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Histopathological studies of selected organs of *Sarotherodon galilaeus* (Linnaeus, 1758) in Igun gold mining reservoir and Opa freshwater reservoir, south western, Nigeria

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Abstract
Histological changes in the gills, fillet and liver of *Sarotherodon galileaus* in Opa and Igun reservoirs were used to evaluate the impact of heavy metals in the fish organs. Fresh fish samples of *S. galileaus* were collected from Opa and Igun reservoirs and identified in the laboratory. Techniques based on histological analyses were done on the tissues and photomicrographs taken using digital binocular compound LED microscope. In Opa and Igun reservoirs, the gills of *S. galilaeus* showed rupture and lifting of epithelium. The gills also showed shortening and hyperplasia of lamellae in Igun reservoir compared to Opa reservoir. The fillet of *S. galilaeus* in both reservoirs revealed muscular atrophy and splitting while necrosis was observed in Igun reservoir compared to Opa reservoir. The liver of *S. galilaeus* in the two reservoirs showed hepatopancreas degeneration while splitting at the wall of central vein, hypertrophy, degeneration and vascular congestion were observed only in the liver of *S. galilaeus* of Igun reservoir. The alterations were comparatively severe in the organs of *S. galilaeus* in Igun reservoir to Opa reservoir.

Keywords: *Sarotherodon galilaeus*, Histopathology, Reservoirs, Opa, Igun

Introduction
The mango tilapia also known as *Sarotherodon galilaeus* (Linnaeus, 1758) is a fish species that belong to the Cichlid family. This fish species is found in lakes, rivers and other fresh water bodies in northern, central and western Africa including Nigeria. Histopathology is the microscopic assessment of altered or diseased structure as described by [1]. [2] noted that the study of histopathology of fish is of main importance in the diagnosis, etiology and prevention of disease. Therefore, fish are commonly considered as organism for assessing the impacts of environmental contamination on aquatic biota [3]. Also, Fish are very vulnerable to environmental changes and respond significantly to aquatic pollution [4]. Histological study provides a rapid process to detect effects of pollutants in various organs and tissues of fish [5]. Similarly, it is useful to have an insight into histological investigation, as they serve as biological indicators to evaluate the toxicity condition of fish [6] [7].
[8] noted that fish are usually use to evaluate the health of aquatic biota because contaminants build up in the food chain may be responsible for adverse effects and death in the aquatic life. Also, water borne metals may alter the biochemical and physiological parameters in fish tissues as described by [9]. The authors were of the view that these heavy metals cannot be destroyed through biological and may cause histological alterations in tissues. The most common sources of heavy metal contamination are mining activities, industrial exploration processing and sewage disposal [10].
Pollution of water bodies with a wide range of contaminants has become a matter of concern [11]. This is because heavy metals discharged from domestic, industrial and other man made activities may pollute the water bodies extensively [12]. Also, heavy metals accumulated in the fish tissues may lead to morphological alterations in the fish [13]. The liver is associated with the detoxification and biotransformation process. It is also one of the tissues affected by pollutants in the aquatic environment [14]. Similarly, the gills are responsible for respiration and transport system involved in osmoregulation. It has also been established that bioaccumulation of metal ions within them may affect their functions [15]. The gills and skin are primary

Markers for aquatic pollution because they exhibited large surfaces which are in direct and permanent contact with potential irritants. Several Studies have been conducted on histopathological changes in the gills, liver and fillet of fish species exposed to various chemicals including pesticide which have been reported to cause pathological alterations [16]. Therefore, the aim of the present study is to evaluate the occurrence of histopathological alterations in the gills, fillet and liver of *Sarotherodon galileaus* of Opa and Igun reservoirs. This is as a result of reported high levels of heavy metals in the fish species of Igun reservoir [17] when compared with that of Opa reservoir [18].

Materials and methods

Study area

The study areas are abandoned gold mine reservoir at Igun village in Atakunmosa West Local Government area of Osun State and Opa freshwater reservoir at Ife central local Government area of Osun State. The abandoned gold mine reservoir extends over longitude $004^{0}30E$-$004^{0}45E$ and latitude $07^{0}35N$-$07^{0}38N$ (figure 1). Streams such as Oika, Eleripon and Osun which serve the community were impounded to form reservoirs in order to meet the mining needs of the Nigerian Mining cooperation which started in December 1941.

The second study area which is Opa reservoir was impounded in 1978. The major tributaries are rivers Opa, Obudu and Esinmirin. The reservoir has a catchment area of about 116km. River Opa is a stream and is located in Ile-Ife, Osun state Nigeria (figure 1). The estimated terrain elevation

Above sea level is 196 meter. The reservoir extends over latitudes $07^{0}21'N$ and $07^{0}35'N$ and longitudes $004^{0}31'E$ and $004^{0}39'E$.

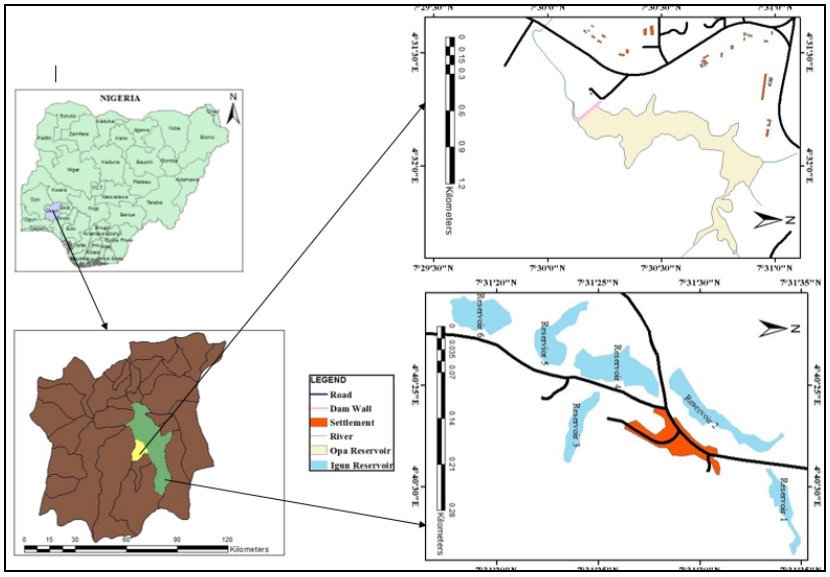

Fig 1: Map of Opa and Igun reservoirs showing its location in Nigeria

Collection of fish samples

Fish samples were collected on a monthly basis using cast net between August 2015 and July 2016. Fishes were identified using standard keys prepared by [19] and [20]. Samples of fish caught were put in a container filled with the reservoir water and dissected in situ.

Preparation of fish tissues and organs for Histological analysis

Each fish specimen was split open anteriorly from the anal pore to the pectoral fin to remove its liver, while the gills were removed from the head region. A piece of fillet was also taken just above the lateral line and before the dorsal fin. Each fish gills, fillet and liver were put in a separate well labelled bottle, fixed in 5% formalin for at least 48 hours and transferred into a sampling bottle rack. The method of [5] was used for tissues processing for histological studies, the tissues were removed from the fixative, and samples of tissue were rinsed in tap water for 5 minutes, dehydrated in ascending ethanol concentrations (70%, 80% and 90% alcohol) for minimum of 2 minutes, cleared or infiltrated in a wax miscible agent (xylene) for 2 minutes and then embedded in paraffin using standard protocols. The fish tissues were then cut into sections of approximately 5 µm thickness from the block using a rotary microtome (Yamato Kohki, Serial no: 75010JO). The cut samples were dried in a hot air oven to remove moisture and each section were mounted on a glass slide. The sections were de-waxed in a wax-miscible agent, rehydrated through descending concentrations of ethanol (90%, 80% and 70% alcohol) for at least 2 minutes. The sections were then stained with haematoxylin and eosin [21], in which the tissues were place in haematoxylin solution for 3 minutes and aqueous eosin for 3 minutes, mounted on a slide and covered with coverslip and labelled appropriately. The tissues were examined, and microphotographs taken using a digital binocular compound LED microscope (model MD827S30L series).

Results

Histopathological alterations in the organs of *Sarotherodon galilaeus* in Opa and Igun reservoirs

Histopathological alterations observed in the gills of *S. galilaeus* of Opa reservoir are curling of secondary lamellae (Figure 2.1a), lifting at the tip of secondary lamellae, epithelial lifting, rupture of gill epithelium (Figure 2.1e). Also, abnormalities in the gills of *S. galilaeus* in Igun

reservoir include epithelial lifting, shortening of secondary lamellae (Figure 2.1d). Similarly, Figure 2.1f showed hyperplasia of secondary lamellae, rupture of chloride cell and rupture of gill epithelium. Lifting and rupture of epithelium were observed in the gills of *S. galilaeus* in the two reservoirs. Also, curling and lifting of lamellae were identified in the fish of Opa reservoir when compared to rupture of chloride cell, shortening and hyperplasia of lamellae observed in the gills of *S. galialeus* of Igun reservoir. Similarly, the alterations revealed in the fillet of *S. galilaeus* in Opa reservoir are atrophy of muscle bundles (Figure 2.2c), splitting of muscle myofibril (Figure 2.2e). Further, the fillet alterations in *S. galilaeus* of Igun reservoir include atrophy of muscle bundles (Figure 2.2b), necrosis of muscle bundles in Figure 2.2d, splitting of muscle myofibrils and muscle bundles (Figure 2.2f). Atrophy and splitting of muscle myofibrils were observed in the gills of the fish in Opa amd Igun reservoirs. In contrast, the fillet of *S. galilaeus* exhibited histopathological changes such as necrosis and splitting of muscle bundles.

Also, the pathological alterations observed in the liver of *S. galilaeus* in Opa reservoir are vascular congestion in central vein (Figure 2.3a), hepatopancreas degeneration (Figure 2.3c). Histopathological alterations observed in the liver of *S. galilaeus* from Igun reservoir include spltting at the wall of central vein, degeneration of liver cells (Figure 2.3b), vascular congestion in portal vein and hepatopancreas degeneration in Figure 2.3d. Nucleus hypertrophy and vascular congestion in sinusoids was also observed as shown in (Figure 2.3f).

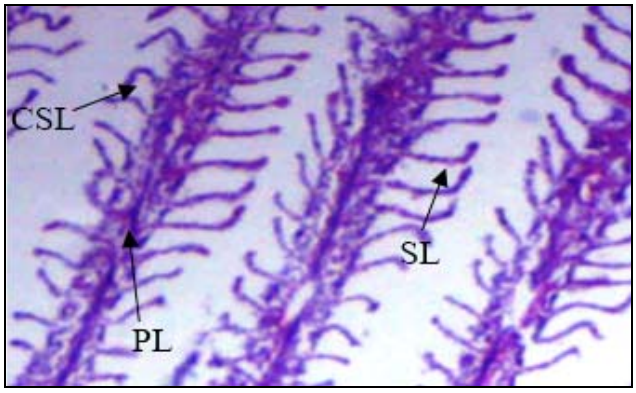

Fig 2.1a: Photomicrograph of gill section

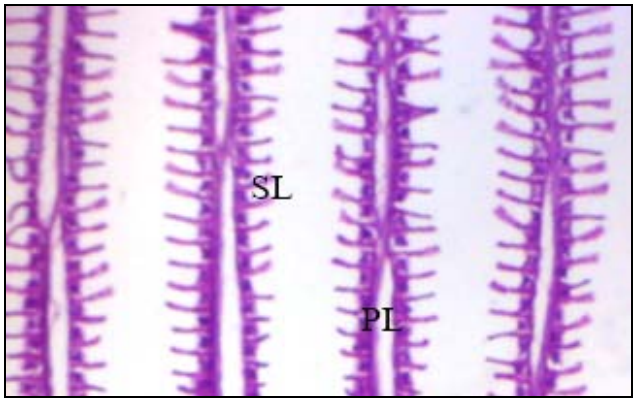

Fig 2.1b: Photomicrograph of gill section in *Sarotherodon galilaeus* of Opa reservoir in *S. galilaeus* of Igun reservoir (Mag. X100) (Mag. X100)

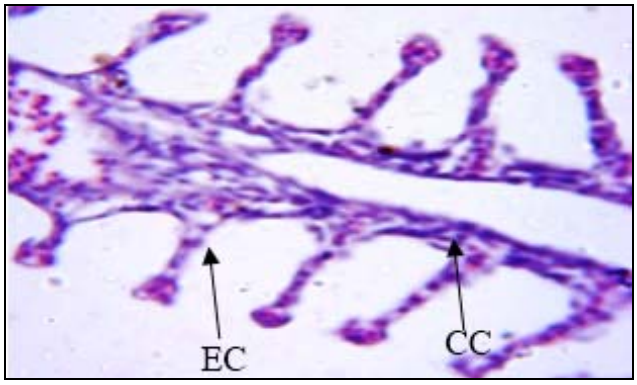

Fig 2.1c: Photomicrograph of gill section

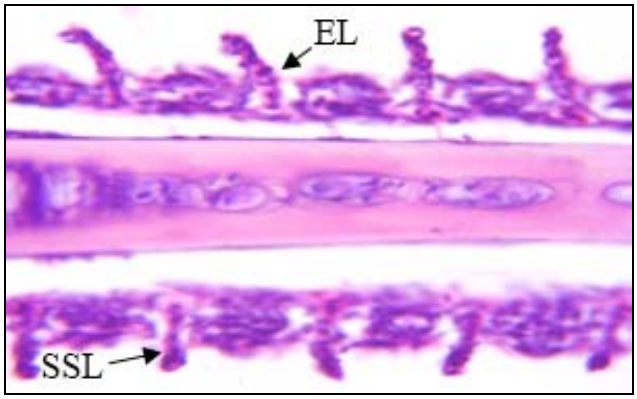

Fig 2.1d: Photomicrograph of gill section in *S. galilaeus* of Opa reservoir (Mag. X400) in *S. galilaeus* of Igun reservoir (Mag. X400)

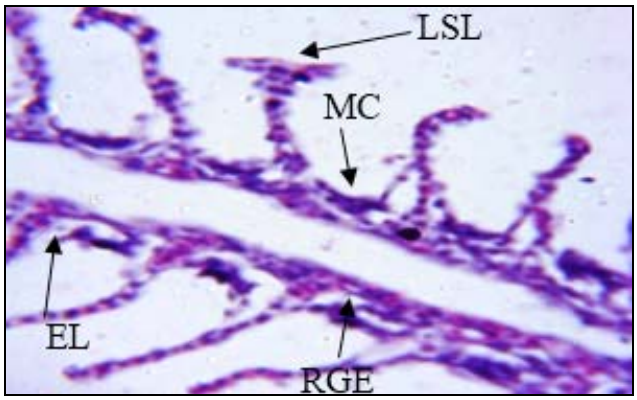

Fig 2.1e: Photomicrograph of gill section

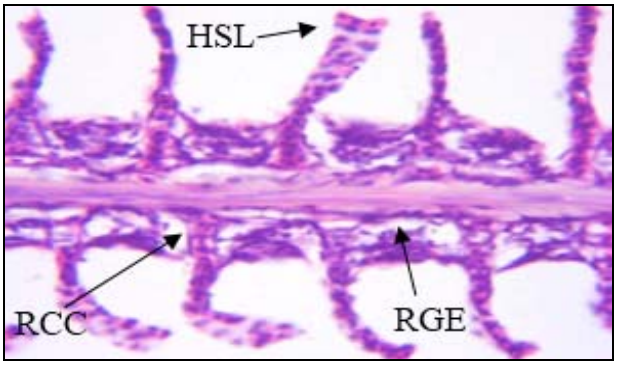

Fig 2.1f: Photomicrograph of gill section in in *S. galilaeus* of Opa reservoir (Mag. X400) in *S. galilaeus* of Igun reservoir (Mag. X400)

Rainy season keys: Curling of secondary lamellae (CSL), primary lamellae (PL), secondary lamellae (SL), epithelial cell (EC), chloride cell (CC), shortening of the secondary lamellae (SSL), epithelial lifting (EL), lifting at the tip of the secondary lamellae (LSL), mucous cell (MC), rupture of chloride cell (RCC) hyperplasia of secondary lamellae (HSL) and rupture of gill epithelium (RGE).
Haematoxylin and Eosin stain.

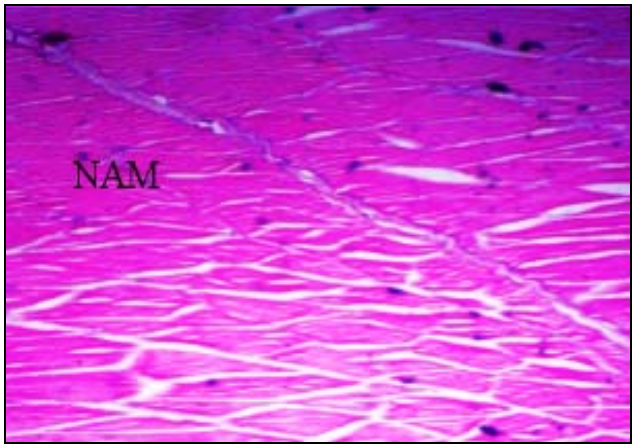

Fig 2.2a: Photomicrograph of fillet section

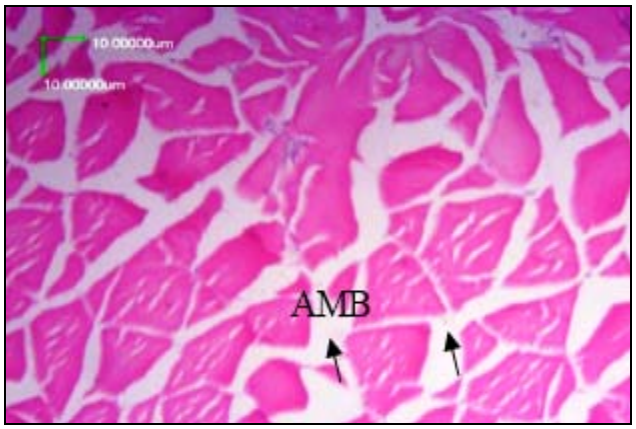

Fig 2.2b: Photomicrograph of fillet section in *Sarotherodon galilaeus* of Opa reservoir in *S. galilaeus* of Igun reservoir (Mag. X40) (Mag. X40)

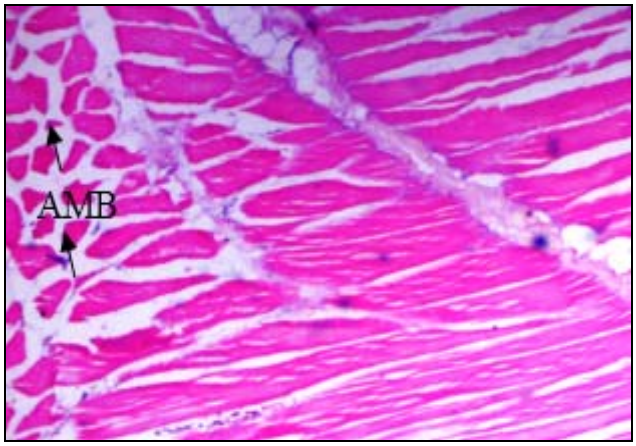

Fig 2.2c: Photomicrograph of fillet section

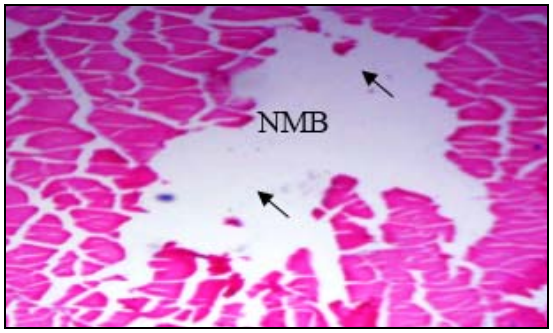

Fig 2.2d: Photomicrograph of fillet section in *S. galilaeus* of Opa reservoir (Mag. X40) in *S. galilaeus* of Igun reservoir (Mag. X40)

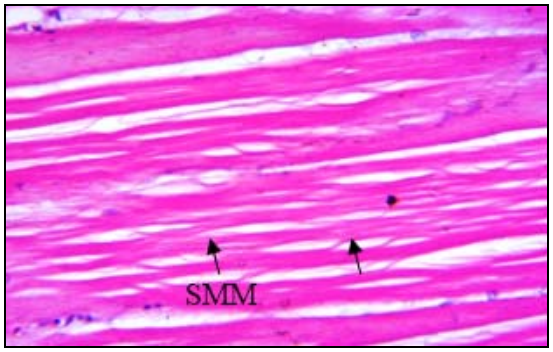

Fig 2.2e: Photomicrograph of fillet section

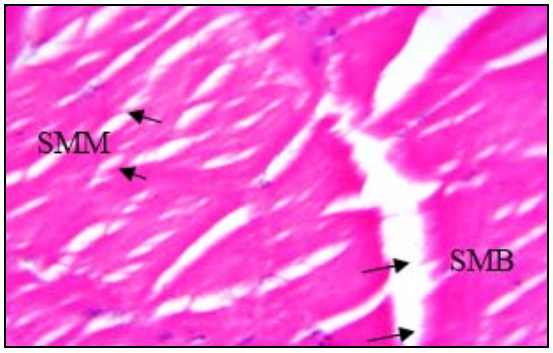

Fig 2.2f: Photomicrograph of fillet section in *S. galilaeus* of Opa reservoir (Mag. X100) in *S. galilaeus* of Igun reservoir (Mag. X100)

Rainy season keys: normal arrangement of muscle bundles (NAM), atrophy of muscle bundles (AMB), necrosis of muscle bundles (NMB), splitting of muscle myofibrils (SMM) and splitting of muscle bundles (SMB).
Haematoxylin and Eosin stain.

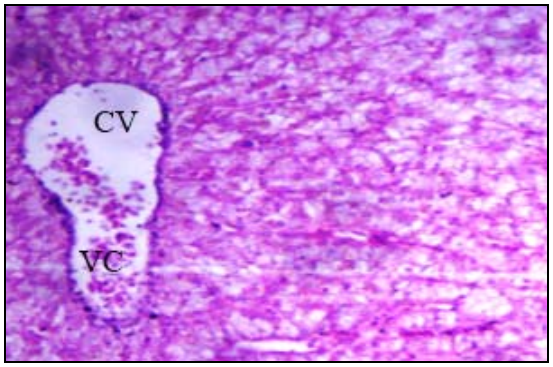

Fig 2.3a: Photomicrograph of liver section

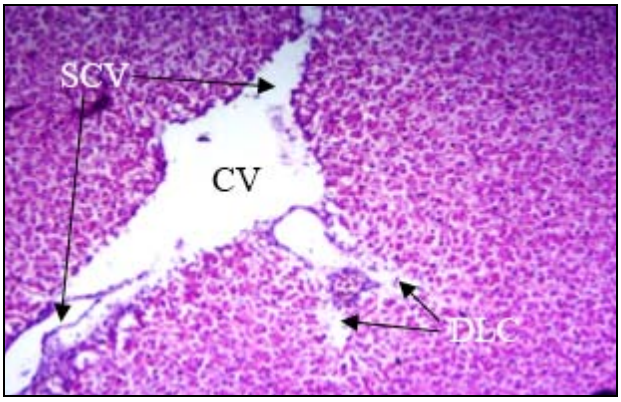

Fig 2.3b: Photomicrograph of liver section in *Sarotherodon galilaeus* of Opa reservoir in *S. galilaeus* of Igun reservoir (Mag. X40) (Mag. X40)

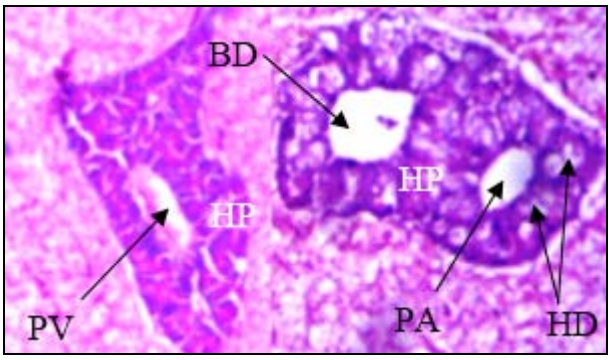

Fig 2.3c: Photomicrograph of liver section

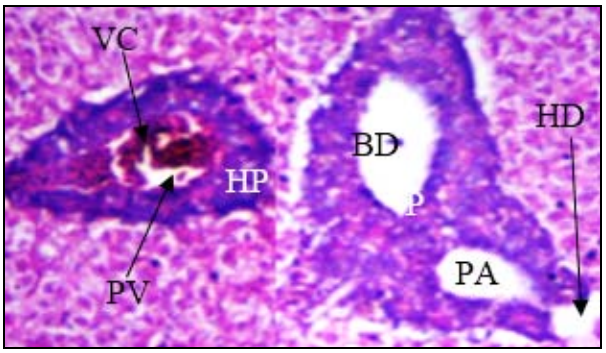

Fig 2.3d: Photomicrograph of liver section in *S. galilaeus* of Opa reservoir (Mag. X100) in *S. galilaeus* of Igun reservoir (Mag. X100)

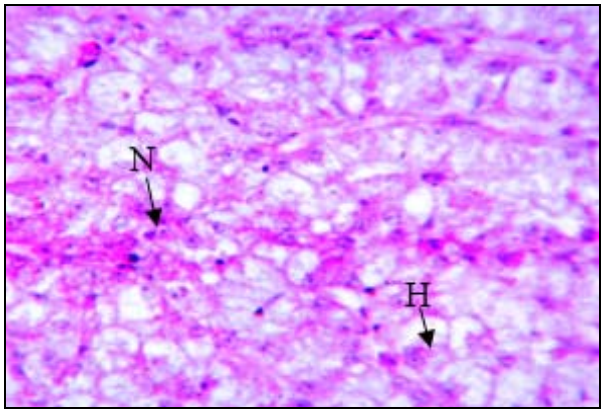

Fig 2.3e: Photomicrograph of liver section

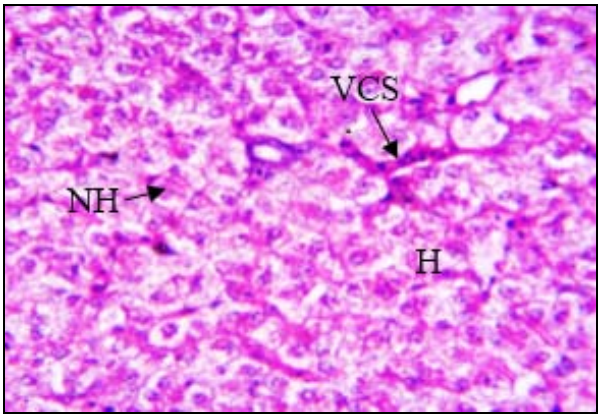

Fig 2.3f: Photomicrograph of liver section in *S. galilaeus* of Opa reservoir (Mag. X400) in *S. galilaeus* of Igun reservoir (Mag. X400)

Rainy season keys: central vein (CV), vascular congestion (VC), splitting at the wall of central vein (SCV), degeneration of liver cells (DLC), nucleus (N), hepatocytes (H), nucleus hypertrophy (NH), vascular congestion in sinusoids (VCS), portal vein (PV), hepatopancreas (HP), bile duct (BD), potal artery (PA) and hepatopanreas degeneration (HD).
Haematoxylin and Eosin stain.

Discussion

The rupture of gill epithelium observed in the gills of *S. galiaeus* in Opa and Igun reservoirs was similar to the review by [22]. According to the review on alterations induced by toxic substances in fish gills, the most common histopathological changes were hyperplasia, hypertrophy and rupture of gill epithelium. Also, epithelial lifting, curling and lifting of secondary lamellae observed in the gills of *S. galilaeus* in Opa reservoir were similar to the results obtained by [23] that reported histopathological changes in gills and liver of *Chana punctatus.* The lifting of lamellar epithelium could probably be induced by incidence of severe edema according to [24]. Hence, it could be suggested that there is an increasing level of pollution in the two reservoirs.

Similarly, shortening of secondary lamellae, hyperplasia of secondary lamellae and rupture of chloride cells were revealed in the gills of *S. galilaues* in Igun reservoir are also in agreement with the result of [23] on *Channa punctatus* under laboratory condition. Hyperplasia of lamellae which is a common responses, is more common for metals than for organics or other pollutants, possibly since metals directly interact with ion transport proteins and inhibit their activity according to [25].

Also, alterations found in the fillet of *S. galilaeus* in Opa and Igun reservoirs were atrophy of muscle bundles and splitting of muscle bundles. This finding is in accordance with the report of [26] who recorded histopathological alterations in *Danio rerio* under laboratory condition. Splitting of muscle myofibrils and necrosis of muscle bundles found in the fillet of *S. galilaeus* in Igun reservoir were similar with alterations recorded by [27] (Begum *et al.*, 2013) on *Heteropneustes fossilis* expose to Arsenic. This result may be explained by the fact that pollution may affect changes in fish tissues.

Furthermore, the liver of *S. galilaeus* in both Opa and Igun reservoirs showed hepatopancreas degeneration. This condition refers to reduction in cell size. Vascular congestion in central vein was observed in the liver of *S. galilaeus* of Opa reservoir. Alterations showed in the liver of *S. galilaeus* in

Igun reservoir corroborates the work of [28]. These are degeneration of liver cells, splitting at the wall of central vein, vascular congestion in sinusoids and portal vein. Cellular degeneration and necrosis may be due to accumulative effect of metals in hepatic tissue according to [27].

Conclusion

The investigation of the organs of *S. galilaeus* in Igun reservoir compared to Opa reservoir has shown that more alterations were found in the organs of *S. galileaus* in Igun reservoir. The results of this research support the notion that accumulation of heavy metals altered fish organs especially in Igun reservoir where there is mining activities.

Appendix

Reagents

1. 5% formalin
2. Xylene
3. 90% alcohol
4. 80% alcohol
5. 70% alcohol
6. Haematoxylin solution
7. Aqueous eosin

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