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Comparative histopathological studies of selected organs of *Oreochromis niloticus* (Linnaeus, 1758) from Igun and Opa Reservoirs, Southwestern Nigeria

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Abstract

Heavy metals have been reported to have negative impacts on the histology of fishes. In this study, the impact of heavy metal bioaccumulation on the gills, muscle and liver of *Oreochromis niloticus* from Opa and Igun reservoirs was determined by histological methods. This was with a view to checking for possible alterations on fish organs. Live fish samples were collected from Opa and Igun reservoirs and identified in the laboratory. Histological analyses were carried out on the organs and their photomicrographs taken using digital binocular compound LED microscope. The gills of *O. niloticus* from Opa and Igun reservoirs showed hyperplasia of secondary lamellae and hypertrophy of primary lamellae while shortening and edema of secondary lamellae were observed in *O. niloticus* from Opa reservoir only. Muscular atrophy and degeneration were revealed in the muscle of fish from the two reservoirs with muscular splitting in *O. niloticus* from Igun reservoir. The liver of *O. niloticus* from Opa reservoir showed vascular congestion in the bile duct compared to *O. niloticus* from Igun reservoir which showed hepatopancreas degeneration, melanomacrophages aggregates, nucleus and hepatocytes hypertrophy. In conclusion, histopathological alterations were more severe in the organs of *O. niloticus* from Igun reservoir compared to that from Opa reservoir.

Keywords: fish organs; histopathology; Igun and Opa Reservoirs

Introduction

The Nile Tilapia, *Oreochromis niloticus* (Linnaeus, 1758), is a member of the family Cichlidae, highly distributed and is found in abundance in the great lakes of Africa (Trewavas, 1983). This species is found in Kainji Lake and lived in shallow inshore and surface waters of the lake and in most rivers of Abuja, the Federal Capital Territory in Nigeria (Arawomo, 1987) and other freshwater bodies in the country. Igun village is known for mining of gold and is located in southwestern part of Nigeria. Mining is the removal of geological materials and the methods vary widely and depend on the location, type and size of mineral resources (Ochieng *et al.*, 2010). Mining activities always disrupt, affect soils, surface water and groundwater, fauna, flora and all alternative types of land-use (Fuggle and Rabie, 1994). Fish are widely used to evaluate the health of aquatic ecosystems because the pollutants which build up in the food chain are responsible for adverse effects and death

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in the aquatic environment since they prey on lower aquatic organisms (Farkas *et al.*, 2002). They are considered as good indicators of environmental quality and are therefore getting special consideration in eco-toxicological analysis (Santos *et al.*, 2011). Water pollution is one of the most recent environmental issues worldwide (Reddy and Rawat, 2013). Fishes frequently have direct contact with highly contaminated water that leads to different alterations ranging from biochemical changes in single cells up to alterations in the population (Bernet *et al.*, 1999).

In water ecosystem, the most important contaminants are the heavy metals, since they are present throughout the environment and are evident in critical amounts (Gaber, 2013). Histopathological alterations in tissues of animals are reliable and direct pointers to stressors in the environment (Malik *et al.*, 2012). The fish gills play a vital function in maintaining whole animal ionic homeostasis (Evans, 1993). Information on gill structural changes as a result of contacts with pollutants have extensively been provided by authors such as Wood (2001) and Au (2004). Histopathological changes of target organs express conditions and represent endogenous and exogenous effects on organisms that originate from damages in cells and tissues (Stebbing, 1985). Histopathological analysis provides accurate data concerning tissue changes prior to external sign (Bhuvaneshwari, 2015). One of the greatest advantages of the use of histopathology as biomarkers in environmental analysis is that this type of biomarkers allows the screening of the target organs (Gernhofer *et al.,* 2001). Pollutants affect function of the fish liver and its distinct metabolic ability (Mohamed, 2009). Several workers used liver histopathology as reliable biomarkers of various pollutants (Marchand, 2009; Reddy and Baghel, 2012). The examination of histological alterations in fish liver is a very sensitive and accurate method to evaluate the impacts of xenobiotic compounds in field and experimental studies (Hadi and Alwan, 2012). Birungi *et al.* (2007) observed that accumulation of heavy metals in a tissue is mostly dependent upon levels of metals in water and exposure period from the field and laboratory experiments.

Although aquatic pollution by heavy metals in Igun reservoirs is considered to be very high which is above WHO recommended limits (Lawal and Komolafe, 2012) compared to Opa reservoir (Olabanji and Oluyemi, 2014), there are no specific records available on the impact of heavy metals on the histopathology of *Orecochromis niloticus* in Igun and Opa reservoirs, hence this study. The study examined and compared histopathological features of the gills, muscle and liver of *O. niloticus* from the two reservoirs. It also established possible linkage between documented heavy metal concentrations in fish and water quality parameters in the two reservoirs.

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°30'E-004°45'E and latitude 07°35'N-07°38'N as shown in 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.

Opa reservoir was impounded in 1978. The major tributaries are rivers Opa, Obudu and Esinmirin. The reservoir has a catchment area of about 116 km. River Opa is a stream and is located in Ile-Ife, Osun state Nigeria. The estimated terrain elevation above sea level is 196 meters. The reservoir extends over latitudes 07^{0}21'N and 07°35'N and longitudes 004°31'E and 004°39'E (Figure 1).

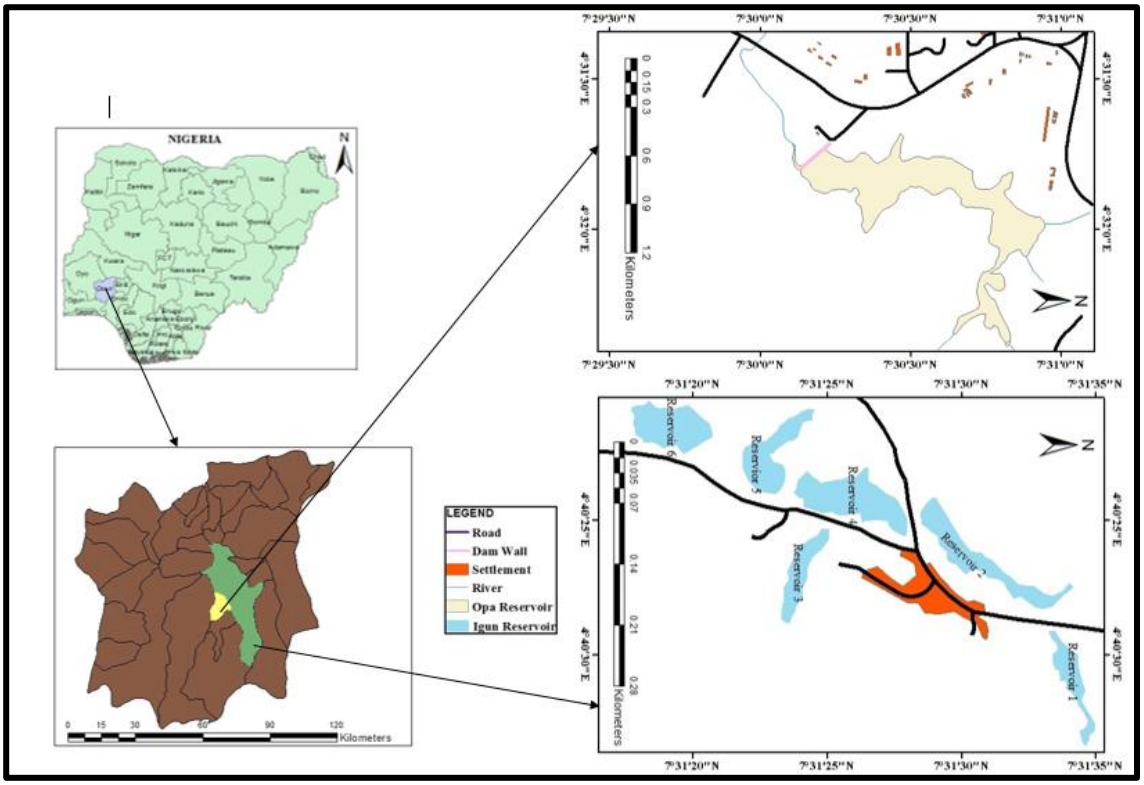

Figure 1. Map of Opa and Igun Reservoirs showing its location in Nigeria

Collection of fish samples

Fish samples were collected on a monthly basis using cast net between August 2015 and July 2016. The fish were identified using standard keys prepared by (Reed *et al.*, 1967) and Adesulu and Sydenham (2007). Samples of fish caught were put in a container filled with the reservoir water and dissected in fishery laboratory in the Department of Zoology.

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 muscle was also taken just above the lateral line and before the dorsal fin. Each fish gills, muscle 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 Bernet *et al.* (1999) was used for tissue processing for histological studies. The tissues were removed from the fixative, and samples 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 was 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 (Bancroft and Cook, 1994), in which the tissues were placed 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).

Ethical issues

The fish used for this experiment followed standard ethical consideration.

Results

Histopathological Alterations in the Organs of Oreochromis niloticus from Opa and Igun Reservoir

Histopathological alterations observed in the gills of *O. niloticus* from Opa reservoir are edema of secondary lamellae (Figure 2.1c), shortening of secondary lamellae, hyperplasia of secondary lamellae and hypertrophy of primary lamellae (Figure 2.1e). Three of the lesions in the fish gills are in first-stage of severity and one lesion being in second-stage of severity (Table 1). The alterations of gills *of O. niloticus* from Igun reservoir include hypertrophy of primary lamellae (Figure 2.1b), hyperplasia of secondary lamellae (Figure 2.1f). As shown in Table 2, anomalies of the fish gills belonged to first-stage of severity.

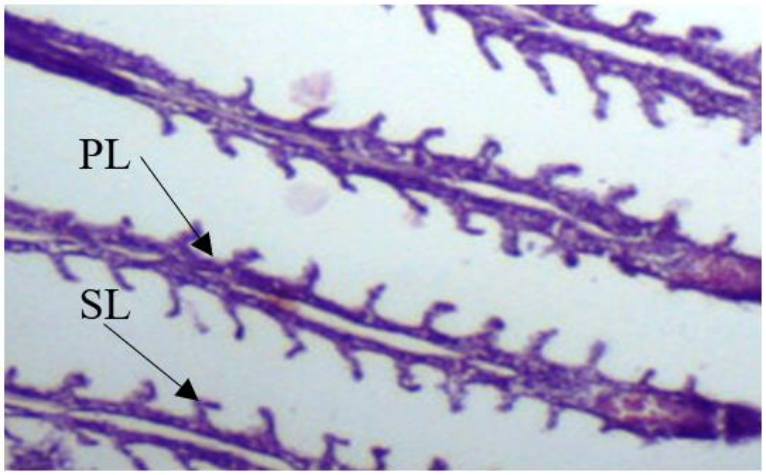

Figure 2.1a. Photomicrograph of gill section of *Oreochromis niloticus* from Opa reservoir (Mag. × 40)
Primary lamellae (PL), secondary lamellae (SL)

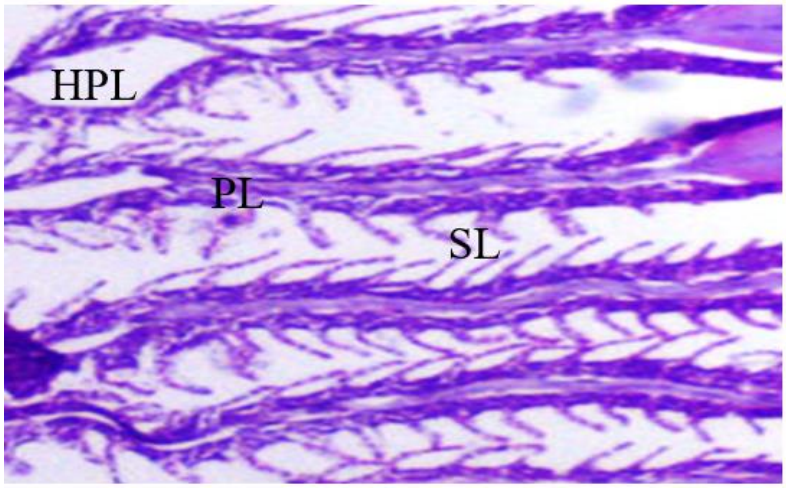

Figure 2.1b. Photomicrograph of gill section of *O. niloticus* from Igun reservoir (Mag. × 40)
Primary lamellae (PL), secondary lamellae (SL), hypertrophy of primary lamellae (HPL)

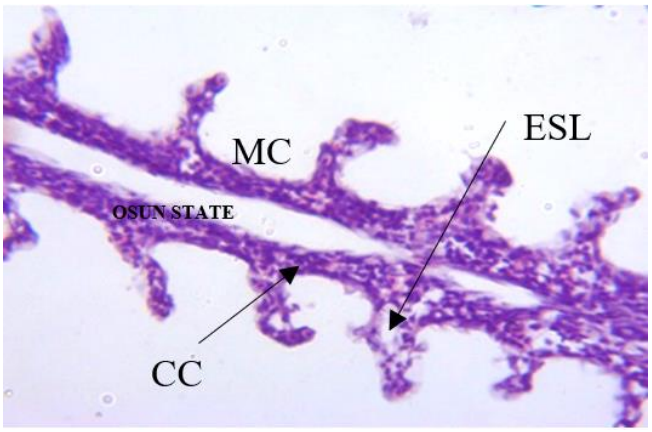

Figure 2.1c. Photomicrograph of gill section of *O. niloticus* from Opa reservoir (Mag. × 400)
Mucous cell (MC), chloride cell (CC), edema of secondary lamellae (ESL)

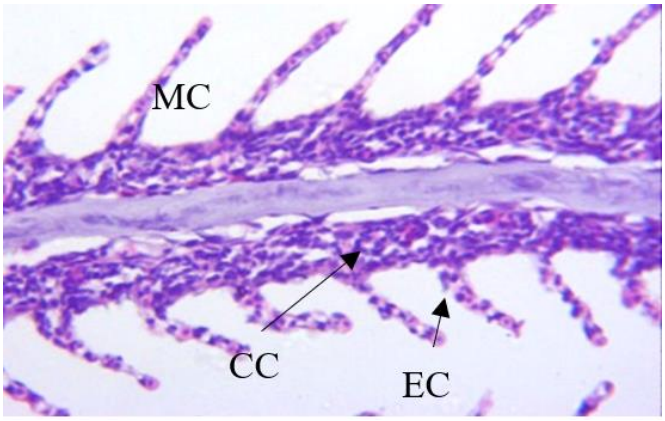

Figure 2.1d. Photomicrograph of gill section of *O. niloticus* from Igun reservoir (Mag. × 400)
Mucous cell (MC), chloride cell (CC), epithelial cell (EC)

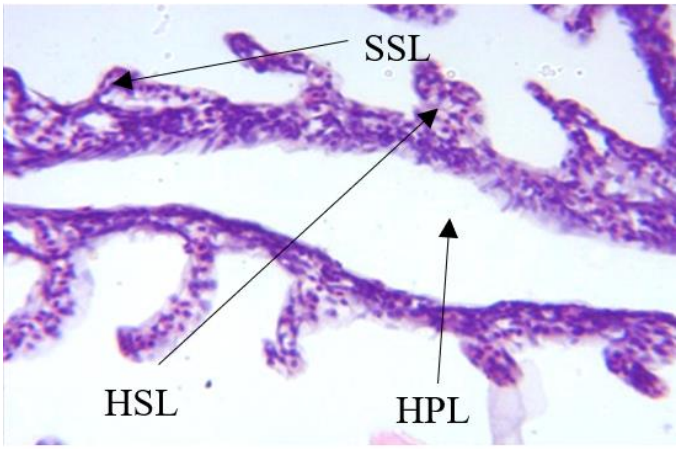

Figure 2.1e. Photomicrograph of gill section of *O. niloticus* from Opa reservoir (Mag. × 400)
Hypertrophy of primary lamellae (HPL), shortening of secondary lamellae (SSL) and hyperplasia of secondary lamellae (HSL).

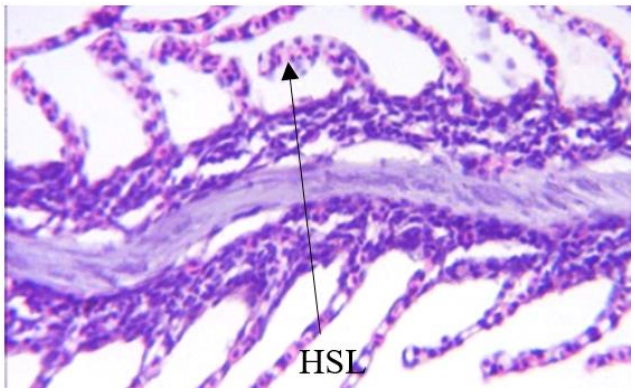

Figure 2.1f. Photomicrograph of gill section of *O. niloticus* from Igun reservoir (Mag. × 400)
Hyperplasia of secondary lamellae (HSL).

Table 1. Histopathological alterations in the organs of *Oreochromis niloticus* from Opa Reservoir and stages of severity of the alterations

Organs	Histopathological alterations	Stage
Gills	Hypertrophy of primary lamellae	I
	Shortening of secondary lamellae	I
	Hyperplasia of secondary lamellae	I
	Edema of secondary lamellae	II
Muscle	Atrophy of muscle bundles	I
	Degeneration in muscle bundles	II
Liver	Splitting at the wall of the central vein	I
	Bile duct with vascular congestion	II

Stage I = slight alteration; Stage II = moderate alteration; Stage III = severe alteration
(Modified from Simonato *et al.,* 2008)

The alterations in the muscle of *O. niloticus* from Opa reservoir are atrophy of muscle bundles (Figure 3.1c) and splitting of muscle bundles in figure 3.1e. These alterations are in first-stage of severity as shown in Table 1. The alterations in muscle *of O. niloticus* from Igun reservoir include atrophy of muscle bundles (Figure 3.1d), degeneration in muscle bundles, splitting of muscle bundles (Figure 3.1f). As shown in Table 2, two of the lesions belonged to first-stage of severity and second-stage of severity had one lesion.

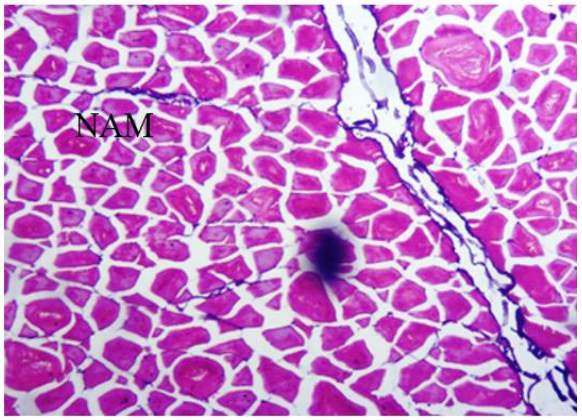

Figure 3.1a. Photomicrograph of muscle section of *Oreochromis niloticus* from Opa reservoir (Mag. ×40)
Abbreviations: normal arrangement of muscle bundles (NAM)

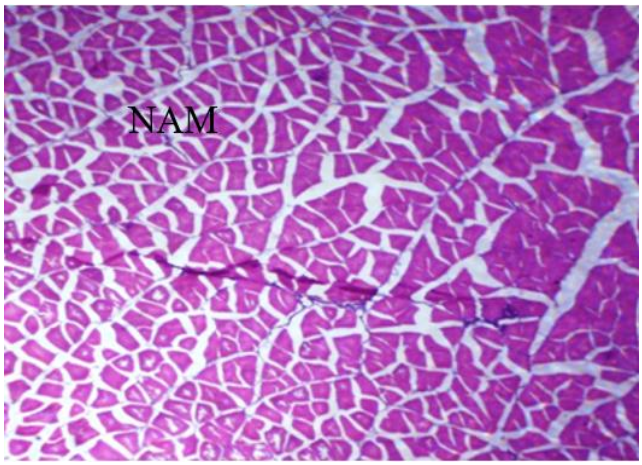

Figure 3.1b. Photomicrograph of muscle section of *O. niloticus* from Igun reservoir (Mag. × 40)
Abbreviations: normal arrangement of muscle bundles (NAM)

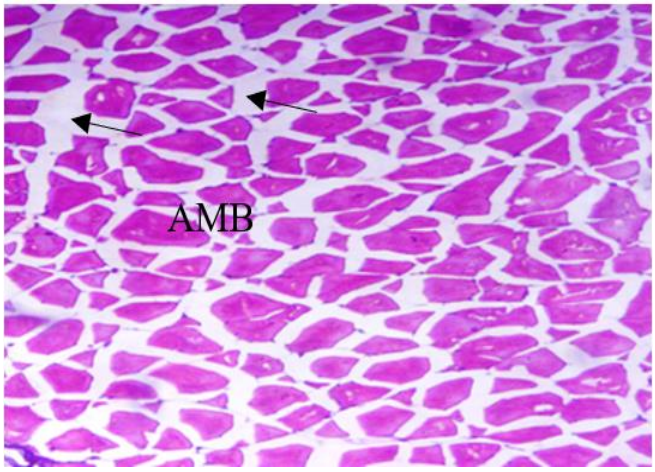

Figure 3.1c. Photomicrograph of muscle section of *O. niloticus* from Opa reservoir (Mag. × 100)
Abbreviations: atrophy of muscle bundles (AMB)

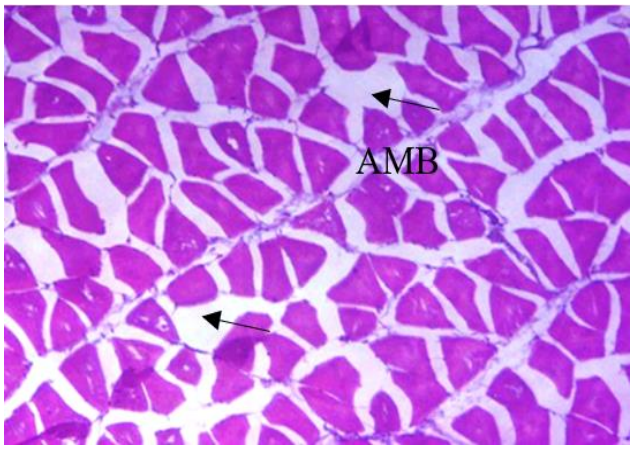

Figure 3.1d. Photomicrograph of muscle section of *O. niloticus* from Igun reservoir (Mag. × 100)
Abbreviations: atrophy of muscle bundles (AMB)

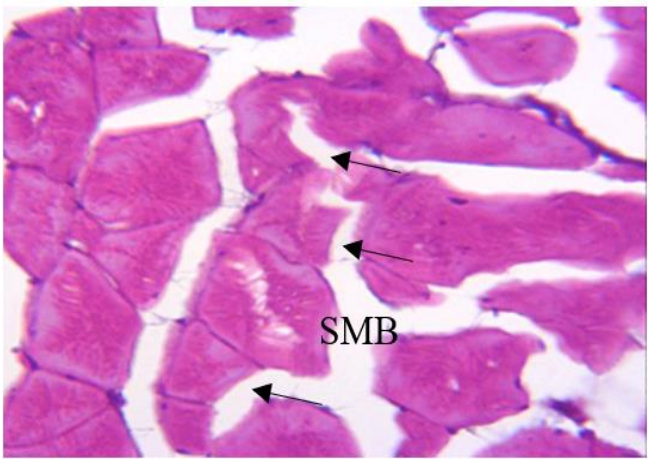

Figure 3.1e. Photomicrograph of muscle section of *O. niloticus* from Opa reservoir (Mag. × 400)
Abbreviations: splitting of muscle bundles (SMB)

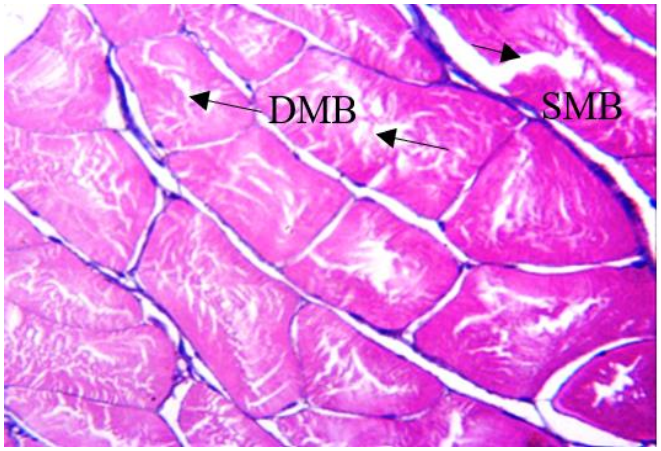

Figure 3.1f. Photomicrograph of muscle section of *O. niloticus* from Igun reservoir (Mag. × 400)
Abbreviations: splitting of muscle bundles (SMB) and degeneration in muscle bundles (DMB).

Table 2. Histopathological alterations in the organs of *Oreochromis niloticus* from Igun Reservoir and stages of severity of the alterations

Organs	Histopathological alterations	Stage
Gills	Hypertrophy of primary lamellae	I
	Hyperplasia of secondary lamellae	I
Muscle	Splitting of muscle bundles	I
	Atrophy of muscle bundles	I
	Degeneration in muscle bundles	II
Liver	Melanomacrophages aggregates	I
	Hepatocytes hypertrophy	I
	Splitting at the wall of the central vein	I
	Nucleus hypertrophy	I
	Hepatopancreas degeneration	II

Stage I = slight alteration; Stage II = moderate alteration; Stage III = severe alteration
(Modified from Simonato *et al.,* 2008)

For fish from Opa reservoir, the alterations in the liver of *O. niloticus* are splitting at the wall of the central vein (Figure 4.1a), and vascular congestion in bile duct (Figure 4.1c). Thus, each of the anomalies are considered to be in first-stage and second-stage of severity (Table 1). Histopathological alterations observed in

the liver of *O. niloticus* from Igun reservoir include splting at the wall of central vein, melanophages aggregates, degeneration of hepatopancreas in figure 4.1d. Hepatocytes hypertrophy and nucleus hypertrophy were also observed (Figure 4.1f).

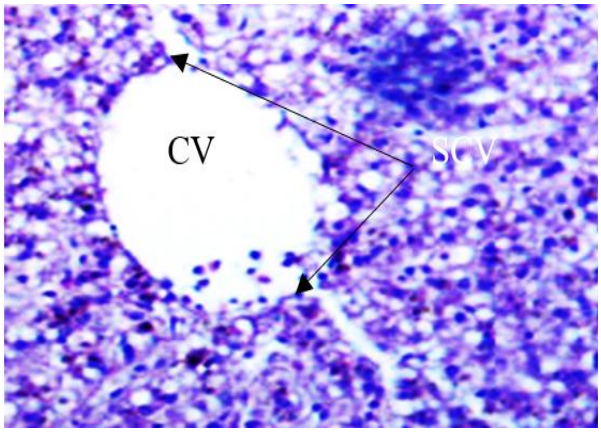

Figure 4.1a. Photomicrograph of liver section of *Oreochromis niloticus* from Opa reservoir (Mag. × 40)
Abbreviations: central vein (CV), splitting at the wall of central vein (SCV)

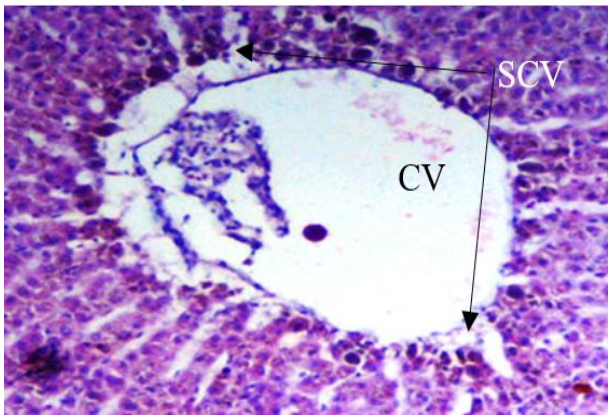

Figure 4.1b. Photomicrograph of liver section of *O. niloticus* from Igun reservoir (Mag. × 40)
Abbreviations: central vein (CV), splitting at the wall of central vein (SCV)

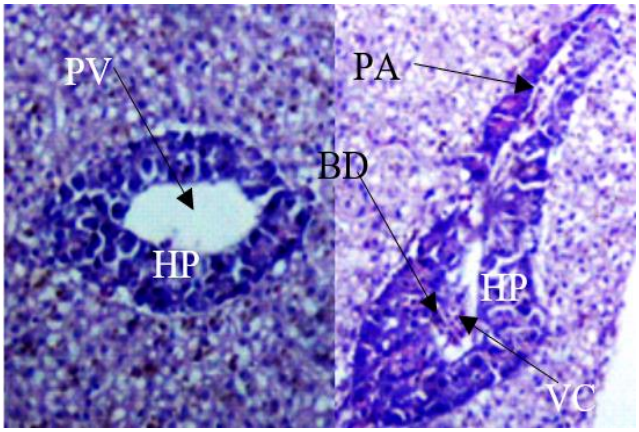

Figure 4.1c. Photomicrograph of liver section of *O. niloticus* from Opa reservoir (Mag. × 100)
Abbreviations: portal vein (PV), hepatopancreas (HP), bile duct (BD), vascular congestion (VC), portal artery (PA)

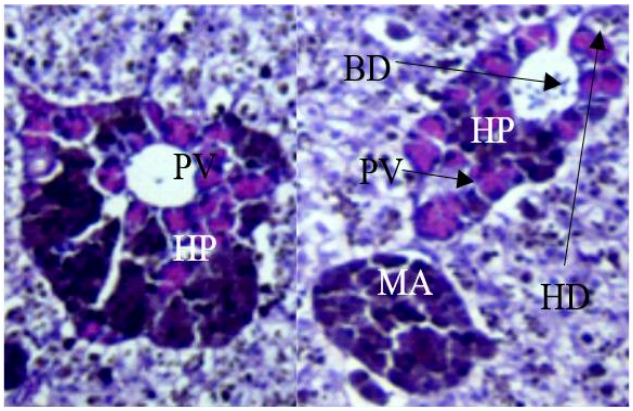

Figure 4.1d. Photomicrograph of liver section of *O. niloticus* from Igun reservoir (Mag. × 100)
Abbreviations: portal vein (PV), hepatopancreas (HP), bile duct (BD), melanomacrophages (MA), hepatopancreas degeneration (HD)

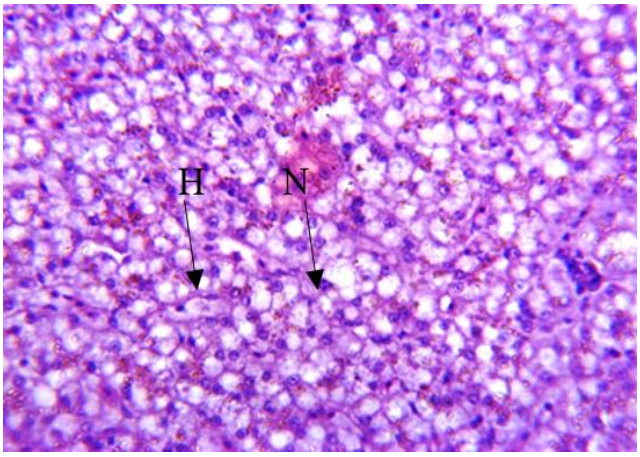

Figure 4.1e. Photomicrograph of liver section of *O. niloticus* from Opa reservoir (Mag. × 400)
Abbreviations: hepatocytes (H), nucleus (N).

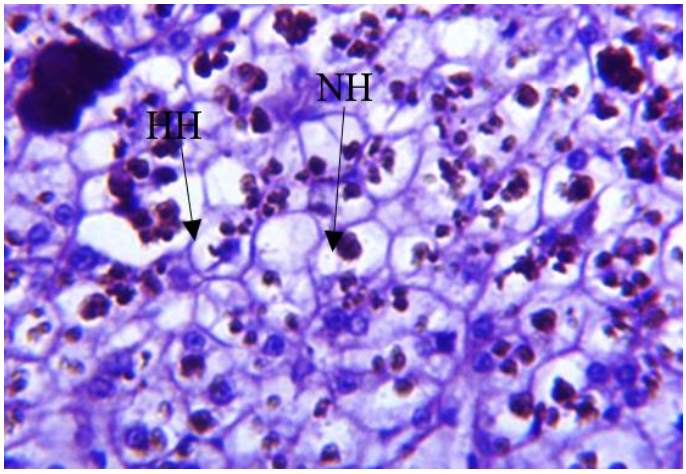

Figure 4.1f. Photomicrograph of liver section of *O. niloticus* from Igun reservoir (Mag. × 400)
Abbreviations: hepatocytes hypertrophy (HH) and nucleus hypertrophy (NH).

Discussion

The gills are involved in many functions in fish such as respiration and osmoregulation. They remain in close contact with the outside environment and particularly quick to respond to changes in water quality. They are regarded to be the primary target of pollutants (Mazon *et al.*, 2002; Camargo and Martinez, 2007). Hyperplasia of secondary lamellae and hypertrophy of primary lamellae were observed in the gills of *O. niloticus* from Opa and Igun reservoirs. These pathological changes in the gills of *O. niloticus* might probably be a reaction to toxicants intake to prevent the entry of pollutants through the gills surface as reported by Mohamed (2009) in Lake Qarun in Egypt. Shortening of secondary lamellae and edema of secondary lamellae were seen only in *O. niloticus* of Opa reservoir. Shortening of secondary lamellae identified in *O. niloticus* of Opa reservoir was similar to those obtained by Simonato *et al.,* (2008). Hyperplasia in the gills as documented by Eller (1975) suggests toxicity in several fish species following exposure to several harmful chemicals. Splitting and atrophy of muscle bundles were identified in the muscle of *O. niloticus* from Opa and Igun reservoirs. This observation is in agreement with the report of Bhuvaneshwari *et al.* (2015). Similarly, these responses indicated that histopathological alterations were severe enough to lead to structural changes at the tissue level.

The liver plays an important role in fish physiology, both in anabolism and catabolism of proteins, lipids and carbohydrates and other detoxication functions (Yogita and Mishra, 2013). Splitting at the wall of central vein were found in the liver of *O. niloticus* from Opa and Igun reservoirs. Vascular congestion in the bile duct were seen in the liver of *O. niloticus* from Opa reservoir while hepatocytes hypertrophy, nucleus hypertrophy, degeneration of hepatopancreas and melanomacropahages were observed in the liver of *O. niloticus* from Igun reservoir only. Melanomacrophage aggregates are associated with a number of fish diseases which are phagocytic in nature (Agius and Robert, 2003). These changes may be as a result of direct effects of harmful chemicals on the liver cells, since the liver is the main organ that removes toxic substances (Osman *et al.*, 2010).

This study has shown that water pollution caused by heavy metals probably affected the organs of *O. niloticus* from Opa and Igun reservoirs. This is because Komolafe and Lawal (2013) recorded high levels of heavy metals in the organs of fish species in Igun reservoir. Also, more histopathological changes were revealed in the organs of *O. niloticus* from Igun reservoir compared to Opa reservoir.

Conclusions

In conclusion, histopathological changes in the organs of *O. niloticus* of the two reservoirs had established earlier studies of the influence of pollution on the fishes in the reservoirs. The findings observed that alterations in the organs of *O. niloticus* in Igun reservoir could be as a direct result of mining activities while agricultural, municipal or domestic waste products could have led to the alterations in the fish organs of *O. niloticus* at Opa reservoir.

Authors' Contributions

Both authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

The fish used for this experiment followed standard ethical consideration.

Acknowledgements

The authors are grateful to the fishermen for their assistance in capturing the fish. Special appreciation to Obafemi Awolowo University and the community leaders of Igun village. Also, the authors thank Mr Gbela for histological preparation of the fish tissues.

Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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