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Seasonal Variations of Heavy Metals Concentrations in Water and Histopathological Evaluation of Tilapia Fish (*Orechromis Mosambicus*) Harvested from Gusau, Kaura - Namoda and Mafara Dams, Zamfara State, Nigeria

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ABSTRACT: The objective of this paper was to investigate the seasonal variation of heavy metal concentration in water and histopathological evaluation of tilapia fish (Orechromis mosambicus) harvested from Gusau, Kaura-Namoda, Tsafe and Talata Mafara dams in Zamfara state, Nigeria using appropriate standard procedures. Results obtained showed that the concentrations [mg/l] of Pb in the Dams were 0.0038±0.64 (Gusau dam); 0.0069±0.23 (Kaura-Namoda dam); 0.0057±0.89 (Tsafe dam), 0.0076±0.75 (Talata Mafara Dam) during the rainy season and 0.0067±0.67(Gusau dam); 0.124±0.88 (Kaura-Namoda dam); 0.1678±0.93 (Tsafe dam), 0.1563±0.07(Talata Mafara Dam) for the dry season respectively. The result revealed a spatial and non-uniform distribution in the concentration of the heavy metals (Zn, Cd, Cr, Pb and Fe) in the water, in all locations studied. The concentrations of heavy metal are generally higher during the dry season than in the wet season. Zn and Cr levels were within international safe limits while Cd, Pb levels were far above the WHO and USEPA limits. 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.

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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 (FAO, 2007). 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 (Abdulrahman *et al.*, 2008; Olaoye; Onilude (2009). Certain environmental conditions such as salinity, pH, and water hardness can play an important factor in heavy metals accumulation up to toxic concentrations in the living organism and cause ecological damage (Garba *et al.*, 2010). Thus, heavy metals acquired through the food chain as a result of pollution are potential chemical hazards that threaten

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consumers (Olaofe *et al.,* 2004). The uncontrolled dissemination of waste effluents to large water bodies has negatively affected both water quality and aquatic life (Abdurahman *et al.,* 2008). A study by Umeh *et al.,* (2004) showed that 48% of the people in Katsina-Ala Local Government Area of Benue State are affected by urinary schistosomiasis, due to increase in water pollution.

Garba *et al.,* (2010) reported a mean arsenic concentration of 0.34 mg/l in drinking water from hand dug wells, boreholes and taps of Karaye Local Government Area, Kano State. The arsenic levels are of serious concern to regulatory agencies because they by far exceed the upper band of 0.01 mg/l recommended by WHO (2008). Nwidu *et al.* (2009) reported that quality of water and prevalent water related diseases in hospitals were casually related to contamination of the ware sources. Several studies have shown that the effects of human activities like agricultural runoffs, sewage and industrial effluents contaminate freshwaters resources (Malik *et al*., 2012). The effects of human activities on water quality differ in magnitude from one place to another. Changes in the physical, chemical, and biological characteristics of water negatively affect both human and ecosystem health (Nwidu *et al.,*2009). 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 (Malik *et al*., 2012).

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 (Reddy et al., 2010; Butchiram *et al*., 2013; Akpolih, 2013). 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. 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 (WHO, 1984). 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 (Staniskiene *et al*., 2006). The assessment of metal burden in fishes and water bodies will create greater environmental awareness regarding the consumption of water and fishes (Adefemi *et al*., 2008). Metals, particularly heavy metals such as lead, mercury, cadmium and arsenic constitute a significant potential threat to human health, both occupational and environmental (Hu, 2000).several workers have reported the use of water as an indicator for metal pollution of oceans, coastal areas, estuaries, rivers and lakes (Chang *et al*., 2009; Garba *et al*., 2010). The values or concentrations attributed to water quality parameters can be used to describe the pollution status of the source, its biotic status or to predict the likelihood or otherwise of particular organisms being present (SAWQG, 1996; Aiyesanmi *et al*., 2006). The menace of water-borne diseases and epidemics still looms large on the horizon of developing countries as a result of lack of accessibility to good quality water (Packham, 1996; WHO, 2002). Polluted water has been the cause of all such cases, in which the major sources of pollution are domestic and municipal wastes from urban and industrial activities and runoff from farmland (Apina, 1999; Baig *et al*., 2010). Hence, the objective of this paper is to investigate the seasonal variation of heavy metal concentration in water and histopathological evaluation of tilapia fish **(***Orechromis mosambicus*) harvested from Gusau, Kaura-Namoda, Tsafe and Talata Mafara dams in Zamfara state, Nigeria

MATERIALS AND METHODS

Sample area and Sampling Points: The dams are located in Gusau LGA (Gusau dam) Kaura -Namoda, and Maru LGA, Tsafe dam, Talata Mafara.

*Sample Collection and Preparation***:** Sample collection: Water samples from the different dams and rivers were collected at three different points in a location to form a composite water solution. The sterile sampling bottle with a rope tied around the neck was dipped into the water source; water was allowed to flow into the bottle and closed immediately. Twelve pieces of Tilapia (*Orechromis mosambicus*) fish samples of 28cm average length and 52g average weight, two from each of four sampling point was collected by a local fisherman. The fish samples were rinsed with distilled water immediately to remove any adhering contaminants and were stored in a frozen pre-cleaned polythene bag. The fishes were immediately transported to a laboratory where they were dissected and parts removed for histopathological analysis and digestion for heavy metal analysis. Metals in the water and fish samples were analyzed by atomic absorption spectrometry.

Digestion of water sample for heavy metal analysis by AAS: A well-mixed acid preserved water sample of about 100ml, was transferred into a 250ml beaker. Concentrated HNO_3 (5ml) was added and heated to boiling. This was then evaporated on a hot plate to 20ml. heating and addition of concentrated HNO_3 continued until digestion was completed as indicated by a light colour, clear solution. The content was then transferred to 100ml pre-cleaned plastic bottles, cooled and diluted to mark with distilled water. Portions of the solution were used for heavy metal digestion.

Histopathological analysis: the gills, skin and liver tissues with diameter of 5mm of six fishes, one from each sampling location were fixed in sera solution (60% ethanol+ 30% formalin + 10% acetic acid) for about 3- 4 hours. The fixed tissues dehydrated at room temperature, embedded in paraffin, sectioned at 4-5 mm by using a microtome (MICROM GmbH, Walldorf, Germany) and stained with haematoxylin and eosin (Bernet et al., 1999). The stained fish samples were then examined under a light microscope (Vickers Ltd, England) and photographed by using the Moticam 1000 camera (Motic, China).

RESULT AND DISCUSSION

From table 1 showing the concentrations of heavy metals in the dams during the dry and wet season, the concentration of Zn ranged from 0.0044±0.93 in Gusau dam during the wet season to 0.0067±0.16 in Talata Mafara dam. Generally, the concentration of all the other heavy metals analyzed in the work followed similar trend from Gusau dam to Talata Mafara dam, a situation which suggest seasonal variation in the concentration of the metals. The concentrations of all the metals followed this pattern: Gusau: Fe > Cd > Pb > Cr > Zn; Kaura-Namoda: Fe > Cd > Pb > Zn > Cr; Tsafe: Fe > Cd > Cr > Pb> Zn; T/Mafara: Fe > Cd > Pb > Cr > Zn . While during the dry season, the distribution of heavy metals follows the below trend: Gusau: Fe > Cd > Pb > Zn > Pb; Kaura-Namoda: Fe > Pb > Zn > Cr > Cd; Tsafe: Cd > Pb > Fe > Zn > Cr; T/Mafara: Cd > Fe >Pb> Zn> Cr respectively.

Table 1: heavy metal concentrations (mg/l) in water from dams in sampling location during the wet and dry seasons
Results are mean ± standard deviation

Location	Zn	Cr	Cd	Fe	Pb
Gusau	0.0044±0.93	0.0041±0.13	0.0254±0.31	0.21±0.27	0.0038±0.64
Kaura-Namoda	0.0057±0.31	0.0033±0.17	0.0411±0.29	0.15±0.32	0.0069±0.23
Tsafe	0.0049±0.47	0.0062±0.59	0.0482±0.32	0.14±0.97	0.0057±0.89
T/Mafara	0.0067±0.16	0.0069±0.17	0.0573±0.69	0.15±0.27	0.0076±0.75
Dry Season					
Gusau	0.0114±0.48	0.0341±0.41	0.0696±0.31	0.24±1.97	0.0067±0.67
Kaura-Namoda	0.0672±0.73	0.0645±0.23	0.0614±0.93	0.17±0.19	0.124±0.88
Tsafe	0.0393±0.62	0.0171±0.73	0.1891±0.78	0.16±0.43	0.1678±0.93
T/Mafara	0.0388±0.17	0.0164±0.07	0.1751±0.17	0.16±1.12	0.1563±0.07

Zn has a biochemical role in the life processes of all aquatic plants and animals; therefore, they are essential in the aquatic environment in trace amounts. Zinc is used in a number of alloys including brass and bronze, batteries, fungicides and pigments. Zinc is an essential growth element for plants and animals but at elevated levels it is toxic to some species of aquatic life (WHO. 2004). The levels of cadmium in the water samples from the six sampling locations were above the (WHO, 2004) standard values of 0.01 mg/l tor the survival of aquatic organism. The concentrations of Cr in water samples from the locations was found to be higher than the permissible limits of 0.1 mg/L set by (WHO, 2004). The highest level of lead was recorded in Tsafe dam when compared with others, while Gusau dam recorded the least value. Hence. Likely source of Pb in these water bodies is from mineral exploitation, soil erosion and runoff water (Winde *et al.*, 2010). Studies done elsewhere in Kenya indicate higher Pb concentration levels for example Ashraf *et al.* (2011) found mean Pb levels ranging from 0.26 - 0.99 mg/l in Lake Victoria. Also, Ashraf *et al.* (2011) recorded lower and higher mean Pb levels at different sites (nd - 0.047 mg/) in surface water of Athi river tributaries. The concentration of Pb in the water samples from this water bodies exceeded the permissible limit of 0.05 mg/l set by (WHO, 2004: USEPA, 2002).

Table 2: correlation analysis for heavy metals during the wet season

	Zn	Cd	Cr	Pb	Fe
Zn	1				
Cd	.394*	1			
Cr	-.130	-.074	1		
Pb	-.021	-.128	-.102	1	
Fe	-.023	.280	.360*	.034	1
N	18	18	18	18	18

**correlation is significant at 0.05 level (2 – tailed)*

Table 3: correlation analysis for heavy metals during the dry season

	Zn	Cd	Cr	Pb	Fe
Zn	1				
Cd	-.023	1			
Cr	.565**	-.247	1		
Pb	.243	-.306	.056	1	
Fe	-.017	.233	.025	-.189	1
N	18	18	18	18	18

correlation is significant at 0.05 level (2 – tailed)
correlation is significant at .001 level (2- tailed)

Generally, the natural sources of heavy metals in rivers and dams, Marine and Coastal water are through land, heavy fresh water inflow, agricultural waste, aquaculture discharge and river run-offs and the mechanical and chemical weathering of rocks (Ashokkumar *et al*., 2009). Correlation analysis showed a significant and positive relationship for Cd and Zn and Fe and Cr during the wet season and a significant positive at the (p>0.05) relationship for Cr and Zn in the dry season. Similar observation was reported on the study of heavy metals in Ureje dam in Ado-Ekiti by Adefemi *et al*. (2007) and in Kanji dam (Amoo *et al*, 2005) and other studies on surface water (Chapman, 1999; Asaolu *et al*. 1997; Karadede *et al*., 2000).

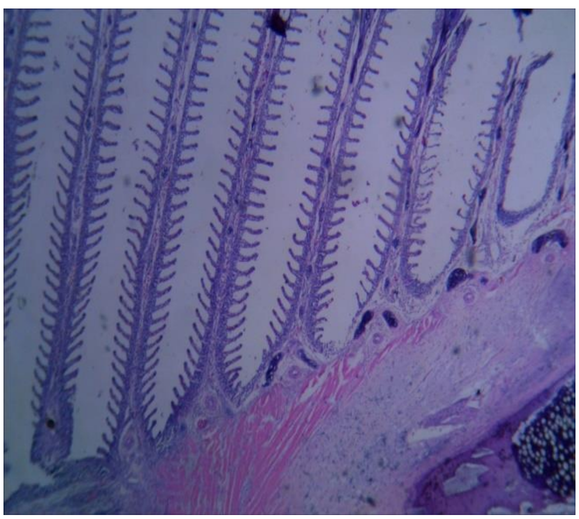

Plate 1: histopathology of control fish gills (H and E; x40)

Histopathological Analysis (plate 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. The fish gills play an important role in maintaining ionic homeostasis in both freshwater and marine environment. Consequently, injury to gill epithelium is a common response observed in fish exposed to a variety of contaminants (Mohamed, 2009). Hypertrophy of the pavement cells is possibly an event associated with necrosis cell swelling. These alterations, are more commonly associated with chronic exposures to metal contaminants than acutely lethal exposures (Rosety-Rodriguez *et al*., 2002). These are the gills lesions in response to a wide range of contaminants, including, petroleum compounds, organophosphates, carbonates, herbicides and heavy metals with a greater reported occurrence (Au, 2004). The same changes have also been reported in the gills of the fishes exposed to organic toxicants and metals and industrial effluent (Rosety-Rodriguez *et al*., 2002).

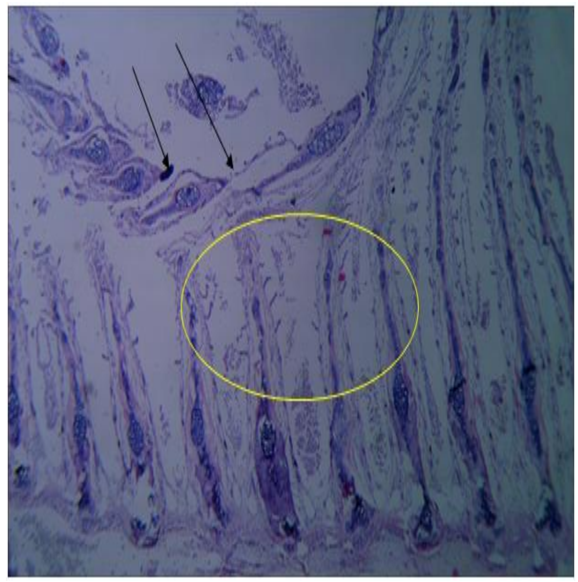

Plate 2: histopathology of Gusau dam fish gills (H & E x40). Report: Photomicrograph of section from gill showing thin and curling secondary lamellae (arrow head); desquamation and necrosis of gill epithelium (yellow circle).

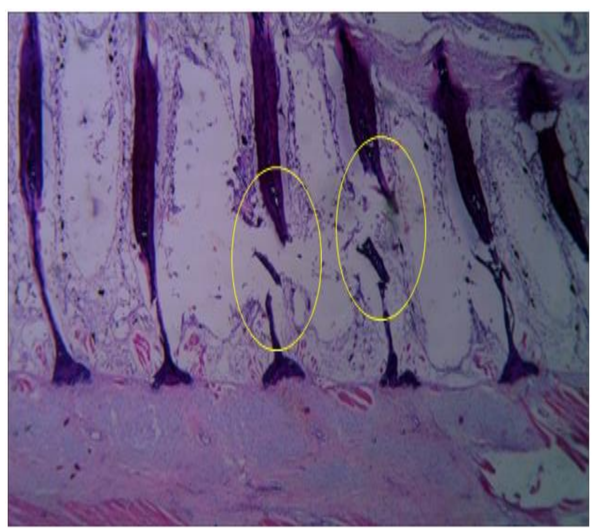

Plate 3: histopathology of Kaura dam fish gills (H&E; x40). Report: Photomicrograph of section of gill showing disappearance of secondary lamellae, disintegration and destruction of gill epithelium (yellow circles).

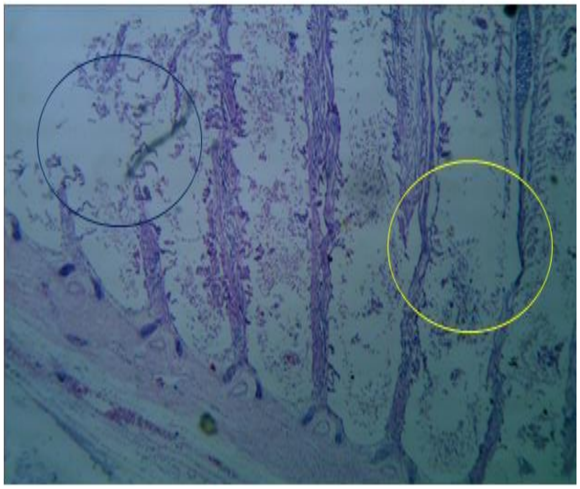

Plate 4: Maru Dam (Gills; H&E; x40). Report: Photomicrograph of section of gill showing disappearance of secondary lamellae, disintegration and destruction of gill epithelium (black circle). Desquamation and necrosis of primary gill epithelial cells (yellow arrow)

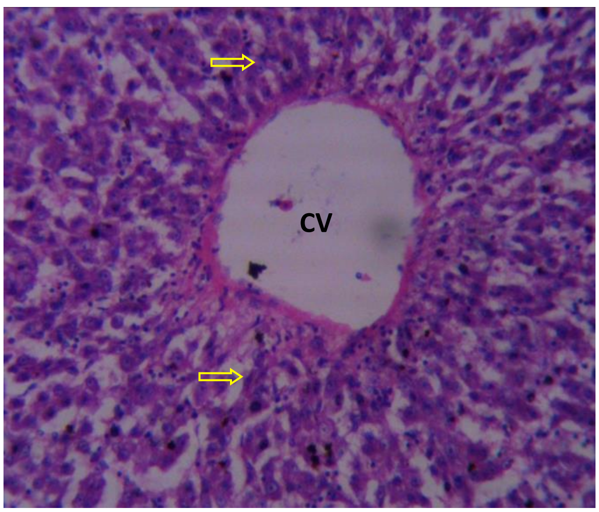

Plate 5: Control Group (Liver; H&E; x250). Report: Photomicrograph of section from liver of *T. mosambicus* showing the normal cellular pattern of the liver histo-architecture. Normal CV (central vein), hepatocytes (yellow arrow heads).

Histopathology Changes of Liver (plate 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 it's pronounced metabolic capacity (Mohamed, 2009).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. All the liver of fish sample showed signs of degeneration (cytoplasmic and nuclear degeneration and nuclear vacuolation) and the focal necrosis in the liver parenchyma. This was similar to the report of Funta *et al*., (2003) who reported such abnormalities in the liver of fish. This was also in close agreement with the report of Reddy *et al*., (2010); Reddy and Baghel (2012) who observed signs of degeneration (cytoplasmic and nuclear degeneration and nuclear vacuolation) and the focal necrosis in the liver parenchyma of fishes exposed to the industrial effluent

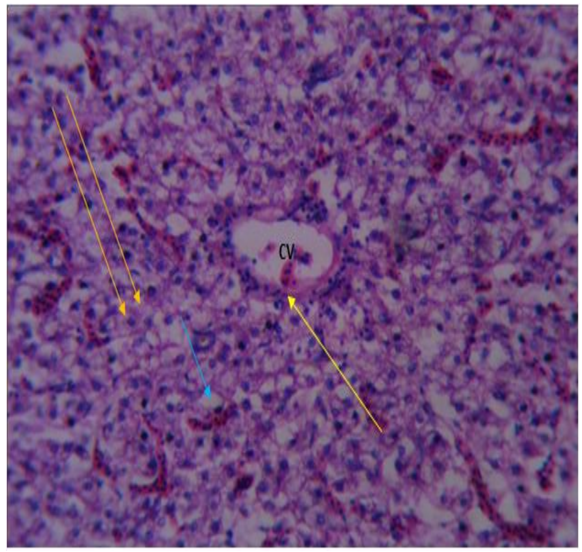

Plate 6: Gusau dam (Liver; H&E x250) Report: Photomicrograph of section from liver showing a distorted cellular pattern of the liver. Hepatocellular microvesicular fatty changes seen (double orange arrow); necrosis of hepatocytes pyknotic nuclei- (short blue arrow) and mononuclear cells infiltration (yellow arrow) was observed.

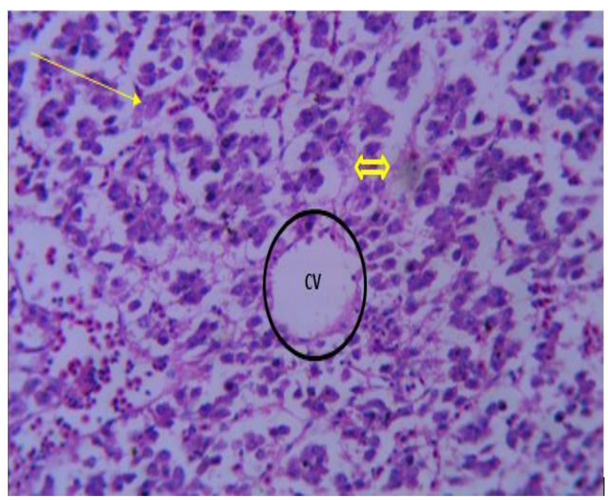

Plate 7: Kaura-Namoda dam (Liver; x250) Report: Photomicrograph of section from liver showing a distorted cellular pattern of the liver. Hepatocellular microvesicular fatty changes seen (double yellow arrow heads); necrosis of hepatocytes pyknotic nuclei- (long arrow); and much reduced central nervous system (black circle) was observed.

Histopathology Changes of Skin (plate 9 – 12).: The observed histological sections of all the fish skin (muscle) from all the dams showed infected epithelium i.e epithelial hyperplasia. The epithelial cells must have been locally irritated by the suction of the contaminants from the investigated rivers thus causing epithelial cellular growth and excess of mucus production on the skin.

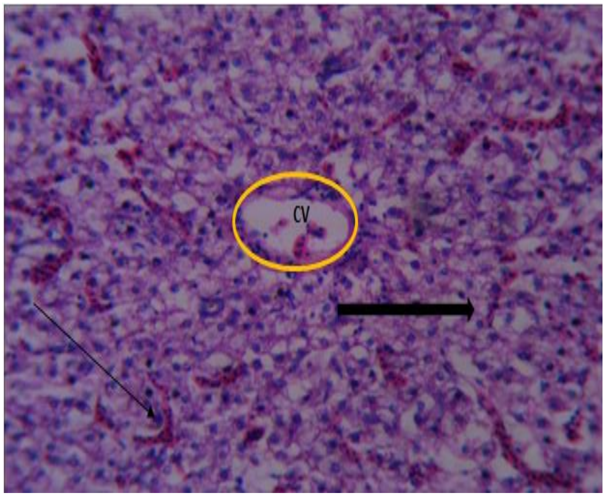

Plate 8: Talata Marara dam (Liver; H&E x250). Report photomicrograph of section of liver showing distorted cellular pattern of the liver with evidence of hepatocellular microvesicular fatty change in hepatocytes; necrosis of hepatocytes (pyknotic nuclei- long black arrow; karyolitic nuclei-thick long black arrow) and a very reduce central nervous system (green circle).

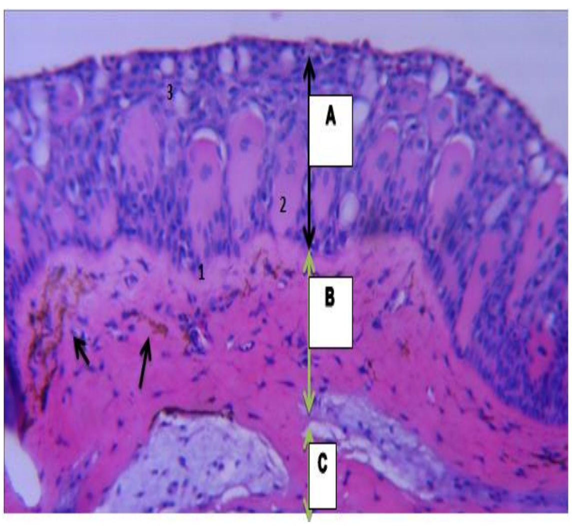

Plate 9: Control Group (Skin, H&E stain; x250). Report: section from *Tilapia mosambicus* showing the normal histo-architecture of the skin and its layers. A (epidermis), B (dermis), C (hypodermis): 1-basal cells; 2- alarm cells; 3-epithelial cells.

The severity of these contaminations may have caused injury like haemorrhage, necrotic skin were increased and seen in dorso-ventral and caudal regions of the fish samples of the examined (muscle) skin for the Tilapia *Mosambicus* showed changes only at the level of the epidermis, without major changes in the dermis and hypodermis. Picnotic nuclei in the matrix layer of the epidermis, were the most severe lesion were recorded. A common feature was erosion with desquamation of epithelium, and rupture (excoriation) of parts of epidermis. The major skin change revealed was a hyperplasia of the epidermal cells including hyperplasia of mucous cells. The same changes have also been reported in the skins of the fishes exposed to organic toxicants**,** metals and industrial effluent (Rosety-Rodriguez *et al*., 2002). The severity of these contaminants causes injury like haemorrhage. This was similar to the report of Funta *et al*., (2003) and (Peck *et al*., 2006). However, the fish sample in this work showed response to the direct effects of the pollutants.

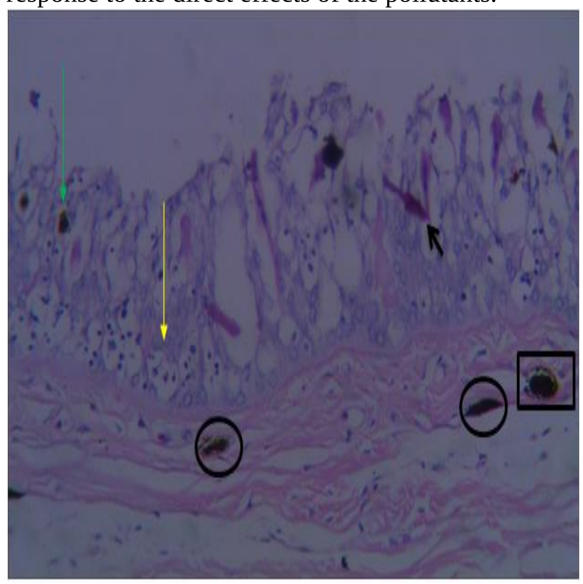

Plate 10: Talata Mafara dam (Skin; H&E; x250). Report: photomicrograph from section of skin (*T. mosambicus*). There was massive necrosis of the epidermis characterized by pyknosis of epithelial cells (black circles); karyolysis of nuclei of alarm cells (black arrow head); hyperplasia of mucus cells (yellow arrow); area of complete necrosis of cells (Black Square); decreased melanin secretion (green arrow).

The gills of a fish comprise 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. The fish gills play an important role in maintaining ionic homeostasis in both freshwater and marine environment. Consequently, injury to gill epithelium is a common response observed in fish exposed to a variety of contaminants. It was observed that all the fish gills from Gusau dam to tsafe dam are injured. The severity of damage to the gills depends on the concentration of the toxicants available in the different water bodies. 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 (Mohamed, 2009). Numerous categories of liver pathology are present as reliable biomarkers of toxic damage (Reddy *et al*., 2012; Deore *et al*., 2012).

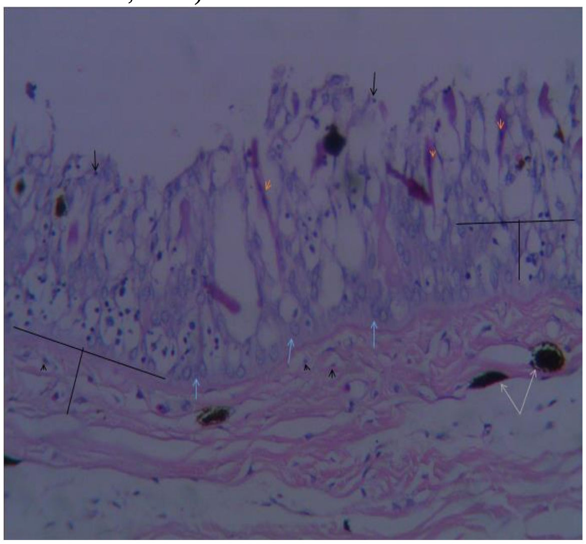

Plate 11: Gusau dam (Skin; H&E; x250): Report: Photomicrograph of section from skin of *T. mosambicus* showing massive destruction of the epidermis with necrosis of epithelial cells (pyknotic nuclei- black arrows); necrosis of alarm cells (pyknotic nuclei-brown arrow heads); hypertrophy of basal cells (blue arrows); hypertrophy and hyperplasia of fat cells (black T); pyknosis of cells of dermis (black arrow heads); condensation & darkening of melanin (green double arrow).

The liver of fish sample in all the four locations showed 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. The same changes were reported by Teh *et al*., (2005) in the liver of 7-day-old larvae of the fish Sarcamento *Splittail*. This was also similar to the report of Funta *et al*., (2003) who reported such abnormalities in the liver of fish. Samples of the examined Tilapia *Mossambis* skin have shown changes only at the level of the epidermis, without major changes in the dermis and hypodermis. Picnotic nuclei in the matrix layer of the epidermis, were the most severe lesion were recorded. The mucous cells of Gusau and Talata Mafara dam were emptied, whereas other samples revealed a lack of this cell type, indicating an overproduction of mucous that has exhausted the capacity of the epidermis to divide and differentiate into mucous cells. The same changes have also been reported in the skins of the fishes exposed to organic toxicants**,** metals and industrial effluent (Rosety-Rodriguez *et al*., 2002). The severity of these contaminants causes injury like haemorrhage. This was similar to the report of (Funta *et al*., 2003; Peck *et al*., 2006).

Conclusion: The distribution pattern of heavy metals levels in the water and fish samples suggests more contribution from anthropogenic influences which include but not limited to mining activities through runoff into the water bodies and also the levels of the metals in the water bodies particularly Cd, Pb, were generally high with exception of Zn metal which should cause trepidation to both the aquatic lives and human health, hence calling for urgent regular monitoring of the dam and control of anthropogenic input into the water bodies while the histopathological analysis of the fish gills, liver and skin sample in this study showed responses to the direct effects of the pollutants. Other samples such as food crops harvested from these areas can be further analyzed to ascertain the extent of contamination of these environments.

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Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability: Data are available from the first author who is also the corresponding author upon request.

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