https://chemclassjournal.com/
ChemClass Journal Vol. 9 Issue 1 (2025); 148-164
e-ISSN:0198-2734 p-ISSN:0198-5680
doi.org/10.33003/chemclas_2025_0901/012

Histopathological Analysis And Heavy Metal Concentration of Tilapia Fish (*Orechromis mosambicus*) Harvested From Rivers Used For Mining Activities

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Abstract

This research work was aimed at determining heavy metal concentrations and histopathological assessment of selected parts of Tilapia fish (*Orechromis mosambicus*) as a biomarker for heavy metal pollution of the rivers located in Anka and Maru LGAs of Zamfara state used for gold mining activities at two different season. Twelve (12) pieces of average size tilapia fish, three from each river were harvested and sacrificed, parts such as the gills, liver and skin (muscle) of the fish samples were processed and digested using standard method for AAS heavy metal determination. Histopathological analysis was also conducted the selected parts using gold standard method. The concentrations of all the heavy metals are generally higher during the dry season than in the wet season. However, Zn and Cr concentrations in the fish were within international safe limits of 5mg/l and 0.1 mg/l respectively, while the highest concentrations were recorded in the dry season for Cd, Pb and Hg (0.963±0.001 – 0.275±0.002), (0.828±0.001 – 0.187±0.002) and (1.349±0.003 – 0.219±0.002) respectively. These recorded values far exceed the WHO and USEPA limits of 0.01mg/l, 0.01mg/l and 0.001mg/l for Cd, Pb and Hg levels respectively in water. Pollution load index were greater than 1 indicating severe contamination. The histology assessment of tissues such as gills, liver and skin (muscle) showed that most common gill abnormalities observed in the fish sample inhabited in all the locations have desquamation of lamellar epithelium, hypertrophy of epithelial cells, lifting up of lamellar epithelium, intraepithelial oedema, aneurysm, hyperplasia, and haemorrhage in the gill filament. Histology of liver revealed the presence of heterogeneous parenchyma characterized by vacuolization, foci of necrosis, hypertrophy of nuclei and degenerated hepatocytes. Histology changes of the skin (muscle) were mostly at the level of the epidermis, without major changes in the dermis and hypodermis. The result of the heavy metal concentration particularly those of Pb, Cd and Hg are exceptionally high and should cause trepidation to regulatory authorities as continuous consumption of both water and fish from this river will cause public health issues.

Key words: Gills, heavy metals, liver, river water, skin and tilapia fish

Introduction

The arbitrary dumping of waste effluents to large water bodies has negatively affected both water quality and aquatic life [1], [8]. Food and Agricultural Organization of U.S.A. revealed that in African countries, particularly Nigeria, water related diseases had been interfering with basic human development [18]. Different aquatic organisms often respond to external contamination in different ways, where the quantity and form of the element in water, sediment, or food will determine the degree of accumulation [1], [30].

Several scientific literatures have reported a series of environmental and biological problems associated with artisanal mining. For example, Heavy metal contaminations and poisoning due to artisanal gold mining activities are widely reported in Ghana, Ecuador, Tanzania (Geita Gold mine) and Brazilian Amazon [14], [15], [26]. In addition, studies of small-scale gold mining sites in the Migori gold belt (Kenya) also demonstrated lead, mercury, and arsenic pollution due to gold processing [27], [31]. High levels of mercury pollution were also found on gold mining and processing sites of Philippines [15]; [13]. During the processing of the ores for gold, poisonous substances such as oxides and sulphides of heavy metal pollutants are released into the environment [11], [5], [12].

Histopathological changes in animal tissues are powerful indicators of prior exposure to environmental stressors and are net results of adverse biochemical and physiological changes in an organism [23]; [33]. For field assessment behavioral studies, histopathology is often the easiest method of assessing both short and long term toxic effects. Histopathological biomarkers can be indicators of the effects on organisms of various anthropogenic pollutants and a reflection of the overall health of the entire population in the ecosystem. Well documented lesions based on experimental data in liver, kidney, gill, ovary, skeleton system and skin have been used as biomarkers [35], [11], [4].

Therefore, histopathology is the gold standard when defining toxicological effects and its evaluation remains an important part of the assessment of the adverse effects of xenobiotics on the whole organism [36], [39]. The worrying high levels of trace metals in foods have prompted several regulatory bodies such as the WHO to establish maximum allowable concentrations for some of the metals in food [42]. Thus the World Health Organization (WHO) as well as the Food and Agriculture Organization (FAO) of the United Nations state that monitoring elements such as Hg, Cd, Pb, As, Cu, Zn, Fe, Sn in fish is obligatory while the monitoring of others though not mandatory may be useful [38], [6]. The assessment of metal burden in fishes and water bodies will create greater environmental awareness regarding the consumption of water and fishes [2], [3].

The aim of the research is to determine the concentration of heavy metal (Cd, Cr, Zn, Pb and Hg) in wet and dry season and histopathological analysis on fish sample (*Orechromis mosambicus*) from three selected rivers in Anka LGA (Bagega, Sunke and Abare) and a major river in Maru LGA of Zamfara State, Nigeria.

Materials and Methods

Collection of Fish Samples: Twelve pieces of Tilapia *(Orechromis mossambicus)* fish samples of 28 cm average length and 52 g average weight, three from each of the four sampling locations was collected by a local fisher man. The fish samples were rinsed with distilled water immediately to remove any adhering contaminants and were stored in a frozen polythene bags. The fishes were immediately transported to a laboratory where they were dissected and the parts removed for histopthalogical analysis and digestion for heavy metal determination.

Digestion of the Fish Sample [14]: Exactly 5 g dry weight fish sample was put into a 50 ml beaker with 5 ml of HNO_3 and 5 ml of H_2SO_4. When the fish stopped reacting with HNO_3 and H_2SO_4, the beaker was then placed on a hot plate and heated at 60°C for 30 min. After allowing the beaker to cool, 10 ml of $HNO_3$was added and returned to the hot plate to be heated slowly to 120°C. The temperature was increased to 150°C, and the beaker was removed from the hot plate when the samples turned black. The sample was then allowed to cool before adding H_2O_2 until the sample was clear. The content of the beaker was transferred into a 50 ml volumetric flask and diluted to the mark with distilled water. Metals were analyzed by Atomic Absorption Spectrometry (HG – AAS, ICE 3000AA01122804v1.30 Series).

Result and Discussion

Table 1: Heavy Metals Concentrations (mg/kg) of Fish Samples for wet and dry Seasons

Sampling location	Zn	Cd	Cr	Pb	Hg
Maru wet	0.013 ± 0.001	0.588±0.001	0.336±0.003	0.577±0.007	0.871±0.003
Dry	0.036±0.003	0.609±0.005	0.697±0.002	0.477±0.002	0.871±0.003
Bagega wet	0.019± 0.002	0.188±0.001	0.216±0.003	0.178±0.002	0.567±0.003
Dry	0.017±0.002	0.275±0.002	0.681±0.004	0.792±0.001	0.581±0.003
Sunke wet	0.049±0.002	0.177±0.002	0.105±0.002	0.177±0.001	0.717±0.001
Dry	0.066±0.001	0.511±0.002	0.397±0.003	0.828±0.001	0.839±0.003
Abare wet	0.334± 0.003	0.250±0.005	0.163±0.003	0.250±0.003	0.900±0.005
Dry	0.447±0.003	0.256±0.005	1.315±0.002	0.277±0.002	1.349±0.003

Key: ND: below detection. Result are mean ± standard deviation.

Table 2: Correlation matrix of Heavy Metals Concentrations (mg/l) in Fish Samples for Wet and dry seasons

	Correlations	For wet				Correlations	For dry			
	Zn	Cd	Cr	Pb	Hg	Zn	Cd	Cr	Pb	Hg
Zn	1					1				
Cd	-.083	1				.228	1			
Cr	.055	.266	1			.823**	-.023	1		
Pb	.162	.633**	.111	1		-.334*	-.206	-.371*	1	
Hg	.118	.763**	.530**	.137	1	-.040	-.828**	.025	.082	1
	36	36	36	36	36	36	36	36	36	36

**.Correlation is significant at the 0.01 level (2-tailed). **.Correlation is significant at the 0.01 level (2tailed).

. *.Correlation is significant at the 0.05 level (2tailed)

Table 3: Heavy Metal Contamination Factors (CF) and Pollution Load Index (PLI) in Fish for Wet and Dry Seasons.

Sampling Location/Wet	Zn	Cd	Cr	Pb	Hg	PLI
Maru	0.0005	23.8000	0.6550	31.2500	476	2.5769
Bagega	0.0003	10.4500	1.7650	18.8000	567	2.2602
Sunke	0.0009	10.0500	1.5050	23.9500	717	2.9768
Abare	0.0065	13.4000	1.5250	30.2000	900	5.1417
Dry						
Maru	0.0006	71.7500	6.8750	33.200	585	5.6886
Bagega	0.0007	35.3500	6.8750	69.8800	400	5.3575
Sunke	0.0015	53.0500	3.9350	72.1500	857.5	7.2398
Abare	0.0124	60	13.0750	33.1500	1349	13.4801

Histopathology Observation of the T. Fish (Orechromis *Mosambicus)* from all the sampling Locations

Histopathological Observation of the Gills (Plates 1 – 4): The gills of a fish comprises of multifunctional organs (respiration, ion regulation, acid-base balance and nitrogenous waste regulation, excretion), which constitute over 50 percent of the total surface area of the fish that make it sensitive to chemicals in water.

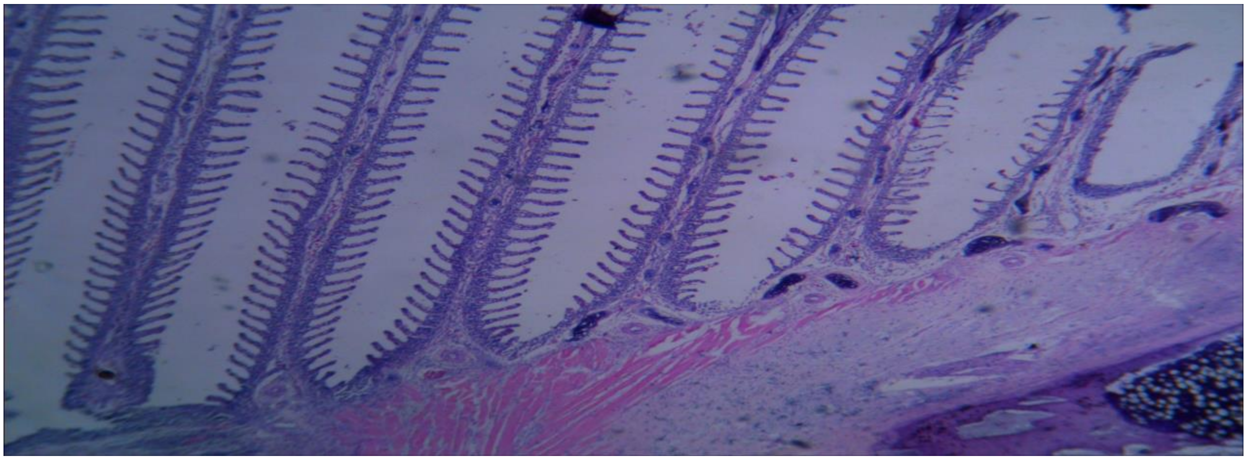

Plate 1: Control Group (Gills; H&E; x40)

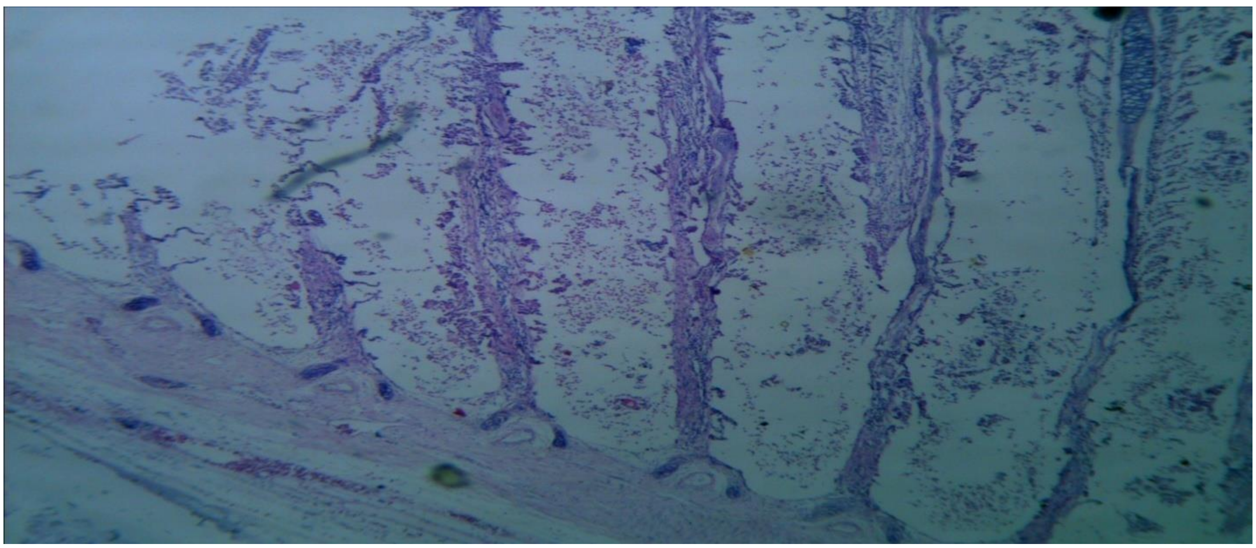

Plate 2: Bagega river (Gills; H&E; x40). Report: Photomicrograph of section of gill showing disappearance of secondary lamellae, disintegration and destruction of gill epithelium (black circles). Desquamation and necrosis of primary gill epithelial cells (black arrow).

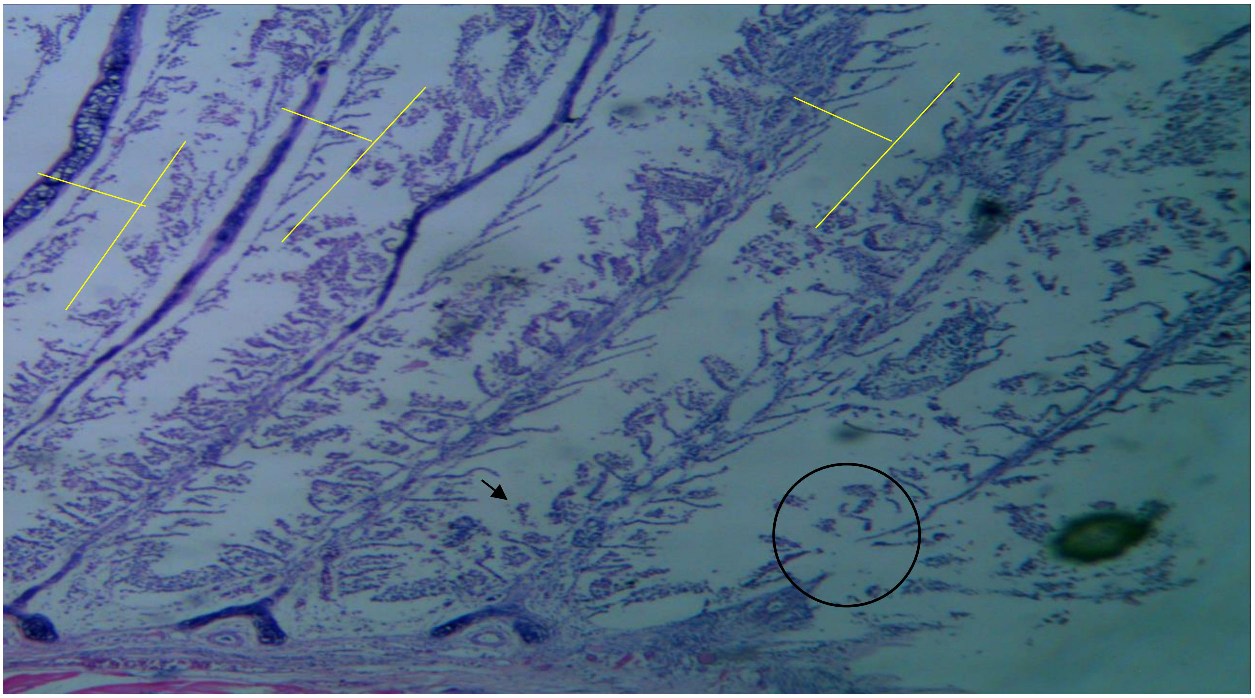

Plate 3: Sunke river **(Gills; H&E; x40**). Report: Photomicrograph of section from gill showing thin and curling secondary lamellae (arrow head); desquamation and necrosis of gill epithelium (yellow arrows & bars); complete disintegration of primary gill and epithelium (black circle)

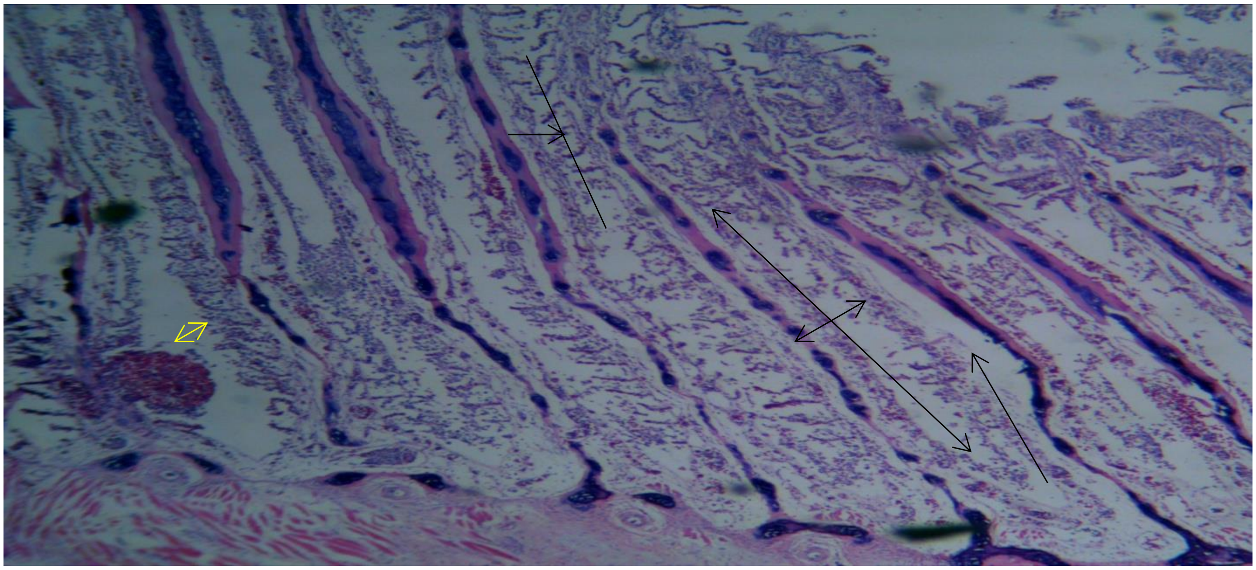

Plate 4: Abare river (Gills; H&E; x40). Report: Thinning and curling of secondary lamellae (arrow & bar); desquamation and necrosis of gill epithelial cells (cross double head arrows); oedematous changes (long arrow) and haemorrhage (yellow arrow heads)

Histopathology Changes of Liver (plates 5 – 8): In general, liver is a target organ due to its large blood supply that causes noticeable toxicant exposure and accumulation and also its clearance function and its pronounced metabolic capacity [24]. The liver of fish samples in all the four locations show sign of abnormalities such as irregular shaped hepatocytes, cytoplasmic vacuolation and nucleus in a lateral position in the siluriform Corydoras paleatus as if they have been exposed to organophosphate pesticides

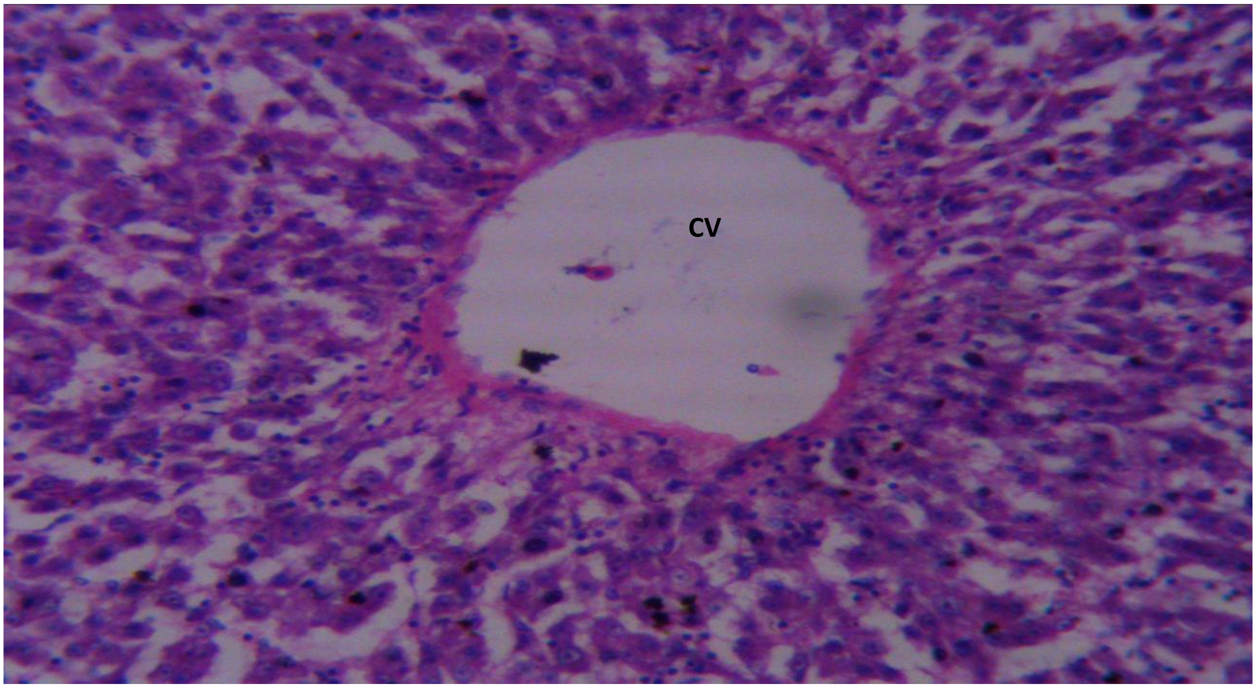

Plate 5: Control Group (Liver; H&E; x250). Report: Photomicrograph of section from liver of *T. mosambicus* showing the normal cellular pattern of the liver histo-architecture.Normal cv (central vein), hepatocytes (arrow heads).

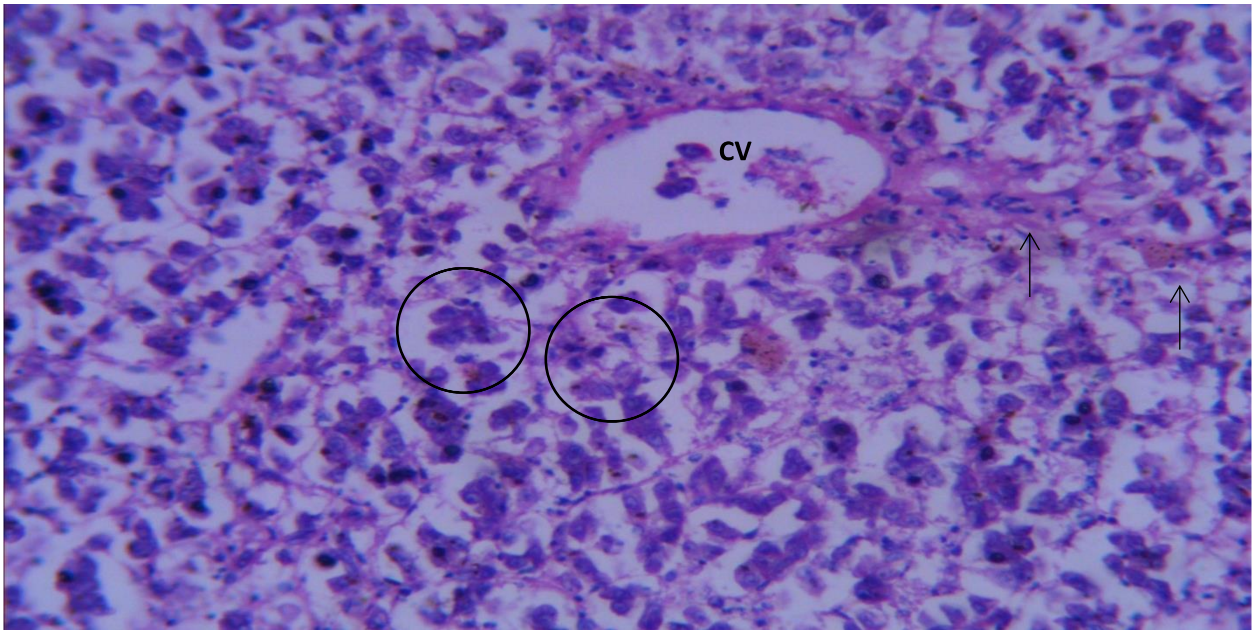

***Plate 6: Bagega river** (Liver; x250). Report: photomicrograph of section of liver showing distorted cellular pattern of the liver with evidence of hepatocellular microvesicular fatty change in hepatocytes; necrosis of hepatocytes (pyknotic nuclei-black circles; karyolitic nuclei-black arrows)*

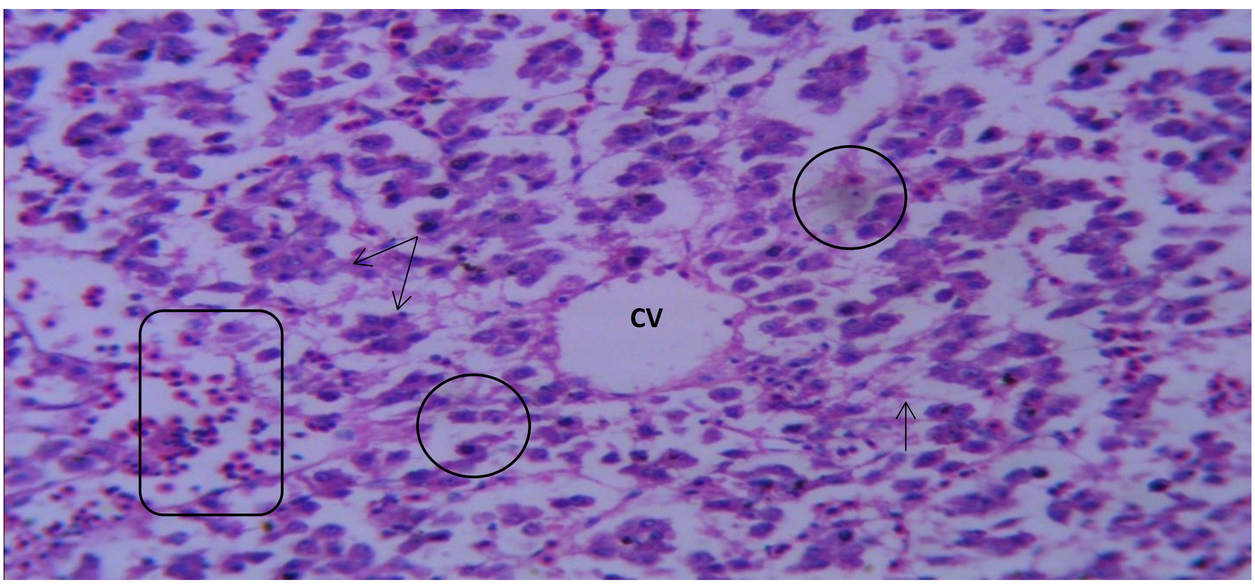

Plate 7: Sunke river (Liver; x250). Report: Photomicrograph of fish liver section showing distortion in the cellular pattern of the liver. Hepatocellular microvesicular fatty change seen in hepatocytes (single and double black arrows); necrosis of hepatocytes (pyknotic nuclei-black circles and karyhexic nuclei-brown arrow); mononuclear immunologic cells infiltration (black rectangle)

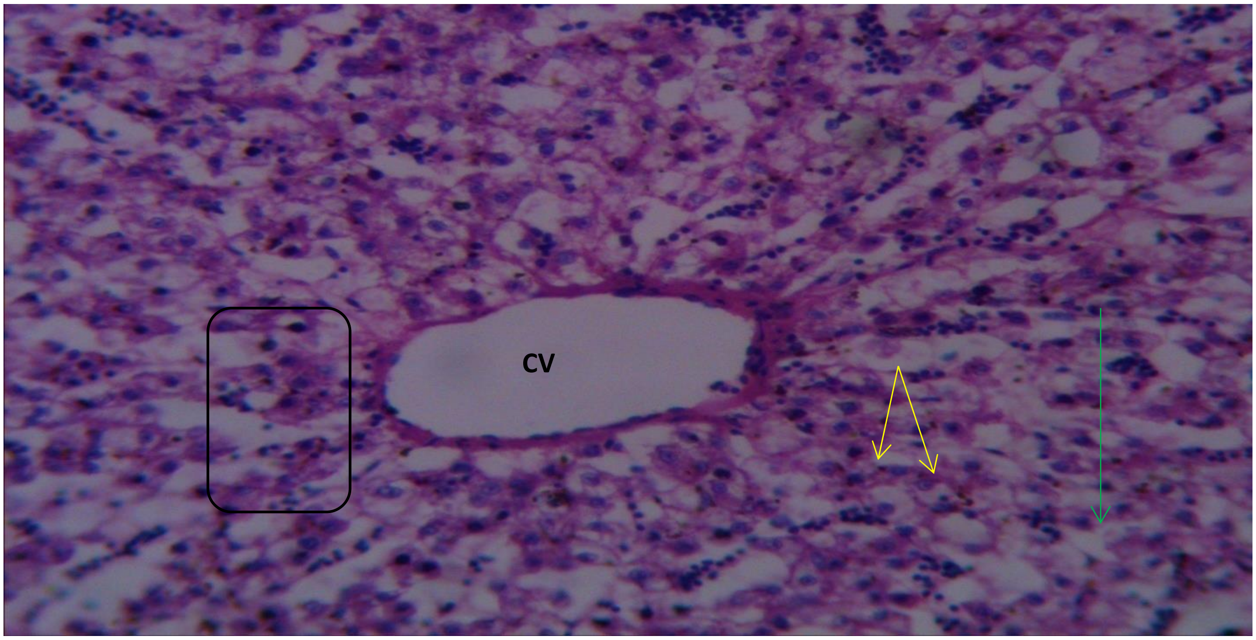

Plate 8: Abare river (Liver; H&E; x250). Report: Photomicrograph of section from liver showing a distorted cellular pattern of the liver. Hepatocellular microvesicular fatty changes seen (rectangular black arrow); necrosis of hepatocytes pyknotic nuclei- (green long arrow); karyolitic nuclei – (arrow head) and mononuclear cells infiltration (double brown arrow) was seen

Histopathology Changes of Skin (plate 9 – 12): From the observed histological sections of all the fish skin, it was revealed that Bagega, Sunke and Abare rivers showed infected epithelium i.e epithelial hyperplasia. However, the fish sample in this work showed response to the direct effects of the pollutants.

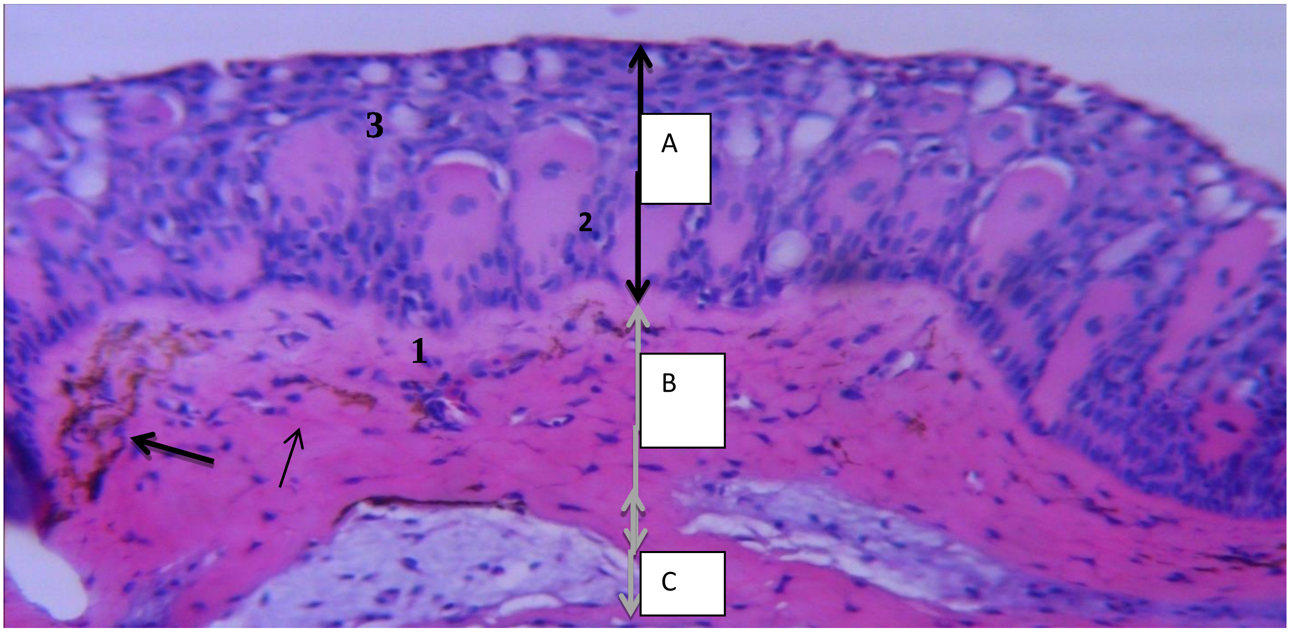

Plate 9: Control Group (Skin, H&E stain; x250). Report: section from *Tilapia mosambicus* showing the normal histo-architecture of the skin and its layers. A (epidermis), B (dermis), C (hypodermis): 1-basal cells; 2- alarm cells; 3-epithelial cells

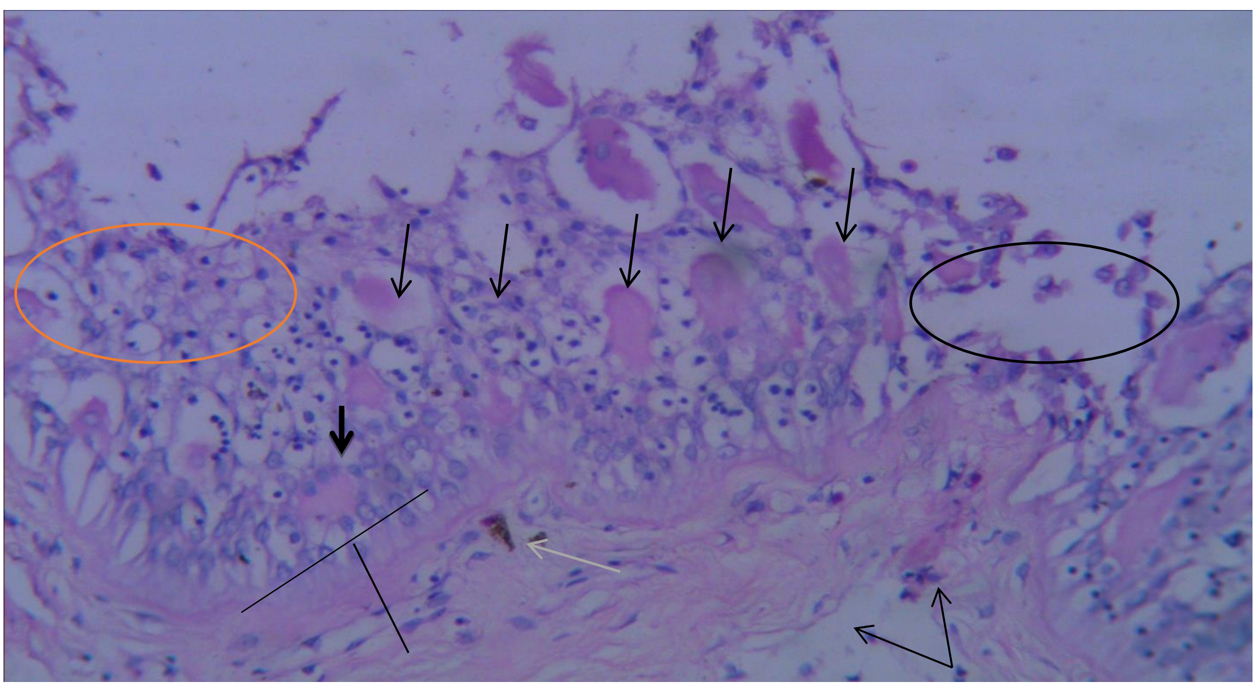

Plate 10: Bagega river (Skin, H&E; x250). Report: photomicrograph from section of skin (*T. masambicus*). There was massive necrosis of the epidermis characterized by pyknosis of epithelial cells (black arrows);

karyolysis of nuclei of alarm cells (black arrow head) hyperplasia of mucus cells (brown circle); area of complete necrosis of cells (black circle); decreased melanin secretion (green arrow) and pyknosis of cells in dermis (black double head arrow).

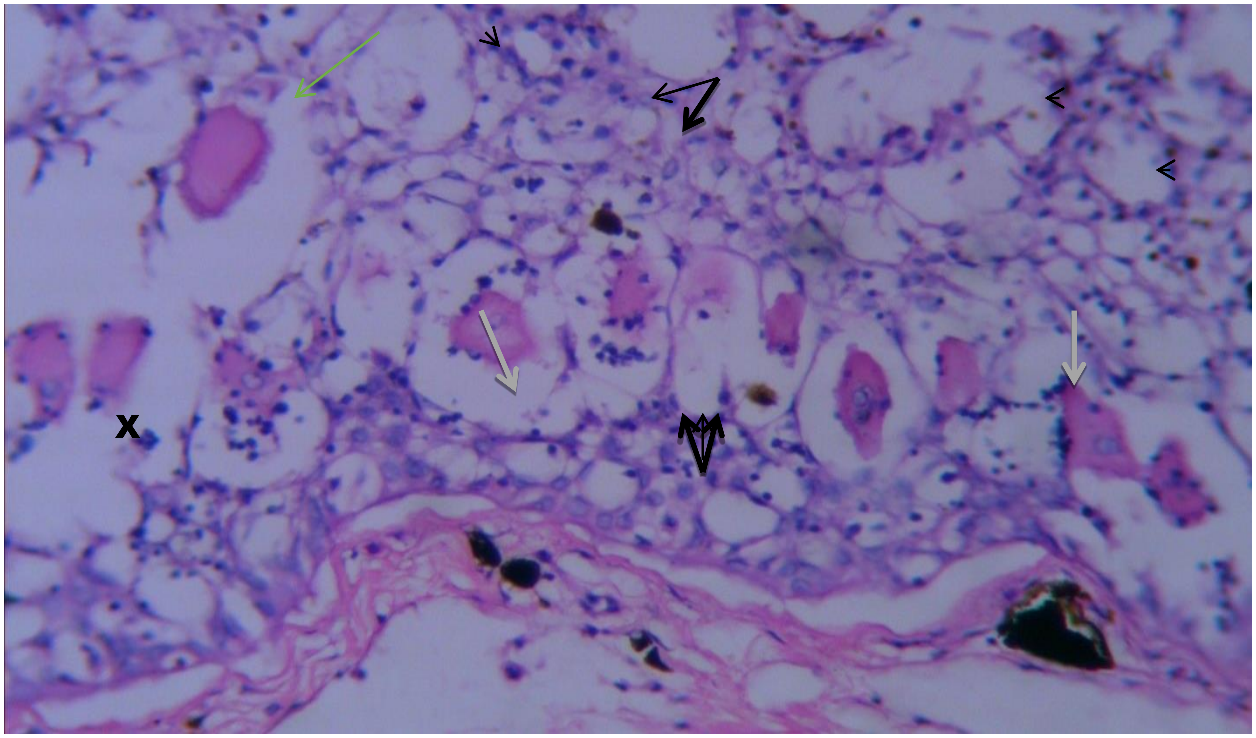

Plate 11: Sunke river (Skin, H&E; x250). Report: micrograph section from *Tilapia mosambicus* showing destruction of the epidermis and dermis of the skin. Necrosis of epithelial cells (pyknosis of epithelial cell-double head arrow); massive vacoulation and hypertrophy of fat cells (black arrow head); hypertrophy of basal cells (triple head black arrow). Necrosis of dermis (x) and darkening/condensation of melanin (green arrow).

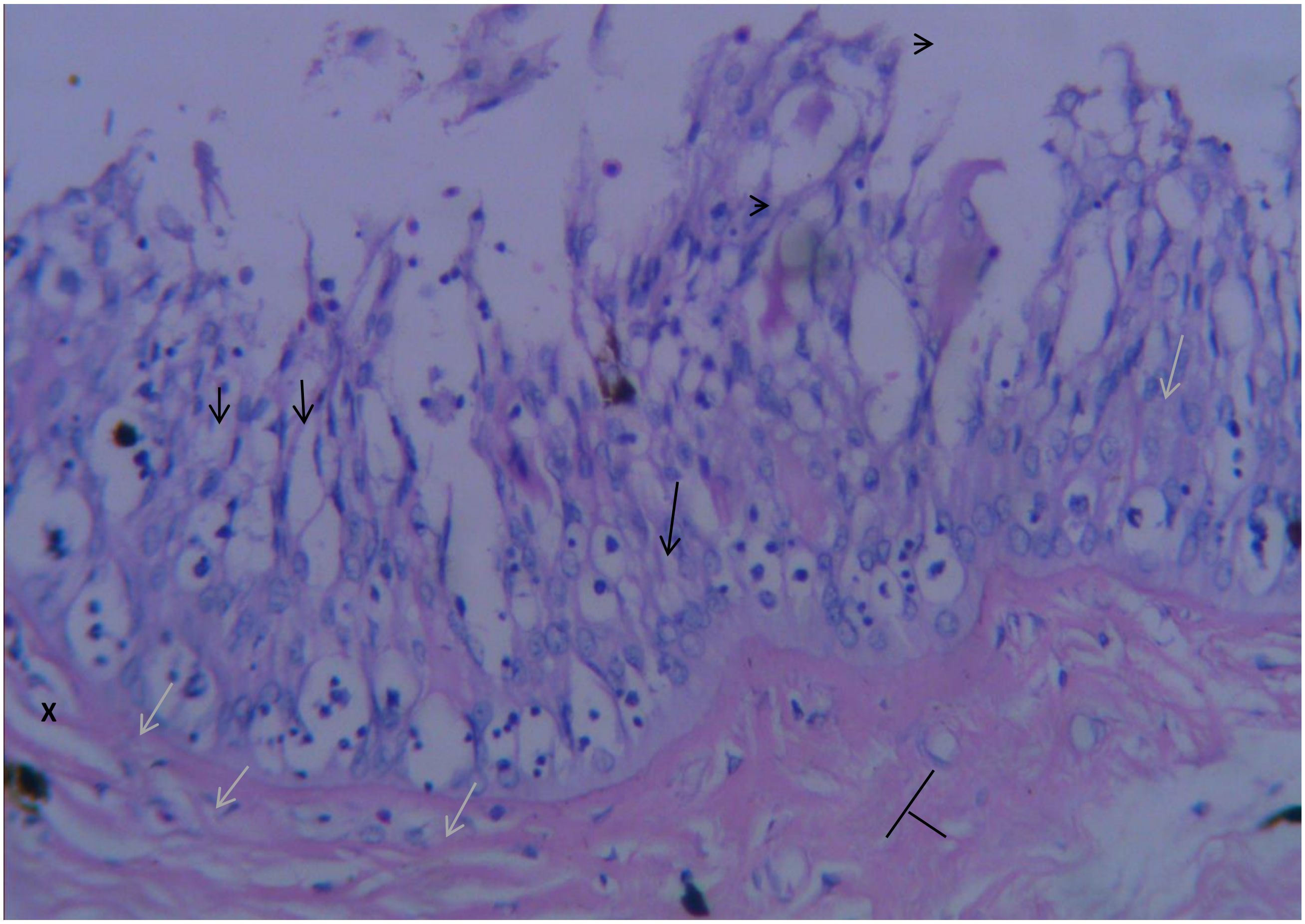

Plate 12: Abare river (skin; H&E; x250). Report: photomicrograph section of skin from *T. mosambicus.* There is massive necrosis of epithelial cells of the epidermis; massive vacoulation and hypertrophy of fat cells (green arrows); complete necrosis of alarm cells (pyknotic basal cell) and hypertrophy of basal cells (black T). Darkened/condensed melanin (x) and necrosis of dermis; 1-2 mononuclear cells sited (black arrow head).

Heavy Metal Content in Fish

Fish is widely acceptable for consumption because of its high palatability, low cholesterol and tender flesh [26]; [9] and also a cheap source of protein. However, the high concentration of non-essential heavy metals in some of the fish evaluated (*T. mosambicus)* is a source of concern. The fish sample in the entire river was analyzed for heavy metals for the period for both wet and dry seasons.

From table 1, the high level of heavy metals in the fish samples could probably be due to the prior exposure of these fishes to toxicants from polluted water body before swimming down to the reservoir since they are pelagic fishes. [23], [37] linked the accumulation of the toxicants in the fish to the possibilities of the fish to accumulate metals and thus could serve as indicators of pollution.

Pearson correlation analysis (Table 2) was conducted to examine whether there is a relationship between the heavy metal concentrations in the fish sample for wet and dry seasons. The result revealed a significant positive relationship for Pb and Cd, Hg and Cd and Hg and Cr respectively for wet seasons. This may explain the consistent variation of these metals in the locations in a particular season. While during the dry seasons, a significant and negative relationship was established for Pb and Zn, Pb and Cr and Hg and Cd then a significant and positive relationship for Cr and Zn respectively. This explain the different sources of these metals in the environmental samples. Generally, the concentrations of heavy metals (Zn, Cd, Cr, Pb and Hg) determined were higher in the dry seasons than in the wet seasons. This situation is expected in view of the reduction in the pollution in the wet season arising from increased dilution and water flow. This observation is also reported in a similar work by Asaolu and Olafe [7] for fishes and crayfish from coastal water of Ondo State. The concentrations of the metal in the fish samples are lower than the one reported by [21] for Niger Delta, Okoye [28] for Lagos lagoon and Asaolu [7] for Ondo State Coastal area. The concentration of Zn in fish in this work, fall below the maximum zinc level permitted for fish (50 mg/kg) according to Food Codex and WHO, 2004. The values recorded for this work are lower compared to Zn values reported for freshwater fishes in China [16]. United States environmental protection agency and the European Commission (US-EPA and EC) have not considered any standards or limits for the zinc concentrations in fish [44]. Cadmium is one of the most toxic elements with reported carcinogenic effects in humans [20]. High concentrations of Cd have been found to lead to chronic kidney dysfunction [32]. The highest mean concentrations of 0.963 mg/kg in this work were recorded at Abare river. This may have come from natural sources, run-off from agricultural soils around the dams and rivers where phosphate fertilizers are in use, and more so, during the dry season when the volume of water of ponds and wells has reduced, miners relocate the mining processing closer to the rivers where there is large volume of water, this was also in agreement to the report of [38]; [16].The concentration recorded in this work are above the 0.01 mg/kg maximum permissible level for fish consumption [42]. The standard level Pb was reported to be 0.5 mg/kg dry weight [18]. Similar to Pb, Cadmium was also high in the fishes and highest in Abare (0.963 mg/kg). The standard permissible level of 0.05 to 5.5 mg/kg fish dry weight was reported for Cd [18]. The concentrations of lead across all the sites are generally high when compared with the 0.01 mg/kg maximum permissible level [25]; [42]. This high concentration of lead might have come from sewage and agricultural and mining wastes discharged into the dams and rivers [17]. Mercury concentrations are high across all locations particularly Abare river (1.349±0.003 mg/kg) is alarming. The observed heavy metal concentrations in this fish are above the 0.001

mg/kg recommended limit for human consumption [14]; [31] and [42].

In conclusion, the result of the heavy metal concentrations particularly those of Pb, Cd and Hg are exceptionally high both in the wet and dry seasons, which are higher than the USEPA and WHO allowable limits in fresh water bodies. The high pollution load index calculated in this work also gave credence to the recorded high concentrations of the heavy metals. The histopathological assessment also confirmed the high concentration as all the fish samples analyzed showed typical skin lesion and internal tissues hyperplasia and haemorrhage. These high concentrations should cause trepidation to regulatory authorities as continuous consumption of both the fish and water from this river will cause health issues.

Acknowledgement: I want to particularly acknowledge the Tertiary Education Trust Fund (Tetfund) for the sponsorship of this research under the Institution Based research grant and the Federal Polytechnic, Kaura-Namoda for opportunity.

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