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Comparative histopathological changes in some organs of *Tilapia zillii* in an abandoned gold mine reservoir of Igun and Opa freshwater reservoir, Ile-Ife, southwestern, Nigeria

*** Komolafe Olusola Olaniyi, Obayemi Oluwadamilare Emmanuel, Lawson Oluwatobi**
Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria

Abstract
Histopathological changes in the organs of *Tilapia zillii* in Opa and Igun reservoir were examined. Fishes were collected using cast nets and traps while the gills, liver and fillet were excised out for histological examination. The result showed the rupture of gill epithelium, hyperplasia and degeneration of secondary lamellae of the gills in fish samples collected from Igun reservoir when compared to Opa reservoir. The fillet of the fish in the two reservoirs showed muscular atrophy while muscular degeneration was observed in the fillet of Igun reservoir fish. Histological examination of liver of *T. zillii* in Igun revealed necrosis, nuclear hypertrophy, hepatocytes hypertrophy and hepatopancreas degeneration. Degeneration of the liver cells was observed in *T. zillii* in Opa reservoir.

Keywords: histopathology, reservoir, Opa, Igun, *Tilapia zillii*

Introduction

Fishes are excellent nutritional source of low fat protein and helps to reduce blood cholesterol [1]. Increasing number and amount of industrial, agricultural and commercial chemicals discharged into the aquatic environment have led to various deleterious effects on the aquatic organisms [2]. Aquatic organisms, including fish, accumulate pollutants directly from contaminated water and indirectly *via* the food chain [3]. Fishes are important biological assays for trace elements in aquatic ecosystem and are useful in evaluating the status of water pollution [4]. Most fresh water bodies contain metals which are natural constituents of the environment. These metals are found in varying concentrations in ground and surface waters [5]. Heavy metals are non-biodegradable and they persist in all parts of the environment, they are considered as major environmental pollutants causing cytotoxic, mutagenic and carcinogenic effects in animals [6]. According to [7, 8] urbanization, civilization, and industrialization had contributed immensely to the pollution of aquatic system, making it the ultimate recipient of heavy metal pollution. Heavy metals entering the aquatic ecosystem originate from different sources such as decay of plants and vegetation, atmospheric particulate, discharge of domestic and municipal wastes etc. [9]. The gills, a very important and multifunctional organ in fish that participate in many important functions in the fish such as respiration, osmoregulation and excretion, remain in close contact with the outside habitat. Similarly, it is mostly sensitive to variations in the quality of the water as well as considered as the primary target of the pollutants [10]. Fish liver serves as a key organ that controls many life functions and plays a prominent role in fish physiology, both in anabolism and catabolism [11].

Several Studies have been conducted on histopathological changes in the gills, liver and kidney of fish exposed to various substances including pesticide which have been described to cause pathological changes in the exposed *Clarias gariepinus* [12]. Histopathological changes integrate the impact of a variety of stressors (pathogens, toxic compounds, and unfavorable nutritional and environmental conditions). One of the great advantages of using histopathological biomarkers in environmental monitoring is that this category of biomarkers allows examination of specific target organs, including gills, kidney and liver that are responsible for vital functions, such as respiration, excretion and the accumulation and biotransformation of xenobiotics in the fish [13]. Monitoring histological changes in fish liver is a highly sensitive and accurate way to assess the effects of xenobiotic compounds in field and experimental studies [14]. Furthermore, the alterations found in these organs are easier to identify than functional ones [15] and serve as warning signs of damage to animal health [16].

Histological changes appear as a medium term response to sub-lethal stressors, and histology provides a rapid method to detect effects of irritants, especially chronic ones, in various tissues and organs [17]. *T. zillii* had been observed to accumulate heavy metals by [18]. Histopathological investigations have long been recognized to be reliable biomarkers of stress in fish [19]. Histopathological changes have been widely used as biomarkers in the evaluation of the health of fish exposed to contaminants, both in the laboratory and field studies [20]. Histopathological alterations can be used as indicators for the effects of various anthropogenic pollutants on organisms and are a reflection of the overall health of the entire population in the ecosystem [21]. The purpose of this study is therefore to further investigate the effect of pollution on *T. zillii* which are economically important fish found on the tables of most people owing to its ability to tolerate favourable environmental factors, it is found in water bodies of rural and urban settlements in Nigeria.

Materials and Methods

Study Area

The study areas are abandoned gold mine reservoir at Igun village in Atakumosa 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 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.

The temperature of the area during the seasons ranged between 23^0C to 33^0C. Igun is characterized with two seasons which are rainy season and dry season. The dry season is from November to February while March to October marks the rainy season. Six reservoirs were impounded in Igun, but one of these reservoirs was used for the present study. Reservoir five is an open water body which receives direct solar radiation. It is wide with a mean depth of 2.75m and accessible by the use of canoe only. It receives high discharge of water during the rainy season; hence its water becomes turbid because of the sediments carried from the catchments area as well as the deposition of wastes from mining activities by local miners. The substratum is made of mud while the shore line is sandy.

The reservoir shoreline vegetation was made of aquatic macrophytes such as *Rhyrchosiabuttner*, *Cyclosoruafer* and *Melantherascanderna*. The surface of the reservoir becomes cleared of some of the higher plants during the rainy season as a result of the influx of water. However, the water surface was covered with macrophytes in the dry season especially in November to March when some of these higher plants were submerged in water.

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 as shown in 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.

The temperature of the area during the seasons ranged between 23.5^0C to 34^0C. Opa is characterized with two seasons which are rainy season and dry season. The dry season is from November to February while March to October marks the rainy season. The vegetation of Opa reservoir is mainly agricultural mosaic, a lot of farmlands and various agricultural activities are particularly noticeable along the banks of the reservoir.

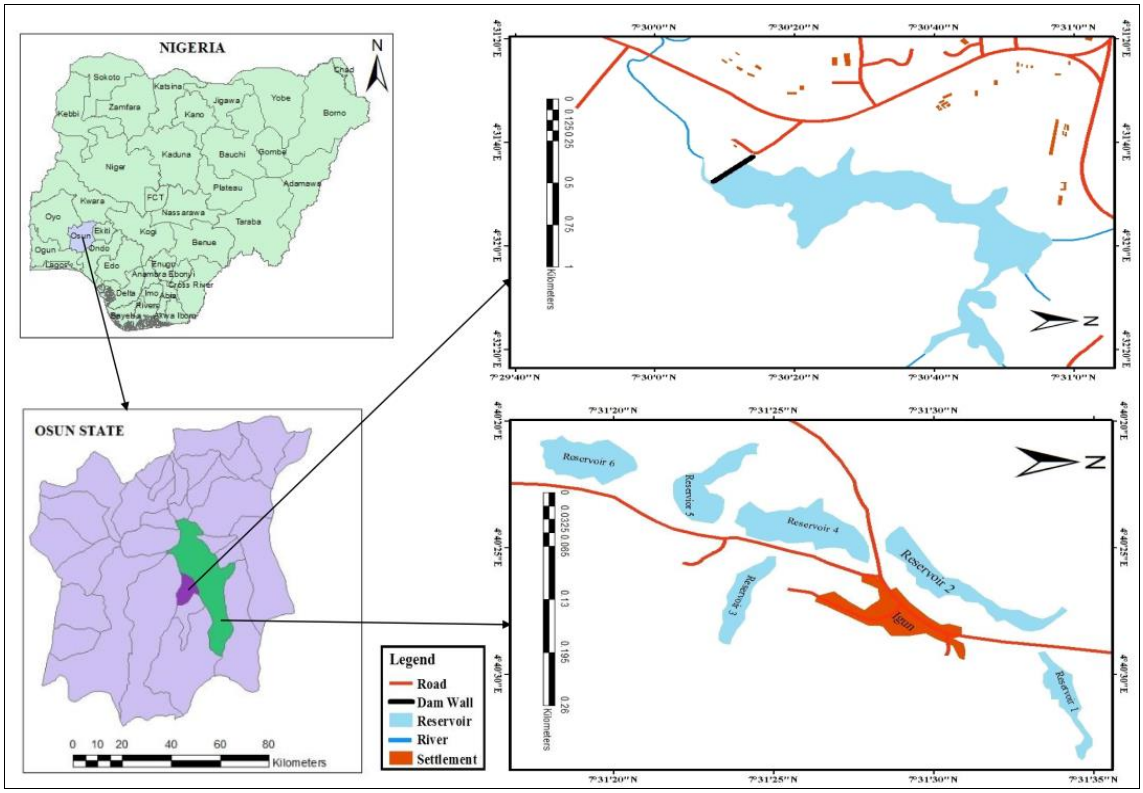

Fig 1: Map of Opa and Igun reservoirs showing its location in Nigeria

Collection of fish samples

Fish samples were collected on a monthly basis using cast net between October 2014 and March 2015. Fishes were identified using standard keys prepared by [22] and [23]. 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 [24] 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 [25], 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 *Tilapia zillii* of Opa and Igun reservoir

The histopathological changes observed in the gills of *T. zillii* of Opa reservoir includes rupture of gill epithelium (Fig. 1.1a), epithelium lifting (Fig. 1.1c) and rupture of chloride cell (Fig. 1.1e). The photomicrograph of the gills of *T. zillii* of Opa reservoir exhibited normal fish gill structure with normal appearance of primary and secondary lamellae. The gills lesion observed in *T. zillii* of Igun reservoir includes rupture of gill epithelium and shortening of secondary lamellae (Fig. 1.1b), hyperplasia of secondary lamellae and curling of secondary lamellae (Fig. 1.1d), degeneration of secondary lamellae and rupture of chloride cell (Fig. 1.1f). There was no indication of shortening, hyperplasia or degeneration of lamellae in the gills of *T. zillii* of Opa reservoir.

Histological sections of *T. zillii* in Opa reservoir showed normal arrangement of muscle fibers with some sections showing muscular atrophy (Fig. 2.1a), splitting of muscle myofibrils in Fig. 2.1c and splitting of muscle fibers in Fig. 2.1e. Histopathological alterations observed in the muscle of *T. zillii* of Igun reservoir exhibited varying degree of alterations from edema between muscle fibers in Fig. 2.1b to splitting of muscle fibers and muscular atrophy (Fig. 2.1d) and muscular degeneration in Fig. 2.2f.

The histological alterations found in the liver of *T. zillii* of Opa reservoir were vascular congetion in the portal vein, degeneration of the hepatopancreas (Fig. 3.1c) degeneration of liver cells (Fig. 3.1e). The alterations observed in the liver of *T. zillii* of Igun reservoir includes focal area of necrosis (Fig. 3.1b), melanomacrophages aggregate close to portal vein, vascular congestion in bile duct and degeneration of hepatopancreas were also identified in Fig. 3.1d, nucleus hypertrophy, hepatocytes hypertrophy (Fig. 3.1f). The main alterations found in *T. zillii* of Igun reservoir compared with that of Opa reservoir includes hypertrophy of nucleus and hepatocytes with some sections showing necrosis of the liver.

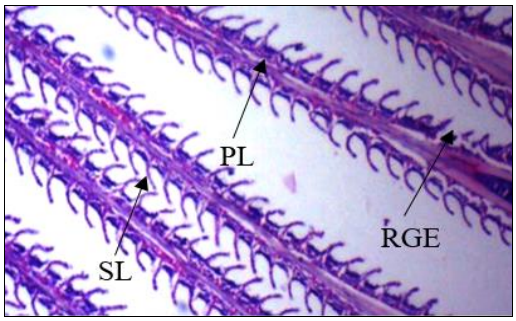

Fig. 1.1a: Photomicrograph of gill section in *Tilapia zillii* of Opa reservoir (Mag. X40)

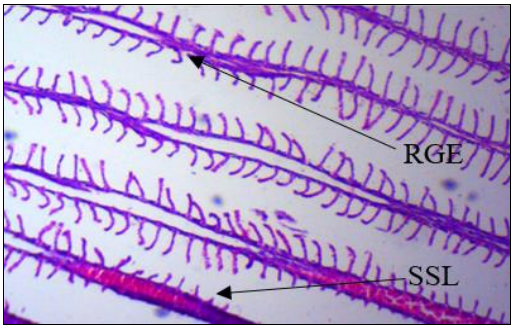

Fig. 1.1b: Photomicrograph of gill section in *Tilapia zillii* of Igun reservoir (Mag. X40)

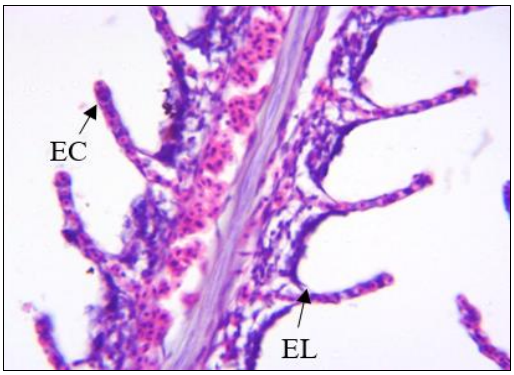

Fig. 1.1c: Photomicrograph of gill section in *Tilapia zillii* of Opa reservoir (Mag. X400)

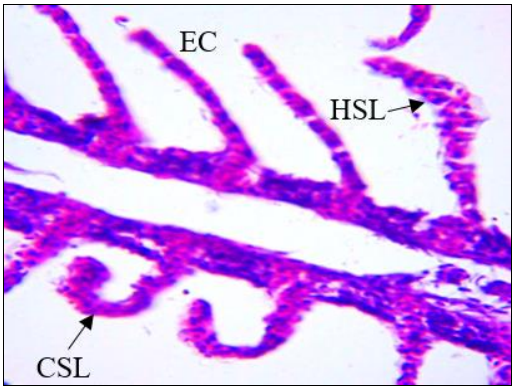

Fig. 1.1d: Photomicrograph of gill section in *Tilapia zillii* of Igun reservoir (Mag. X400)

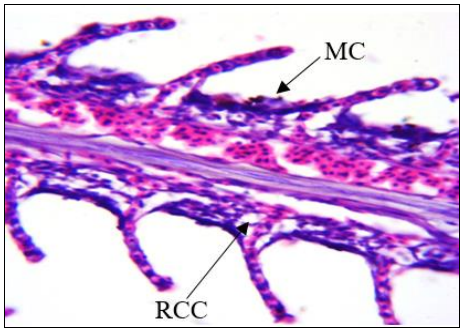

Fig. 1.1e: Photomicrograph of gill section in *Tilapia zillii* of Opa reservoir (Mag. X400)

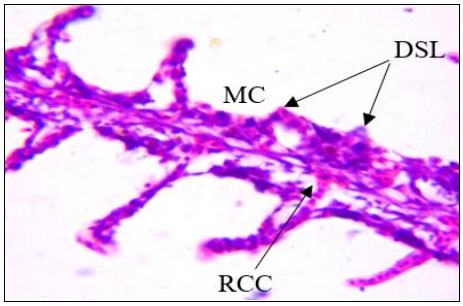

Fig. 1.1f: Photomicrograph of gill section in *Tilapia zillii* of Igun reservoir (Mag. X400)

Keys: secondary lamellae (SL), primary lamellae (PL), rupture of gill epithelium (RGE), shortening of the secondary lamellae (SSL), epithelium cell (EC), epithelium lifting (EL), curling of secondary lamellae (CSL), hyperplasia of secondary lamellae (HSL), mucous cell (MC), rupture of chloride cells (RCC), and degeneration of secondary lamellae (DSL).

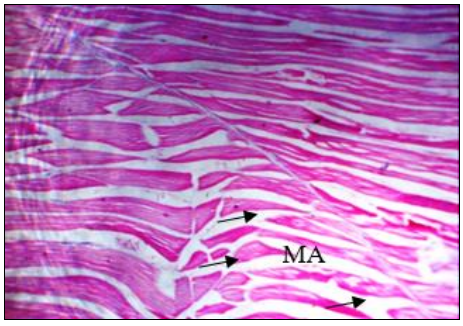

Fig. 2.1a: Photomicrograph of fillet section in *Tilapia zillii* of Opa reservoir (Mag. X40)

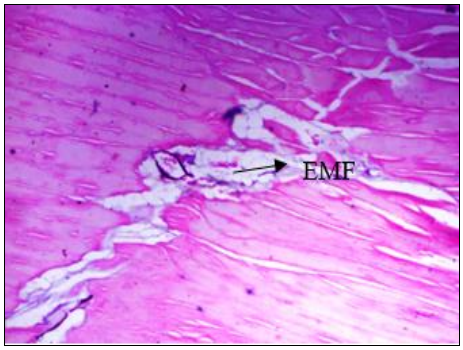

Fig. 2.1b: Photomicrograph of fillet section in *Tilapia zillii* of Igun reservoir (Mag. X40)

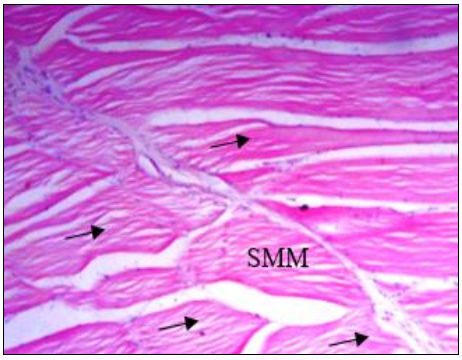

Fig. 2.1c: Photomicrograph of fillet section in *Tilapia zillii* of Opa reservoir (Mag. X100)

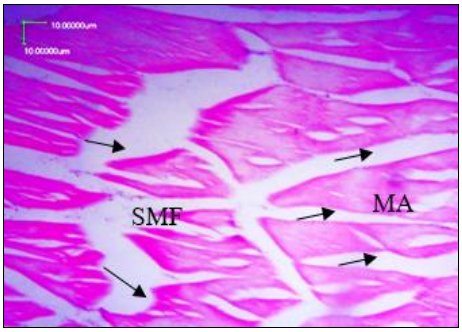

Fig. 2.1d: Photomicrograph of fillet section in *Tilapia zillii* of Igun reservoir (Mag. X100)

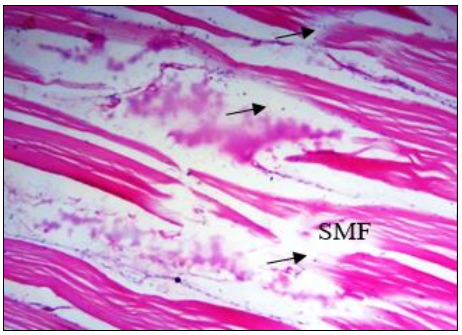

Fig. 2.1e: Photomicrograph of fillet section in *Tilapia zillii* of Opa reservoir (Mag. X400)

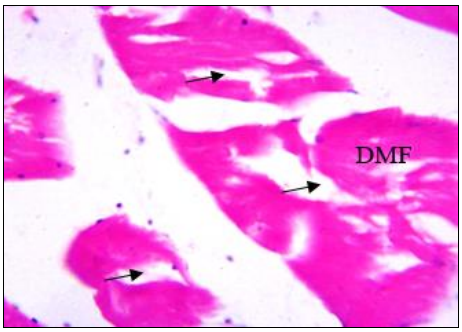

Fig. 2.1f: Photomicrograph of fillet section in *Tilapia zillii* of Igun reservoir (Mag. X400)

Keys: musclar atrophy (MA), edema between muscle fibers (EMF), splitting of muscle myofibrils (SMM), splitting of muscle fibers (SMF), degeneration in muscle fibers (DMF). Haematoxylin and Eosin stain.

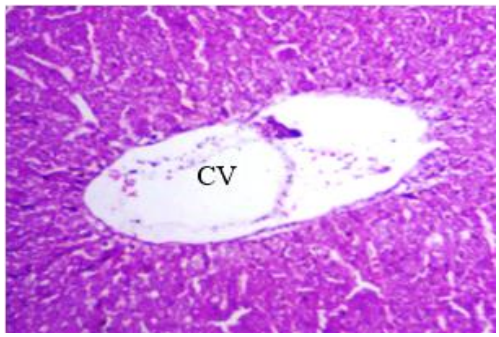

Fig. 3.1a: Photomicrograph of liver section in *Tilapia zillii* of Opa reservoir (Mag. X40)

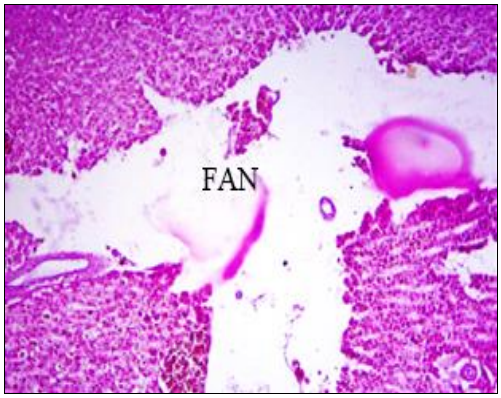

Fig. 3.1b: Photomicrograph of liver section in *Tilapia zillii* of Igun reservoir (Mag. X40)

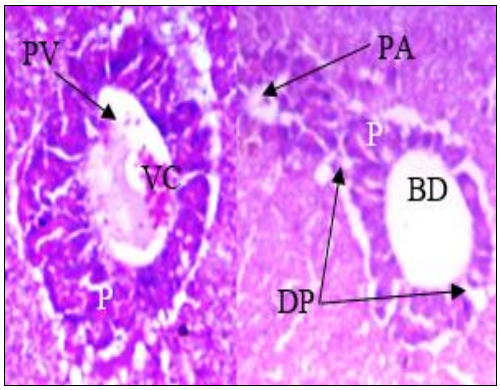

Fig. 3.1c: Photomicrograph of liver section in *Tilapia zillii* of Opa reservoir (Mag. X100)

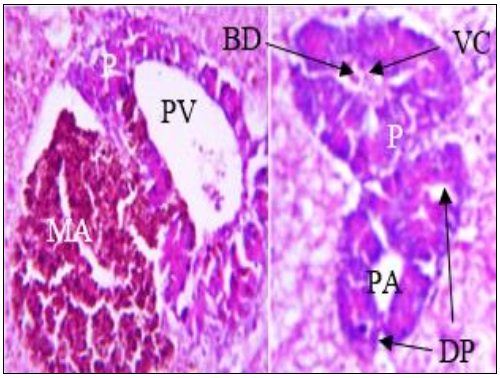

Fig. 3.1d: Photomicrograph of liver section in *Tilapia zillii* of Igun reservoir (Mag. X100)

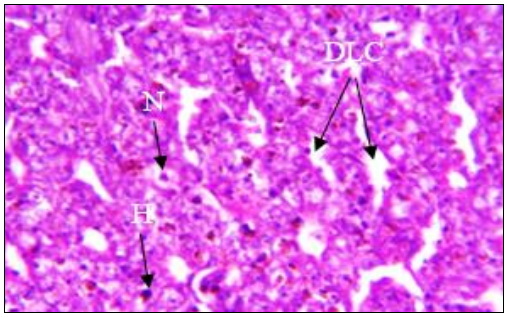

Fig. 3.1e: Photomicrograph of liver section in *Tilapia zillii* of Opa reservoir (Mag. X400)

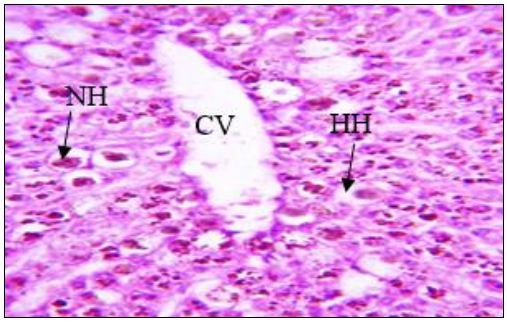

Fig. 3.1f: Photomicrograph of liver section in *Tilapia zillii* of Igun reservoir (Mag. X400)

Keys: central vein (CV), focal area of necrosis (FAN), portal vein (PV), hepatopancreas (P), portal artery (PA), vascular congestion (VC), degeneration of hepatopancreas (DP), hepatopancreas (P), bile duct (BD), melanomacrophages aggregates (MA), nucleus (N), hepatocytes (H), degeneration of liver cells (DLC), nucleus hypertrophy (NH) and hepatocytes hypertrophy (HH), Haematoxylin and Eosin stain.

Discussion

This study showed that Epithelial lifting, rupture of gill epithelial and rupture of chloride cells were the changes observed in the gills of *Tilapia zillii* of Opa reservoir while rupture of gill epithelial, shortening of secondary lamellae, curling of secondary lamellae, hyperplasia of secondary lamellae, rupture of chloride cells and necrosis of secondary lamellae were identified in the gills of *T. zillii* of Igun reservoir. The alterations found in *T. zillii* of Opa reservoir were similar to the study carried out by [26] on histopathological alterations in some body organs of adult *Clarias gariepinus* exposed to 4-Nonylphenol. [27] had reported that hyperplasia of gill might increase the thickness of the epithelial in order to retard into the blood stream. Alterations like curling of secondary lamellae, shortening of secondary lamellae, rupture of secondary lamellae and degeneration of secondary lamellae which were observed only in *T. zillii* of Igun reservoir differed a little with previous report by [10] that examined histopathology of gills, kidney and liver of a Neotropical fish (*Prochilodus lineatus*,) caged in an urban stream in which the authors observed hyperplasia, hypertrophy and fusion of the gill epithelium.

Alterations identified in both gills of *T. zillii* of Opa and Igun reservoir, epithelial lifting, rupture of gill epithelium and rupture of chloride cells were similar to the works observed by

[28]. Epithelial lifting could lead to dysfunctional or even non-functional gills, and may lead to sudden death of the fish [29]. Splitting of muscle fibers and muscular atrophy were observed in both fillet of *T. zillii* of Opa and Igun reservoir [21]. Also reported similar findings with this present result. Fillet alterations examined in the *T. zillii* of Opa and Igun reservoir such as atrophy between muscle bundles and edema between muscle bundles with degeneration in muscle bundles was as a result of heavy metal toxicant leading to many pathological changes in different fish tissues as observed by [30] worked on *Labeo rohita* exposed to mercury chloride and *Chana punctatus* exposed to phenyl-mercuric acetate.

The liver of fishes is associated with biotransformation and detoxification process due to its function, position and blood supply [31]. It is also one of the organs most affected by pollutant in the water [10]. The liver of *T. zillii* of Opa and Igun reservoir shows similar alterations, which were vascular congestion and hepatopancreas degeneration, these listed damages indicated possible injuries to the liver hepatic metabolism as reported by [15]. Nucleus hypertrophy, melano-macrophages close to the bile duct, focal area of necrosis and hepatocytes hypertrophy observed in the liver of *T. zillii* of Igun was also similar to the findings with of [32]. Melano-macrophage aggregates are associated with a number of fish diseases and which are phagocytic cells in nature as reported by [33]. Focal area of necrosis have been reported as severe changes, which are more commonly associated with the exposure of fishes to pollution by various metals [34]. Varying degree of alterations in the organs of *T. zillii* in Igun reservoir compared to that of Opa reservoir could be as a result of harmful chemicals released from the gold mines close to the reservoir thereby altering the normal structure of the organs [18]. Had reported the presence of heavy metals such as As, Cr and Zn in the fillet and gills of *T. zillii*, *Hemichromis fasciatus* and *Sarotherodon galilaeus* at unacceptable level in the habitat. The high degree of histopathological changes in the *T. zillii* of Igun compared to Opa was probably as a result of environmental pollution due to mining activities in the environment thereby altering the normal structure of the organs to cause various changes in the tissues.

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