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HISTOPATHOLOGY STUDIES OF SELECTED ORGANS OF *Hemichromis fasciatus* INHABITING IGUN GOLD MINING AND OPA RESERVOIRS, OSUN STATE, NIGERIA: A COMPARATIVE STUDY

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ABSTRACT

This study examined the histopathological alterations in the gills, fillet and liver of *Hemichromis fasciatus* in Igun reservoir (located in an abandoned goldmine area) compared to those of Opa reservoir. Life fish species were collected from Opa and Igun reservoirs and identified in the laboratory. Techniques based on histological analyses were done on the organs and photomicrographs taken using digital binocular compound LED microscope. Epithelial lifting and hypertrophy of lamellae were observed in the gills of *H. fasciatus* in Opa reservoir and compared to rupture of gill epithelium, rupture of chloride cell, fusion, hyperplasia, curling of lamellae in *H. fasciatus* of Igun reservoir. The fillet of *H. fasciatus*in Opa and Igun reservoirs revealed splitting and atrophy of muscle bundles. Also, parasite cyst and necrosis were observed in the fillet of *H. fasciatus* of Igun reservoir compared to degeneration in muscle bundles in the fish of Opa reservoir. Similarly, the liver of *H. fasciatus* in Igun and Opa reservoirs showed splitting at the wall of central vein, hepatopancreas and liver cells degeneration. Moreover, nucleus hypertrophy was also identified in the liver of *H. fasciatus* in Opa reservoir compared to vascular congestion in the central vein, bile duct, portal vein and portal artery of *H. fasciatus* in Igun reservoir. The study therefore concluded that *H. fasciatus* specimens in Igun reservoir were histopathologically unhealthy as compared with those of Opa reservoir probably due to the high level of pollution resulting in bioaccumulation of heavy metals in Igun reservoir samples.

Keywords: *gills, fillet, liver, Hemichromis fasciatus, histopathology, reservoir*

INTRODUCTION

The Cichlid, *Hemichromis fasciatus* (Peters 1857) commonly called banded jewel fish is an ornamental fish which occurs in various freshwater bodies in Africa. An increased pollution of the aquatic environment has caused severe alterations of tissues and organs in aquatic organisms (Mazon*et al.*,1999). The authors are of the view that in a disturbed environment, especially where pollutants occur in chronic and sublethal concentrations, changes in the structure and function of aquatic organisms are more frequent than mass mortality. Poleksic and Mitrovic-Tutundzic (1994) noted that one of the possible methods of evaluating the effects of pollutants in fish is to examine their organs for morphological changes.

Raskovic (2010) reported that fish can be used to evaluate the health of aquatic ecosystems because contaminants build up in the food chain and are responsible for adverse effects and death in the aquatic systems. Also, studies carried out on various fishes have shown that heavy metals altered the physiological activities and biochemical parameters in organism tissues as observed by Popov *et al.* (2002), Golovanova (2008), Mary *et al.* (2015). The toxic effect of heavy metals in fish includes bioaccumulation, histopathological changes in tissues as reviewed by Usha Rani (2000); Adami, *et al.* (2002) andSehar *et al.* (2013)

Similarly, histopathological changes have been widely used as bio-indicators in evaluating the health of fish exposed to contaminants, both in the laboratory and field studies. Hence, histopathological alterations can be used as indicators for monitoring the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem as reported by Mohammed (2009).Drishya *et al.* (2016) stated that one of the advantages of using histopathological bio-indicators in environmental monitoring is that this category of bio-indicators allows examining specific target tissues which includes: gills, kidney and liver that are responsible for vital roles. These functions are respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish.

Furthermore, the alterations found in these organs are normally easier to identify than functional ones (Karthigayani*et al.* 2014) and serve as warning signs of damage to animal health (Hinton and Laurén 1990). According to Yildiz*et al.* (2010), increasing exposure to toxic elements in fresh water organisms such as fish and river birds which are often used to monitor the presence of contaminants can have adverse toxicological effects. Available records have shown that fish species in Igun reservoir bioaccumulated more heavy metals compared to fish species in Opa reservoir (Lawal and Komolafe 2012; Olabanji and Oluyemi, 2014).

This study therefore aims to compare histopathological alterations in the gills, fillet and liver of Igun and Opa 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 004030E-004045E and latitude 07035N-07038N. 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 is located in Ile-Ife, Osun State, Nigeria. Opa reservoir was impounded in 1978. The major tributaries are rivers Opa, Obudu and Esinmirin. The reservoir has a catchment

area of about 116km. The reservoir extends over latitudes07^{0}21'N and07^{0}35'N and longitudes004^{0}31'E and 004^{0}39'E (figure 1).

COLLECTION OF FISH SAMPLES

Fish samples were collected on a monthly basis using gill nets, cast net. They were identified using standard keys prepared by Paugy, *et al.* (2003) and Adesulu and Sydenham (2007). 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 gill, 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 Bernet*et al.* (1999) 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

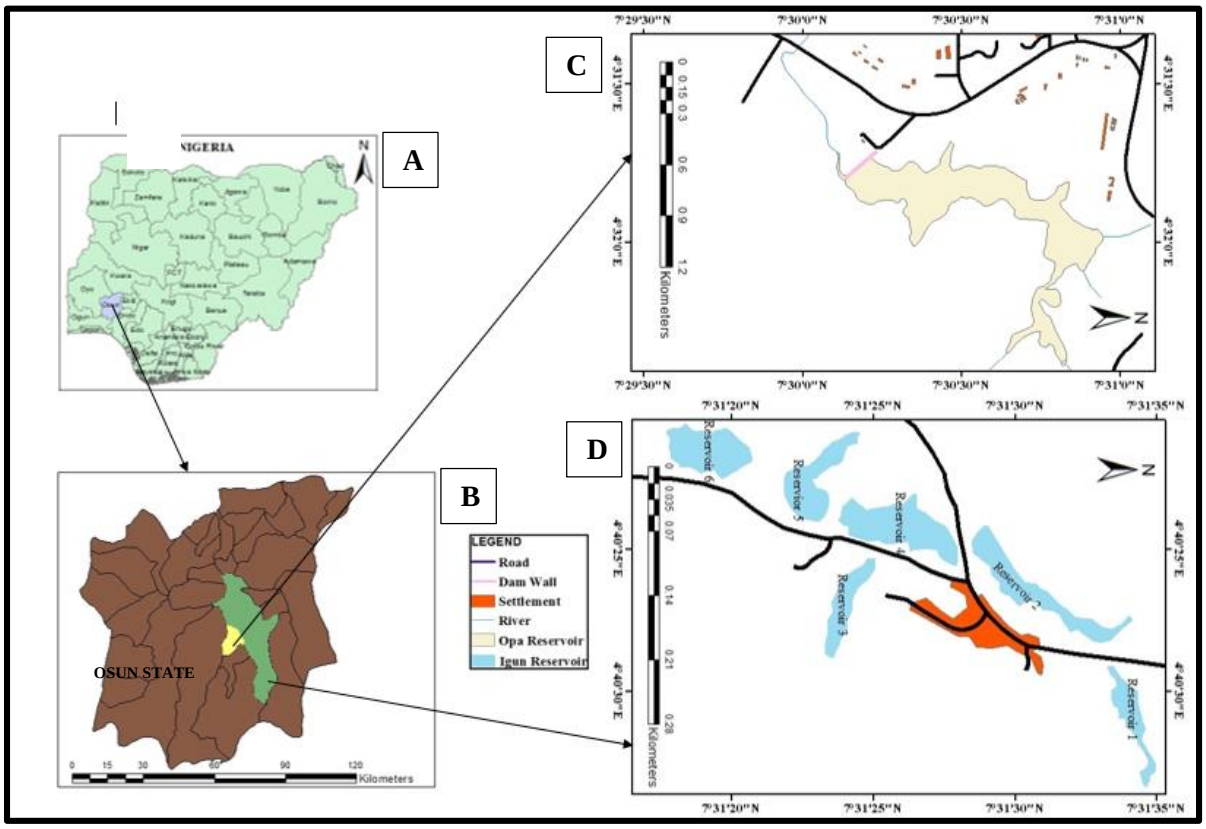

Figure 1: Map of Opa and Igun reservoirs showing its location in Nigeria
A = Nigeria, B = Osun State, C = Opa Reservoir, D = Igun Reservoir

(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 (Bancroft and Cook 1994), 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 *Hemichromis fasciatus*in OPA AND IGUN RESERVOIRS

The gills of *Hemichromis fasciatus* in Opa reservoir showed normal primary and secondary lamellae. However, hypertrophy of primary lamellae was observed as shown in Plate 1.1a and epithelial lifting (Plate 1.1e). The photomicrograph of gill section in *H. fasciatus* of Igun reservoir showed fusion of secondary lamellae (Plate 1.1b), hyperplasia of secondary lamellae (Plate 1.1d), rupture of gill epithelium and curling of secondary lamellae (Plate 1.1f).

The histopathological changes observed in the fillet of *H. fasciatus* of Opa reservoir includes atrophy of muscle bundles (Plate 1.2a); degeneration in muscle bundles in Plate 1.2c and splitting of muscle bundles (Plate 1.2e). The photomicrograph result of fillet section in *H. fasciatus* of Igun reservoir showed parasite cyst and atrophy of muscle bundles (Plate 1.2d); splitting of muscle bundles and splitting of muscle myofibrils (Plate 1.2f).

Result of the histopathological examination of *H. fasciatus* liver in Opa reservoir include splitting at the wall of central vein as shown in (Plate 1.3a); hepatopancreas degeneration and degeneration in liver cells (Plate 1.3c) and nucleus hypertrophy (Plate 1.3e). Histopathological alterations in the liver of *H. fasciatus*in Igun reservoir are shown in Plate 1.3b, with vascular congestions in central vein, splitting at the wall of central vein; vascular congestion in the portal vein, bile duct, and portal artery. Degeneration of liver cells and hepatopancreas degeneration was also observed (Plate 1.3d) as well as nucleus hypertrophy as shown in (Plate 1.3f).

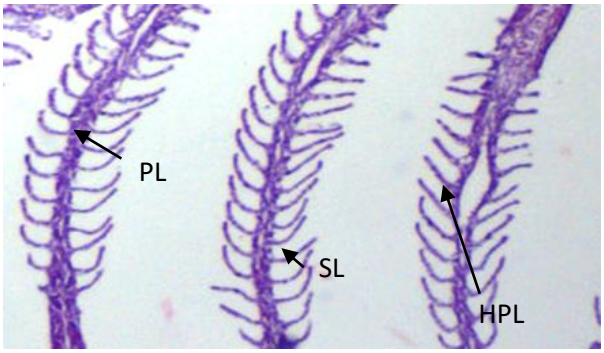

Plate 1.1a: Photomicrograph of gill section in Hemichromis fasciatus of Opa reservoir (Mag. X40)

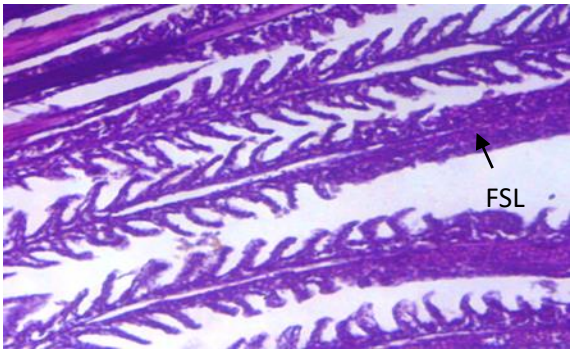

Plate 1.1b: Photomicrograph of gill section in H. fasciatus of Igun reservoir (Mag. X40)

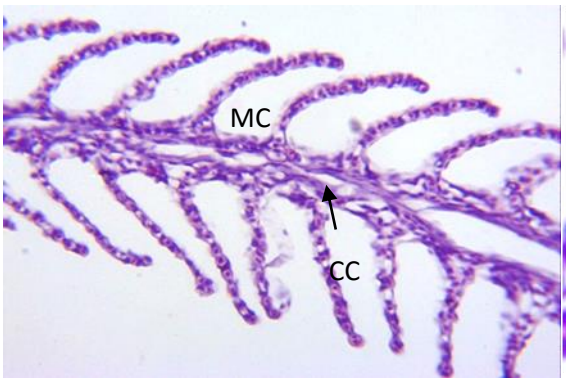

Plate 1.1c: Photomicrograph of gill section in *H. fasciatus* of Opa reservoir(Mag. X400)

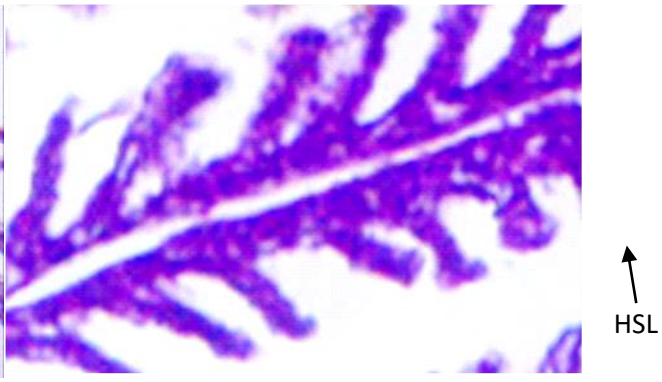

Plate 1.1d: Photomicrograph of gill section in *H. fasciatus* of Igun reservoir(Mag. X400)

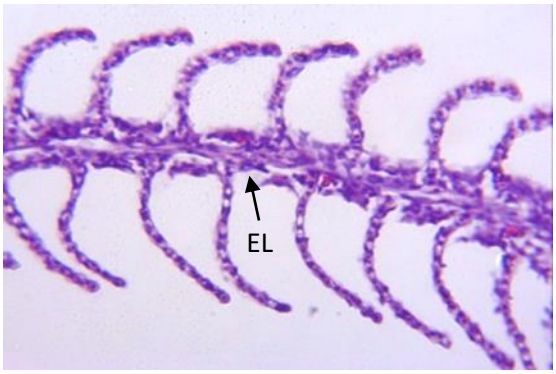

Plate 1.1e: Photomicrograph of gill section in *H. fasciatus* of Opa reservoir(Mag. X400)

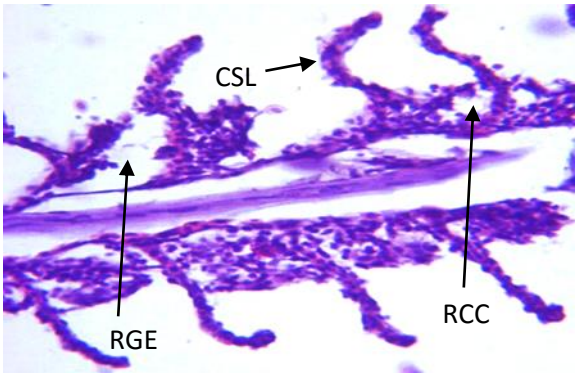

Plate 1.1f: Photomicrograph of gill section in *H. fasciatus* of Igun reservoir(Mag. X400)

Keys:*primary lamellae (PL), secondary lamellae (SL), hypertrophy of primary lamellae (HPL), fusion of secondary lamellae (FSL), mucous cell (MC), chloride cell (CC) hyperplasia of secondary lamellae (HSL), epithelial lifting (EL), rupture of gill epithelium (RGE), curling of secondary lamellae (CSL) and rupture of chloride cells (RCC).*

Haematoxylinand Eosin stain.

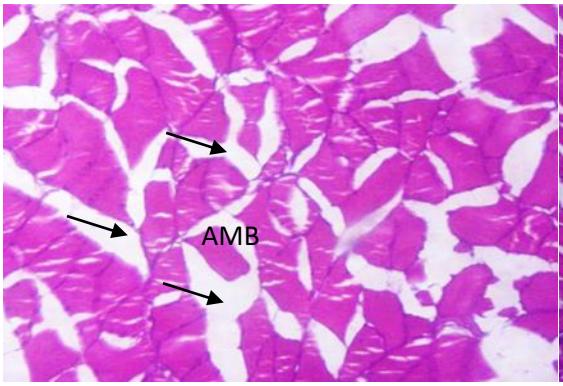

Plate 1.2a: Photomicrograph of fillet section in *Hemichromis fasciatus* of Opa reservoir (Mag. X40)

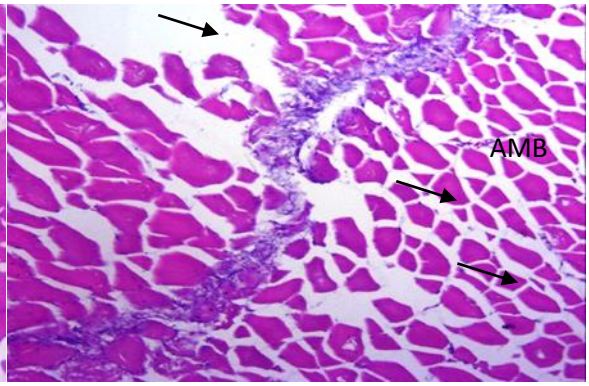

Plate 1.2b: Photomicrograph of filletsection in *H. fasciatus* of Igun reservoir(Mag. X40)

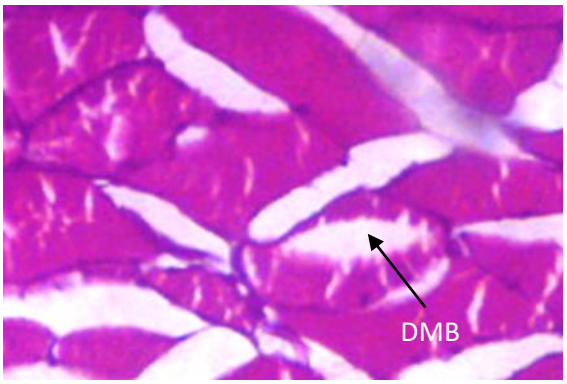

Plate 1.2c: Photomicrograph of fillet section in *H. fasciatus* of Opa reservoir(Mag. X100)in

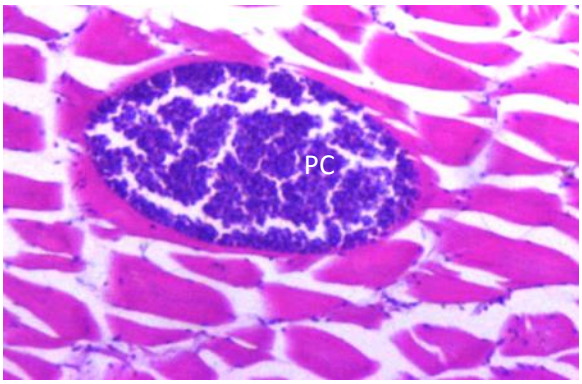

Plate 1.2d: Photomicrograph of filletsection *H. fasciatus* of Igun reservoir(Mag. X100)

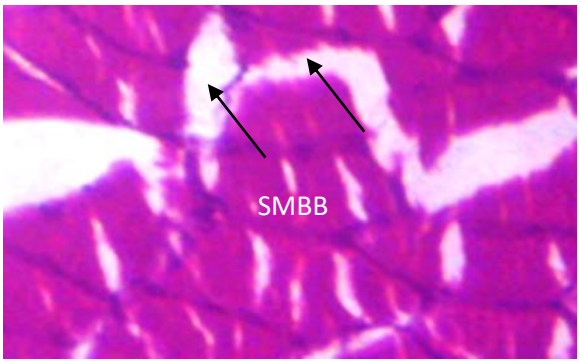

Plate 1.2e: Photomicrograph of fillet section in *H. fasciatus* of Opa reservoir(Mag. X400)

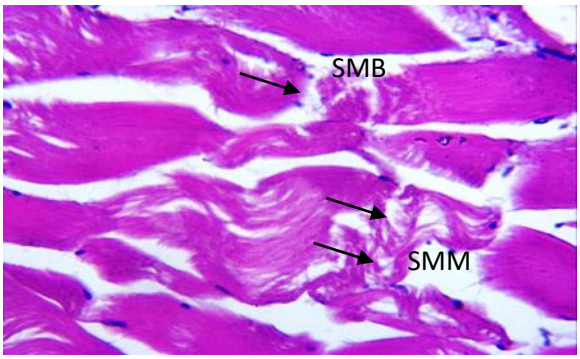

Plate 1.2f: Photomicrograph of filletsection in *H. fasciatus* of Igun reservoir(Mag. X400)

Keys: *atrophy of muscle bundles (AMB), degeneration in muscle bundles (DMB), parasite cyst (PC), splitting of muscle bundles (SMB), splitting of muscle myofibrils (SMM).*

Haematoxylinand Eosin stain.

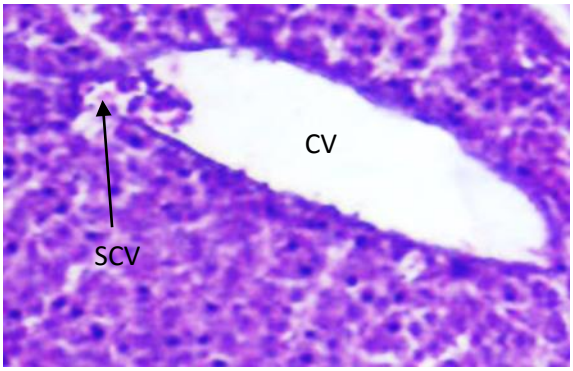

Plate 1.3a: Photomicrograph of liver section *in Hemichromis fasciatus* of Opa reservoir (Mag. X100)

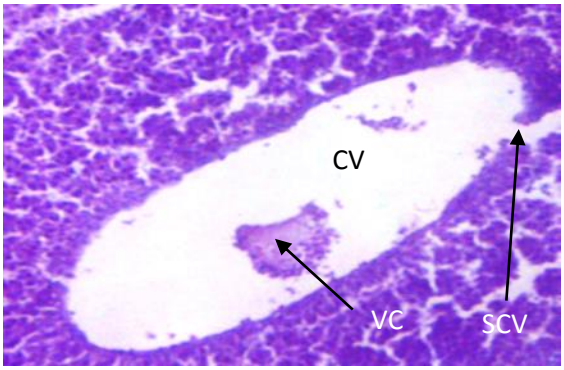

Plate 1.3b: Photomicrograph of liversection in *H. fasciatus* of Igun reservoir(Mag. X100)

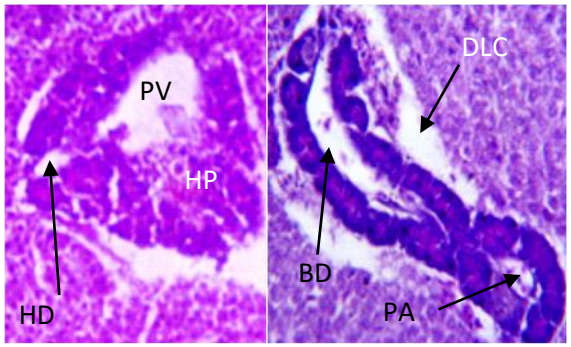

Plate 1.3c: Photomicrograph of liver section in *H. fasciatus* of Opa reservoir(Mag. X100)

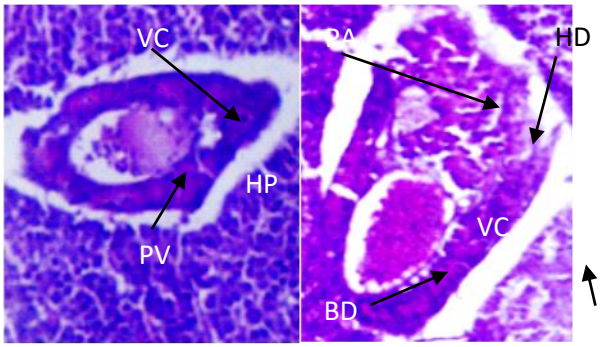

Plate 1.3d: Photomicrograph of liversection in *H. fasciatus* of Igun reservoir(Mag. X100)

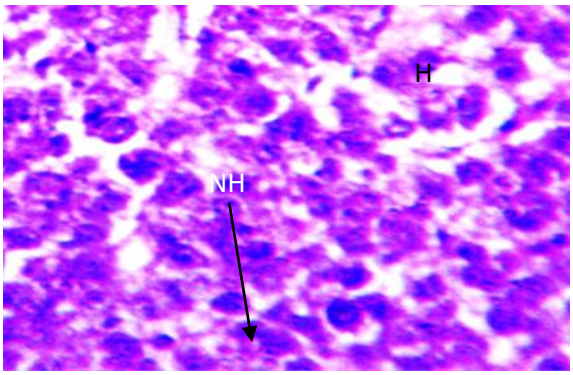

Plate1.3e: Photomicrograph of liver section in *H. fasciatus* of Opa reservoir(Mag. X400)

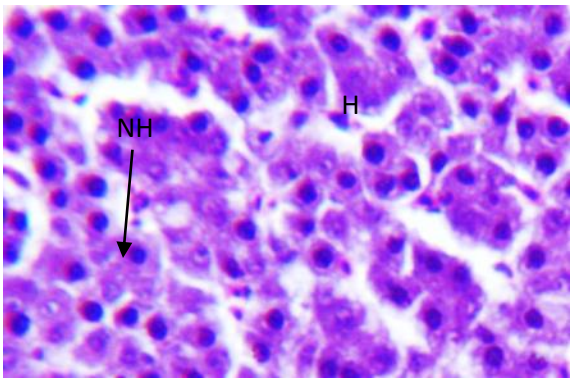

Plate 1.3f: Photomicrograph of liversection in*H. fasciatus* of Igun reservoir(Mag. X400)

Keys: *splitting at the wall of the central vein (SCV), central vein (CV),vascular congestion (VC), portal vein (PV), hepatopancreas degeneration (HD), hepatopancreas (HP), bile duct (BD), degeneration of liver cells (DLC), portal artery (PA), nucleus hepatocytes (NH), hepatocyte (H).*

Table 1: Histopathological Alterations in the Organs of *Hemichromis fasciatus*in Opa Reservoir and Stages of Severity of the Alterations

Organs	Histopathological alterations	Stage
Gills	Hypertrophy of primary filament	I
	Epithelial lifting	I
Fillet	Atrophy of muscle bundles	I
	Splitting of muscle bundles,	I
	Degeneration in muscle bundles	II
Liver	Splitting at the wall of the central vein	I
	Nucleus hepatocytes	I
	Hepatopancreas degeneration	II
	Degeneration of liver cells	II

Stage I = slight alteration, Stage II = moderatealteration, Stage III = severe alteration

(Source: Simonato *et al.*, 2008)

Table 2:Histopathological Alterations in the Liver of *Hemichromis fasciatus*in Igun Reservoir and Stages of Severity of the Alterations

Organs	Histopathological alterations	Stage
Gills	Fusion of secondary lamellae	I
	Hyperplasia of secondary lamella	I
	Curling of secondary lamellae	II
	Rupture of gill epithelium	II
	Rupture of chloride cells	II
Fillet	Atrophy of muscle bundles	I
	Splitting of muscle myofibrils	I
	Splitting of muscle bundles	I
	Parasite cyst	I
Liver	Splitting at the wall of the central vein	I
	Nucleus hypertrophy	I
	Hepatopancreas degeneration	II
	Central vein with vascular congestions	II
	Portal vein with vascular congestion	II
	Portal artery with vascular congestion	II
	Vascular congestion in the bile duct	II
	Degeneration of liver cells	II

Stage I = slight alteration, Stage II = moderatealteration, Stage III = severe alteration

(Source: Simonato *et al.*, 2008)

DISCUSSION

The gills of a fish play a vital role in maintaining of aquatic organism ionic homeostasis (Evans, 1993). Subsequently many contaminants come in close contact with gill epithelium and causes injury. The damages could depend on the level and period of exposure of the pollutants. Hypertrophy of primary lamellae and epithelial lifting observed in the gills of *H. fasciatus* in Opa reservoir were similar to the results of Yogitaand Mishra (2013). The authors noted that epithelial lifting could lead to dysfunctional or even non-functional gills, and sudden death of the fish. Similarly, histopathological alterations observed in the gills of *H. fasciatus* in Igun reservoir were similar to the findings of Abdullah (2001) who observed epithelial necrosis and rupture of the gill epithelium induced by zinc ions in *Cyprinus carpio*. The epithelium lifting is considered to be one of the initial reactions of the gill to variety of pollutants according to Al-Mansoori (2006). The histopathological alterations in this organ are a response to exposure to non-specific pollutants (Au, 2004).

Also, atrophy and splitting of muscle bundles revealed in the fillet of *H. fasciatus* in Opa and Igun reservoirs was likewise recorded by Ramesh and Nagarajan (2013) in the muscle of *Clarias batrachus*. The degeneration of muscle bundles seen in the fillet of *H. fasciatus* in Opa reservoir was also reported by Kaoud and El-Dahshan (2010) in the muscle of *O. niloticus*. It can thus be suggested that different alterations observed in the fillet of *H. fasciatus* in Opa and Igun reservoirs could be due to the presence of various contaminants in the reservoir.

Histopathological alterations observed in the liver of *H. fasciatus*in both reservoirs were similarly recorded by Chavan and Muley (2014) in the liver of *Cirrhinus mrigala*. These lesions are hepatopancreas degeneration,

splitting at the wall of central vein, degeneration of liver cells and nucleus hypertrophy. It seems possible that these alterations are due to heavy metals pollution in the two reservoirs. Furthermore, hepatopancreas degeneration observed in the liver of *H. fasciatus* in Opa reservoir was similar to the report of Naeemi *et al.* (2013) on histopathological changes of gills, kidney and liver of *Caspian kutum*exposed to Alkylbenzene Sulfonate. Also, vascular congestion in central vein, portal vein and bile duct seen in the liver of *H. fasciatus* of Igun reservoir were similar to the alterations observed in the liver of *Anabas testudineus*in Ban Pu Reservoir according to Saenphet*et al.* (2009). A possible explanation for congestion in the liver cells could be as a result of injury to the cells. The results of this study is in agreement with the work of Osman *et al*. (2009) who reported congestion in liver cells of *Oreochromis niloticus* exposed to polluted water. This study has shown that the organs of *H. fasciatus* in Igun reservoir was severely damaged compared to the organs of *H. fasciatus* in Opa reservoir. The findings of this study suggest that aquatic pollution resulting from mining activities in Igun reservoir may have resulted to severe changes recorded in the organs of *H. fasciatus* in Igun reservoir.

CONCLUSION

The results of this study have shown that more alterations were found in the tissues of *H. fasciatus* in Igun reservoir compared to Opa reservoir. The evidence from this study suggests that bioaccumulation of heavy metals in the tissues of fish from Igun reservoir could have resulted in severe changes in the tissue.

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APPENDIX

Reagents

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