side effects on fishes includes: vertebral fractures, anemia, decreased digestive efficiency, osmo-regulatory issues, and growth deficiencies, as well as hematological and biochemical effects (Hosnia et al., 2015). The presence of agricultural activities, smoke from vehicles and sewage effluents among others contribute to the Cu concentration, however, its low concentration in Ureje water might be due to lack of widespread industries along the river tributaries that involve the production of oils and fats, perfumes, quarrying and cement-making, which releases Cu as their by-product as reported by Agarwal et al. (2007) and Dimari et al. (2008). According to WHO (2009) standards, the acceptable range of magnesium in water should not exceed 50 mg/L. In this study, magnesium levels ranged from 0.89 to 13.60 ppm - all falling below the WHO standard limit. Similar findings were reported by Soylak et al. (2001) in drinking water from Turkey. Magnesium is crucial for the proper functioning of living organisms and is naturally found in minerals such as dolomite and magnetite.

The evaluation of live and organ weight of female catfishes from the different fishing point of Ureje River revealed that live weight of fishes caught at Aduloju was the highest, and this was comparable to those from the Earthen Pond, and this was responsible for the higher organ weight for samples caught from these two locations. However, fishes from Earthen Pond had the highest dressed weight. Exposure of fishes to different metals has been found to affect the weight gain of fish (Ali et al., 2003). The fish growth results are in line with the findings of Shafique et al. (2012) and Hayat (2009) who reported significant weight differences in different fish species under exposure metal mixture. The gonadosomatic index is used to predict their reproductive ability. From the findings of this study, fish samples from Earthen Pond and Waterworks had very low gonadosomatic index compared to other fishing points. The same trend was observed for the egg count. This outcome might be due to the age of the fishes caught at the fishing points compared to the ones from Earthen Pond, and also exposure of those from the fishing points to heavy metals (Jia et al, 2017).

The uptake of heavy metals by fishes are through either guts or gills from where it gets into the bloodstream through to the various organs (Al Taee et al., 2020). Therefore, it is essential to investigate the blood parameters essential to fish health. Serum biochemical parameter is one of the important indicators used in evaluating fish health as affected by nutrition, physiological state, toxins or disease and habitat (Coskun et al., 2016).

Glucose levels serve as specific indicators of sympathetic activation during stress conditions, with the body's homeostatic mechanisms regulating blood glucose within a defined range (Skulkarni and Bedjargi, 2016). In the sampled fish, the highest glucose content was observed at Ajebamidele (29.07 mmol/L), and similar trends in serum glucose levels have been reported in Oncorhynchus mykiss (Coskun et al., 2016). Lower glucose content in polluted waters may result from impaired gluconeogenesis. Serum cholesterol, an essential steroid metabolite in cell membranes and blood plasma, plays a critical role in the proper functioning of the body and serves as an indicator for the synthesis of sexual hormones and corticosteroids in fish (Ruqaya et al., 2012). Serum urea levels in this study align with those reported in *Clarias gariepinus* (Mozanzadeh et al., 2015).

AST, ALT, and ALP are sensitive biomarkers in ecotoxicology, which provides early warning signs of potential harm to contaminated aquatic organisms (Nel et al., 2009). In this study, there was a significant increase in serum enzyme activities (AST, ALT, and ALP) compared to the Earthen Pond at other sampling points. Increased AST and ALT activities in Asian walking catfish (*Clarias batrachus*) have been linked to enhanced synthesis of liver enzymes or hepatic cell injury, as noted by Akter et al. (2023).

Lipid peroxidation serves as a valuable indicator for assessing oxidative stress in cells and tissues. It is a well-established method for describing cellular damage caused by oxidative processes. (Leong et al., 2010). Glutathione is pivotal in antioxidant protection and in managing pathways that maintain cellular balance. It acts as a cleanser of internal and external substances and is involved in various cellular processes such as cell growth and programmed cell death, gene regulation, immune response, and the metabolism of cellular components (Javed et al., 2016). The highest levels of Lipid Peroxidation were observed in fish from Emirin (42.10 x 10-5 MDA/mg protein), indicating significant cellular damage. Ajebamidele fishes had the highest SOD activity (2.46 U/mm/mg protein), suggesting an increased defensive response to oxidative stress, while Emirin fish showed the highest GPx levels (8.22 GSH/min/mg protein), and catalase activity (8.22 nm H2O2/min/mg protein) reflecting an adaptive response to neutralize peroxides and active response to detoxify hydrogen peroxide. Erinfun fish had the highest total antioxidant activity (1.60 mmol/L), while Aduloju had the lowest (0.55 mmol/L), suggesting varying capacities to counteract oxidative damage. Hence, fish in more polluted areas exhibited higher oxidative stress markers, reflecting the impact of pollutants on cellular functions (Valko et al., 2005; Mustafa, 2012)..

Histological alterations were noted in the gill, liver, heart and kidney of the sampled fish from most of the sampled points, indicating that the fish are experiencing stress and damage due to the polluted water (Shahid et al., 2022). The gills of fishes caught at most of the sampled points showed necrosis of the epithelia, loss of secondary lamellae and oedema except at Emirin, Aduloju and Earthen Pond. Fish use their gills as their primary respiratory organ (Roberts and Smith, 2011). The thin epithelium covering these organs regulates acid-base and ionic balance, facilitates gas exchange, and excretes nitrogenous waste (Evans et al., 2005). Therefore, cases of lamellar fusion, oedema and hyperplasia, gill bridging, necrosis, and epithelial lining is likely to occur in those exposed to waste water (Javed and Usmani, 2012) especially heavy metals (Abdel-Khalek, 2015). The primary metabolic organ in fish where detoxification takes place is the liver (Bruslé and Anadon, 2017); hence, it may be more susceptible to deterioration. The liver of fishes from the Earthen Pond showed mild mononuclear infiltration and mild vacuolation of hepatocytes however, in the liver of fishes from other sampling points connected to Ureje river, severe macrophage and lymphocytes infiltration, coagulative necrosis of the hepatocytes, vacuolation of hepatocytes, congestion, loss of architecture, severe steatosis and presence of giant cells were noticed. These histological alterations are connected to how hepatocytes react to toxins (Paulino et al., 2014). The livers of several fish species have been shown to have similar histological report on heavy metal contamination (Velma and Tchounwou, 2010). Also, for fishes analysed from the Earthen Pond, the heart had no visible changes, and the kidney only showed mild necrosis and moderate infiltration of the interstitium. However, the kidney analysis from other sampled points showed severe alteration. Necrosis or flattening of tubular epithelial, lymphocytes and macrophage infiltration in the interstitial and tissue damage have been linked with metals contaminated water (Tetreault et al., 2012).

The result of biochemical and histopathological examination in this study indicates that fish exhibit both adaptive and stress responses to pollution. Elevated stress markers and enzyme activities reflect the physiological burden of pollution (Stoliar and Lushchak, 2012), while some adaptive mechanisms (e.g., increased total protein and reproductive adjustments) are also evident. Heavy metals contaminations in river are capable of inducing alterations likely to affect the wellbeing of catfish (Adewuyi et al., 2010; Łuszczek-Trojnar et al., 2014). These are likely to be accountable for changes in live weight and organ weight, oxidative stress biomarkers, histopathological changes in gill, liver, heart and kidney of the catfishes under study from Ureje River. The contamination of fish with heavy metals and the observed histopathological alterations raises concerns about the safety of consuming fish from the Ureje River. Chronic exposure to heavy metals through fish consumption can lead to serious health issues in humans, including neurological, cardiovascular and developmental problems (Jaishankar et al., 2014; Isangedighi and Gift, 2019).

This study underscores the need for stringent environmental regulations and pollution control measures to protect the Ureje River. This includes better waste management practices for industrial and agricultural activities to prevent the discharge of harmful substances into the river. Regular monitoring of water quality and aquatic life health is crucial for detecting and addressing pollution issues promptly (Rini, 2011; Risjani et al., 2020). Implementing and enforcing policies that limit the use of hazardous chemicals and promote sustainable practices is also essential.

However, it should be noted that industrial activities and use of Ureje water differs around the sampled points, and therefore, the inconsistency of some parameters measured. Further research to closely monitor the industrial activities and assessment of environmental pollution around these selected sampled points of Ureje River on total fauna is underway.

Conclusion

This study highlights the significant impact of environmental pollution on the Ureje River's water quality and the health of aquatic organisms. Pollutants from industrial, agricultural, and domestic sources are contributing to the degradation of the river's ecosystem. Fish in more polluted areas exhibited higher oxidative stress markers and organ pathologies, reflecting the impact of pollutants on cellular functions.

This study recommends crucial efforts to mitigate these issues, implementation of effective pollution control measures, enhance environmental regulations, and promote sustainable practices to protect the river ecosystem and ensure the safety of fish for human consumption. Regular monitoring and assessment of water quality and aquatic health are essential for the sustainable management of the Ureje River. Additional studies are recommended to explore long-term impacts of pollution on aquatic ecosystems and to develop strategies for mitigating these effects.

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