HISTOPATHOLOGICAL ANALYSES OF LIVER AND GILL OF *COPTODON ZILLII* OBTAINED FROM OKERENKOKO WATERFRONT, DELTA STATE, NIGERIA

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ABSTRACT

Pollutants and untreated anthropogenic effluents in surface water can cause abnormal changes in internal organs of fishes. Liver and gill are the most affected fish tissues due to their constant contact with toxins. Histopathological biomarkers are identifiable abnormalities in tissues of organisms predisposed to wide range of environmental pollutants. Information on histopathological biomarkers of fish from the Okerenkoko Waterfront (OWF) is limited. Hence, this study was carried out to investigate the effects of untreated effluents on the liver and gill of Coptodon zillii obtained from OWF. Samples of C. zillii were collected for four months using gill net of mesh size 24 mm. Liver and gill of farmed C. zillii were used as control. Harvested liver and gill of C. zillii were fixed in Bouin's fluid and taken to laboratory for histopathological assay using standard procedures. The C. zillii harvested from OWF showed significant damage in selected organs. Liver tissues showed hepatocyte vacuolization, hepatocyte degeneration, necrosis, inflammation and sinusoids dilation, while gill tissues revealed uplifting of the primary epithelium, fusion, vacuolation, hypertrophy and necrosis. Liver and gill tissues of control C. zillii did not show any histopathological abnormalities in its liver and gills. Results from this study showed a positive correlation between the extent of tissue damage and surface water pollution. Findings from this study serve as baseline for future studies, while regular monitoring of pollutants in the OWF should be encouraged to protect its fish stock.

Keywords: Anthropogenic effluents, Pollution, Surface water, Fish, Histopathology, Biomarkers

INTRODUCTION

Fish histopathological examination involves the collection and preservation of fish tissues, followed by histological processing, staining procedures and microscopic examination (Shahid *et al.,* 2020). It has been reported that histopathological assessments can assists in identifying specific diseases or conditions affecting fish populations and provide insights into their etiology, physiological progression, and impacts on overall fish stock (Kalaiyarasi *et al.,* 2017). Histopathological analyses serve as diagnostic tools to detect the presence of pathogens, parasites or toxins in fish tissues (Sultana *et al.,* 2016). Furthermore, histopathological data can be used to monitor long-term health trends of fish populations, assess the effectiveness of management measures and evaluate the potential risks of pollutants in aquatic ecosystems (Santos *et al.,* 2014).

Histopathological changes in fishes have been associated with the presence of heavy metals in the aquatic ecosystem of east Berbice-Corentyne, Guyana (Rajeshkumar *et al.,* 2015).

Introduction of untreated effluents into surface water has the potential to raise the levels of pollutants above the natural concentrations (Ewutanure and Olaifa, 2021). This act encourages the accumulation of pollutants in water, sediment, fish tissues and eventual bio-magnification in the aquatic food chain. Increase concentration of pollutants in water and sediment represents danger to the aquatic biota and poses negative effect on human health through the aquatic food chain (Ewutanure *et al.,* 2022). The pollution status of any aquatic ecosystem is a function of the contamination of its sediment which is the final repository of pollutant (Batlle and Tejero, 2017), while the presence of heavy metals could lead to serious environmental damage, destruction of aquatic habitats, reduction of biodiversity and contamination of sediments/soil and groundwater (Aghoghovwia *et al.,* 2016).

In histopathological studies involving the use of fish samples, the most frequently used tissues are liver, kidney, intestine, stomach, gills, skin and spleen (Alyahya *et al.,* 2018). These tissues are selected based on their physiological significance, susceptibility to toxins, diseases and their ability to reflect systemic responses (Abdel-Moneim *et al.,* 2012). The responses of fish tissues to varying degrees of exposure to various levels of pollutants have been reported (Ekpete *et al.,* 2019). Physiological changes in gill histoarchitecture that are exposure-dependent include lamellar fusion, epithelial lifting and necrosis as evidence of pollutants toxicity in the fishes have been reported (Amadi, 2012).

According to FishBase (2023)*Coptodon zillii* Gervais, 1848 (Cichliformes: Cichlidae) has its origin from the marine but has also been widely found in freshwater environment in different regions of West Africa such as Nigeria, Guinea, Sierra Leone, Liberia, and Ivory Coast. According to FishBase (2023), *C. zillii* has a laterally compressed body form, possesses a prominent mouth which is a characteristic of species that feed on algae and other plant material. They are sexually dimorphic species with males typically displaying brighter colour and possessing longer fins compared to females. *C. zillii* exhibits parental care strategy with the male guarding the eggs and fry after spawning (Fish Base, 2023). Meristically, *C. zillii* possesses a total of 14 – 16 dorsal spines, 12 – 13 dorsal soft rays, 3 anal spines and 8 – 10 anal soft rays (FishBase, 2023).

Okerenkoko is a community located in the Gbaramatu Kingdom along the Escravos Estuary. Studies have shown that Okerenkoko and other communities in the Gbaramatu Kingdom have suffered severe consequences of oil spillages (Ewutanure *et al.,* 2022; Michael-Olomu and Udeh, 2022; Sam *et al*., 2023). Information on the histopathological changes in its fish species from Okerenkoko Waterfront is limited. This study was carried out to investigate the impacts of untreated effluents on the liver and gill of *C. zillii* obtained from Okerenkoko Waterfront, Delta State, Nigeria.

MATERIALS AND METHODS

Study Area: Okerenkoko Waterfront is located on latitudes 5°37′62″ N and 5°37′16″ N of the equator and longitudes 5°23′69″ E and 5°23′12″ E of the Greenwich meridian (Figure 1), while the exact locations of all sampling stations were determined using Garmin GPSMAP eTrex10 type sensors. It has a common link with the Escravos River, situated in the Niger Delta Region, Delta State, Nigeria. It is located in a mangrove swamp forest. The major species of mangrove identified were the red (*Rhizophora racemose*) and white (*Avicennia africana*) mangroves, while the flood plain borders the Waterfront and its adjoining creeks. Okerenkoko dwellers are majorly fishers (Ewutanure *et al.,* 2022).

The study area has humid climate, with wet season occurring from March – October and dry season from November – February. The climate is influenced by two prevailing winds such as the South-West monsoon wind from the Atlantic Ocean and the North-East trade wind from the Sahara Desert. The South-West monsoon wind causes wet season, while the North-East wind is responsible for the dry season (Ewutanure and Binyotubo, 2021). Rainfall peaks in June/July and September, with a short break period in August, while the average annual temperature was 27 ± 4°C (Ewutanure and Olaifa, 2021).

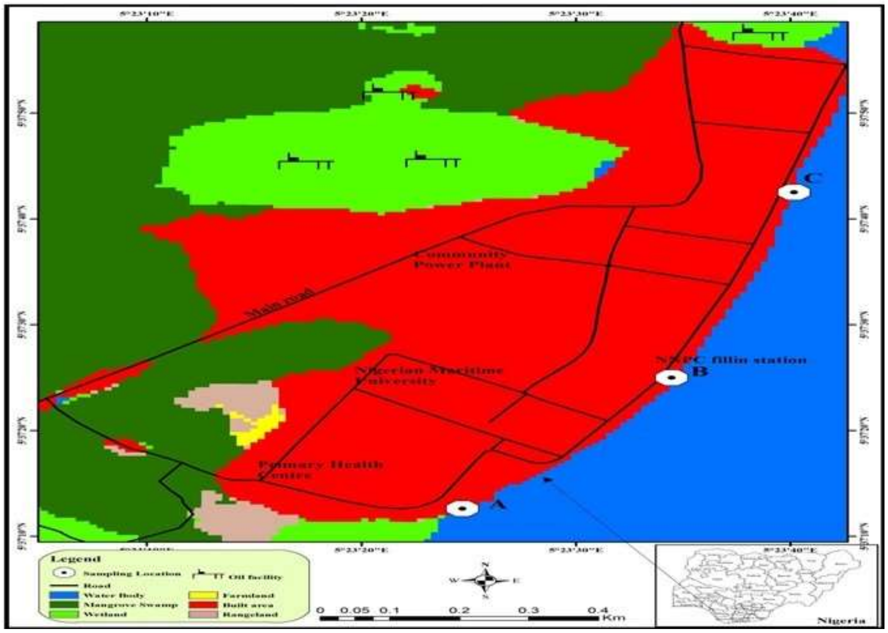

Figure 1: Map of Okerenkoko Waterfront showing the sampled sites (A, B and C) (QGIS, 2023)

Sampling Techniques and Procedures: Okerenkoko Waterfront was spatially stratified into three stations (A, B and C) based on proximity to key anthropogenic activities. Monthly stratification covered April – July 2023. *C. zillii* samples were collected on monthly basis using a lift net and identified to species level by using standard keys (FishBase, 2023).

Analytical Procedures

Histopathological procedures: Liver and gills samples of *C. zillii* were collected and fixed in Bouin's solution. The fixed samples were dehydrated in 60% to absolute alcohol, cleared in xylene, infiltrated with molten wax, embedded in paraffin wax, sectioned and stained with Haematoxylin and Eosin (H&E) stain as described by Drury *et al.* (1967). The stained tissues sectioned were examined under a light microscope as described by Camargo and Martinez (2007). Cellular details, tissue organization and pathological changes commonly specific to fish liver and gill were observed and documented. Photomicrographs were taken to observe these features and compare them with the normal gill and liver histology of tilapian fishes. The observed histological features were interpreted in the context of fish health and histopathological conditions (Dhevakrishnan and Zaman, 2012). The presence of abnormalities, inflammation and other pathological changes were identified and characterized (Ezemonye and Ogbomida, 2010). The histopathological results obtained were quantified and compared with standard fish histological atlases (Takashima and Hibiya, 1995; Genten *et al.*, 2009) to draw conclusions about the impacts of pollutants on the gill and liver of *C. zillii* populations.

RESULTS AND DISCUSSION

Observed histopathological changes in liver and gill of *C. zillii* are presented in Figures 2 and 3 respectively. When compared with the normal photomicrograph of *C. zillii*, histological lesions observed in the liver were hepatocyte degeneration, necrosis, inflammation and fibrosis. When compared with the normal photomicrograph of *C. zillii*, histological lesions observed in the gill were hyperplasia of lamellae, lamellae fusion, aneurysm, lifting of respiratory epithelium and curving of secondary lamellae.

Histopathological biomarkers can be sensitive indicators of sub-cellular stress in organisms that are predisposed to a wide range of pollutants over a certain period of time (Ibrahim, 2013). These biomarkers embody tissue lesions arising as a result of previous or current exposure of the organism to one or more toxins (Liebel *et al.*, 2013).

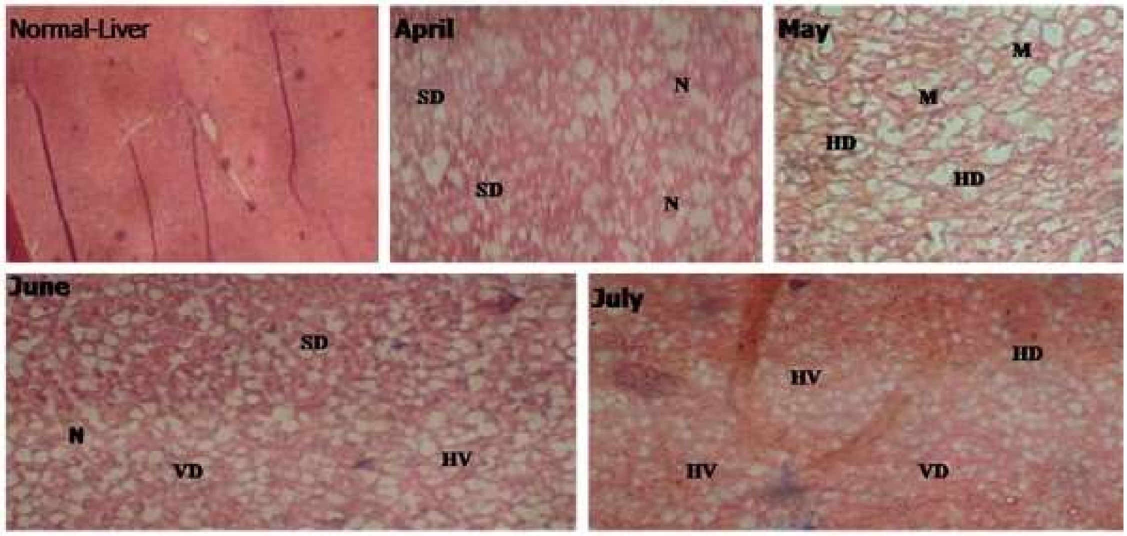

Figure 2: Photomicrograph of *Coptodon zillii* liver showing sinusoid dilation (SD), Necrosis (N), Mitochondrial granular hepatocytes (M), Hepatocytes degeneration (HD) and Hepatocytes vacuolation (HV). H&E, Mag. = x 400

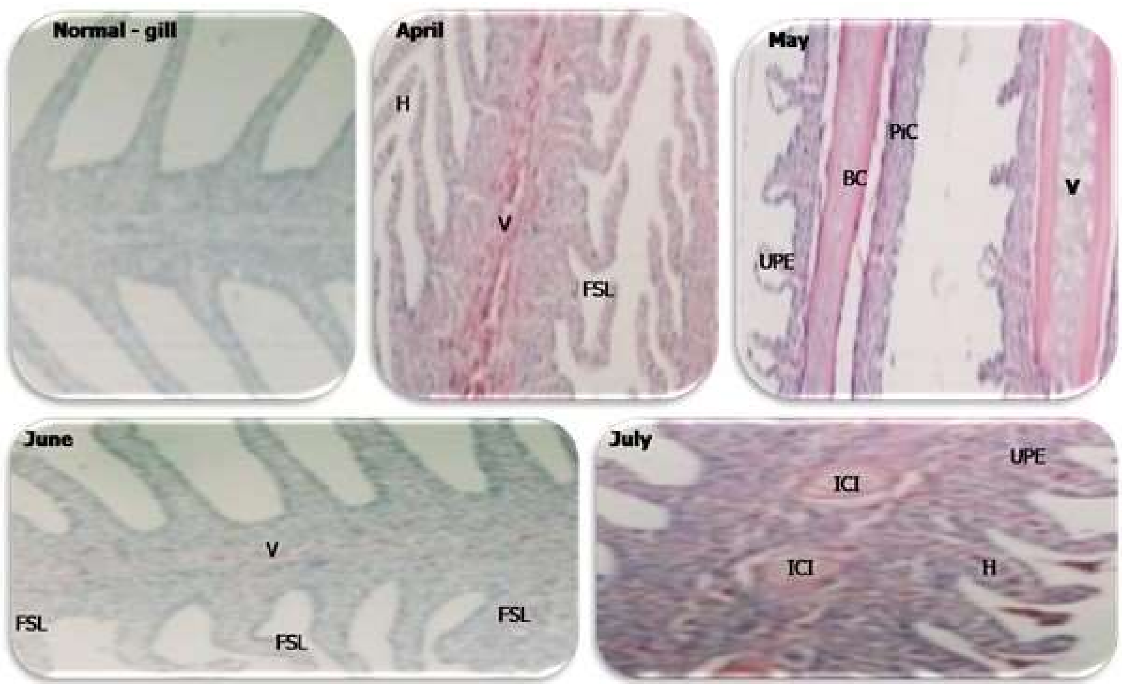

Figure 3: Photomicrograph of *Coptodon zillii* gill showing Hypertrophy (H), Vacuolation (V), Fussed secondary lamellae (FSL), Pillar cell (PiC), Blood capillary (BC), Uplifting of primary epithelium (UPE) and inflammatory cell infiltration (ICI) H&E, Mag. = x 400

Well documented lesions based on environmental studies on liver, gill, ovary, skeleton system and skin have been used as biomarkers till date (Marchand *et al.*, 2012).

The histological lesions observed in the photomicrograph of the liver of *C. zillii* were hepatocyte degeneration, necrosis, inflammation, hepatocyte vacuolization, sinusoids dilation and fibrosis. Results obtained corroborated the report on *Arius maculatus* (Kalaiyarasi *et al.,* 2017). Similar results were also reported in *Mugil cephalus* because of heavy metal pollution in Ennore Estuary (Vasanthi *et al.,* 2013). The findings of this study were also in agreement with the findings of Rajeshkumar *et al.* (2015) on the use of histological and ultra-structural biomarkers in *M. cephalus* for assessing heavy metal pollution. Abdel-Moneim *et al.* (2012) also adduced that hepatocyte vacuolization and necrosis in parenchymal tissues of *Oreochromis niloticus* could be due to polluted surface water with heavy metals. According to Topić Popović *et al.* (2023), the liver is a very vital organ in fish and it is the principal detoxification organ. It also assists in the buildup of blood plasma, storage and release of glycogen (Paithane *et al.,* 2012). Inflammation distorts the flow of portal venous blood that is responsible for sinusoid dilation (Patnaik *et al*., 2011).

The accumulation of pollutants in the liver can results to changes in its morphology, histology and histopathology (Santos *et al.,* 2014). Certainly, the effects of pollutants were very visible, severe and damaging to the liver cells of *C. zillii*. This observation corroborated earlier study carried out on the effects of heavy metals on *O. niloticus* obtained from Warri River which clearly indicated focal and toxic necrosis, oedema in the sub-capsule, melanomacrophage center reduction and accumulation of toxic fluids in the liver (Aghoghovwia *et al.,* 2016).

However, fused gill lamellae, inflammatory cell infiltration, hypertrophy, lifting of primary epithelium, fusion and vacuolization of gills reported in the present study corroborated with the findings of Capaldo *et al.* (2019) in *Anguilla anguilla*. According to Vasanthi *et al.* (2013), gills are sensitive organs which are easily damaged by various pollutants even at very low concentrations. Since the gills function as a source of respiration, osmoregulation, excretion and possess a large surface area in contact with the external environment, they are particularly sensitive to chemical and physical changes of surface water (Capaldo *et al*., 2019). This makes it a target organ by aquatic pollutants (Liebel *et al*., 2013).

Ezemonye and Ogbomida (2010) reported vacuolation, hyperplasia and lamellar fusion in gill of *Clarias gariepinus* exposed to Gammalin 20. Uplifting of lamellae observed may be as a result of infiltration of fluid between epithelium and basement membrane that supports diffusion of gases during gaseous exchange. Results of this study were equally in agreement with the findings of Santos *et al.* (2014) for gills of two fish species in Jansen Lagoon, Brazil. Similar findings were also reported by Liebel *et al.* (2013) and Shahid *et al.* (2020) in *O. niloticus* and *Ictalurus punctatus* obtained from polluted surface waters. According to Shahid *et al.* (2020) vacuolization may be a defense mechanism against wounds that alter the health condition of the fish.

Consequently, the presence of toxic substances in an aquatic environment causes alterations in the vital functions carried out by the gills of fishes and alterations in their morphologic structures (Aghoghovwia *et al.,* 2016). Previously, researchers have used the gills of fish as an organ for evaluating the presence of pollutants in natural environments (Flores-Lopes and Thomaz, 2011; Liebel *et al.,* 2013; Shahid *et al.,* 2020). Field studies are very essential in evaluating, understanding the biology and ecological effects of chemical agents under natural conditions (Ewutanure *et al.,* 2022).

Conclusion: Histopathological changes in the liver and gill of *C. zillii* obtained from Okerenkoko Waterfront indicated that *C. zillii* responded to some environmental stressors whose exact nature was not determined in this study. However, the extent of liver and gill damage was generally mild to moderate except for liver hepatocyte degeneration and hyperplasia in gill which should be a cause of concern. It has been established that hyperplasia gills can cause massive mortalities of fishes in surface water due to accumulation of pollutants. As current impacts of climate variability on the aquatic environment persist, it provides an avenue to adopt routine limnological monitoring programmes of Okerenkoko Waterfront. The availability of such data will be very beneficial for present and future sustainable management of its fish stock.

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