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Histological Aberations in *Heterobranchus Longifilis* and *Clarias buthupogon* Obtained from Polluted Asa River, Nigeria

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ABSTRACT

Histological aberrations were evaluated in *Clarias buthupogon* and *Heterobranchus longifilis* sampled from Asa River Nigeria. Impact of water borne contaminants on aquatic organisms has become a great concern to public health. Since fish gills are indirect contact with the environmental medium and are very sensitive to changes in water quality while fish liver serve as gate-keeper to the entire body of fish. Therefore, the aim of this study was to evaluate the effects of Asa River water quality on the gills and liver histology of the two selected sampled fish. The sample included a total of 55 individuals (28 for *C. buthupogon* and 27 for *H. longifilis*). There was a recorded moderate to intense alterations of gill tissues. The most notable alterations were congestion of blood vessels, desquamation, swelling of pillar cell, hyperplasia and hypertrophy of the gill epithelium. Epithelial lifting of the basement membrane wascommon in vertually all sampled fish species, while circulatory disturbances were slightly pronounced. The liver showed congestion of central vein, degeneration of hepatocyte, sinusoidal distortion, cellular inflammation and necrosis. However, all histological alterations recorded in the gill and liver tissues were most likely to be caused by increased concentrations of certain pollutants from anthropogenic premises. Therefore, proper assessment of Asa River is necessary with strict vigilance in view of ensuring the protection and conservation of the organisms there-in.

Keywords: Histology, Contaminant, *Clarias buthupogon*, *Heterobranchus longifilis,* Asa River and Histopathology

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INTRODUCTION

Decline in the populations of important fish species have become a severe problem in many Nigerian rivers over the past few decades and the reasons for this remained unclear. Water contaminants has been suspected to be possible contributors of visible abnormalities in aquatic organisms. Asa River in Nigeria is a typical example of a river in which fish populations have declined over the past years (Ogundiran and Fawole, 2014 and Bernet *et al.*, 2000). The fresh water ecosystem_is threatened by increasing levels of various pollutants originating from anthropogenic activities, urban, agricultural and industrial discharges. A considerable number of chemicals have already been released into the environment and persist in sediment, water and biota (Ogundiran and Fawole, 2017).

Antropogenic activity has had a significant impact on the hydrologic regime, water and sediment quality of river Asa, main sources of pollution to this river are irrigation channels which are not maintained properly, sewage effluents, effluents from industrial premises, fish ponds and livestock farms and so on (Adewoye, 2003; Ogundiran and Fawole, 2014, Ogundiran and Fawole, 2017).

Histopathological biomarkers are valuable indicators of the harmful effects of pollutants and potential pathogens. These markers are intermediate biomarkers in terms of ecological importance, response time and level of biological organisation, and as such are very suitable for the assessment of potentially harmful effects of various pollutants (Van der Oost *et al.,* 2003). Histopathological evaluation is a sensitive tool in toxicant impact assessment to indicate the effects of toxicants on fish health and also allows for early warning signs of disease and injury in cells, tissues, or organs. Such structural changes in fish as biomarkers in various tissues in different species have also been studied by many researchers (McDonaldand Wood, 1993; Greco, *et al.,* 1995; Ogundiran, *et al.,* 2009; Magar and Bias, 2013). Therefore, this present study aimed at evaluating the histological alterations in gill and liver tissues of *Clarias buthupogon* and *Heterobranchus longifilis* obtained from Asa River, Nigeria.

MATERIALS AND METHODS

Samples of *Clarias buthupogon* and *Heterobranchus longifilis* were collected separately from the downstream portion of Asa River using standard fishing device. The samples include a total number of 55 fish, selected from the pool of fish collection, 28 for *Clarias buthupogon* and 27 for *Heterobranchus longifilis*. They were transported in pre-treated plastic containers to laboratory for histological analysis. The samples were sacrificed and dissected immediately; the second left gill arch from the left side was excised and the gill tissue obtained was immediately fixed in 10% formaldehyde. After 24 hours the fixed tissues were taken for histological evaluation using the modified method of Bernet *et al., (*1999). Sections were made at 5-6µm thickness and stained with Haematoxylin and Eosin, stained slides were examined under light microscope and photographed (Labomed). A qualitative histological assessment was done to identify histological aberrations in the gill and liver tissues of the sampled fish population. These results were assessed and analysed using a protocol developed by Takashima andHibiya, (1995) and Bernet *et al.*, (1999).

RESULTS AND DISCUSSION

The gill structure of the two fish species sampled showed varying proportion of histological degradations ranging from hypertrophy, desquamation, oedema, swelling of pillar and chloride cells, disappearance or obliteration of secondary lamellae and cellular necrosis (Plates 1 and 2). In addition to these changes, lifting of secondary lamellae epithelium (desquamation) and a pronounced fusion of adjacent lamellae observed in almost all gill structure examined was probably as a result of the pollution load of the surrounding medium.

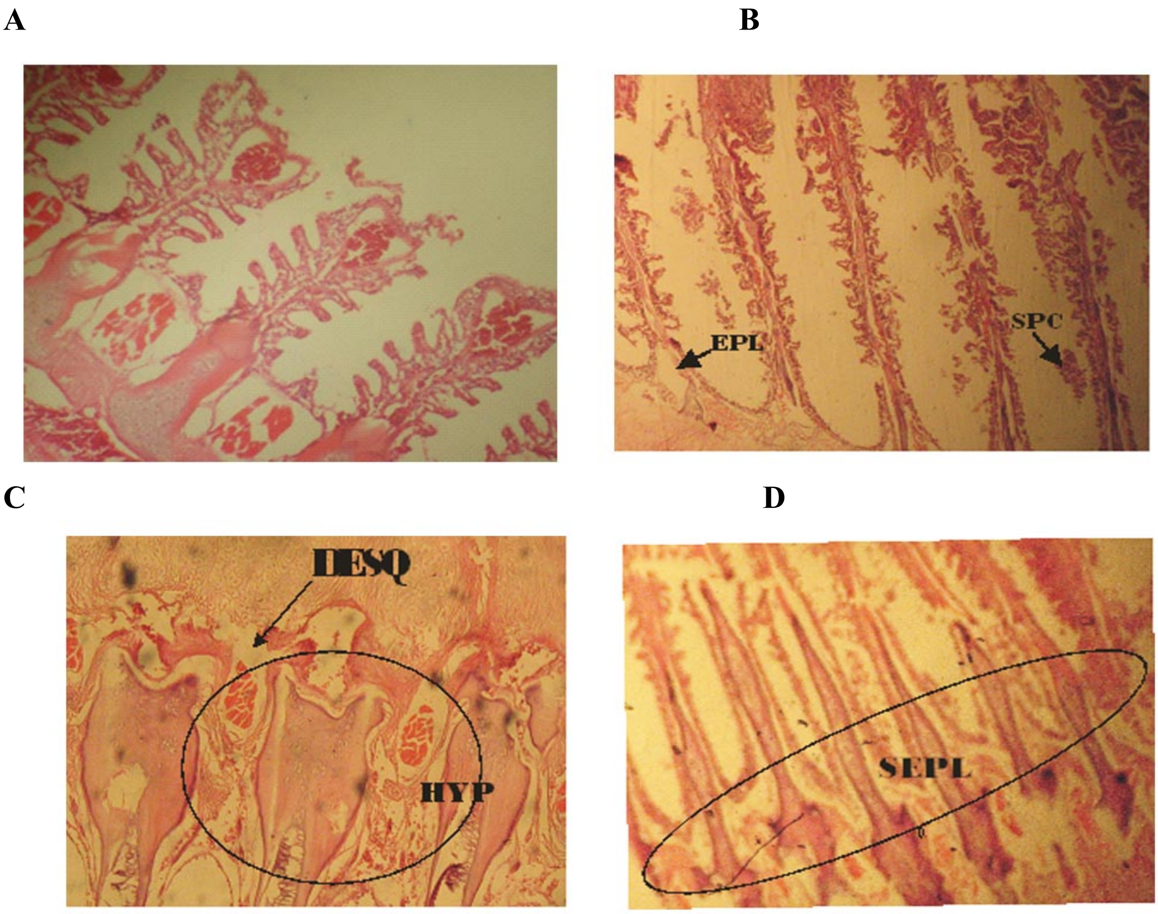

Plate 1: (A) Photomicrograph of a fairly normal gill tissue of *C. buthupogon* from a polluted portion of Asa River with mild congestion of blood vessels. **(B)** Gill tissue of *C. buthupogon* from the polluted portion of Asa River showing severe epithelial lifting of secondary lamellae and swelling of pillar cell (SPC). **(C),** Gill tissue of *C. buthupogon* from the polluted portionof Asa River with severe desquamation of secondary lamellae and hypertrophy.**(D),**Gill tissue of *C. buthupogon* from the polluted portion of the river showing severe epithelial lifting of secondary lamellae (X400 mg).

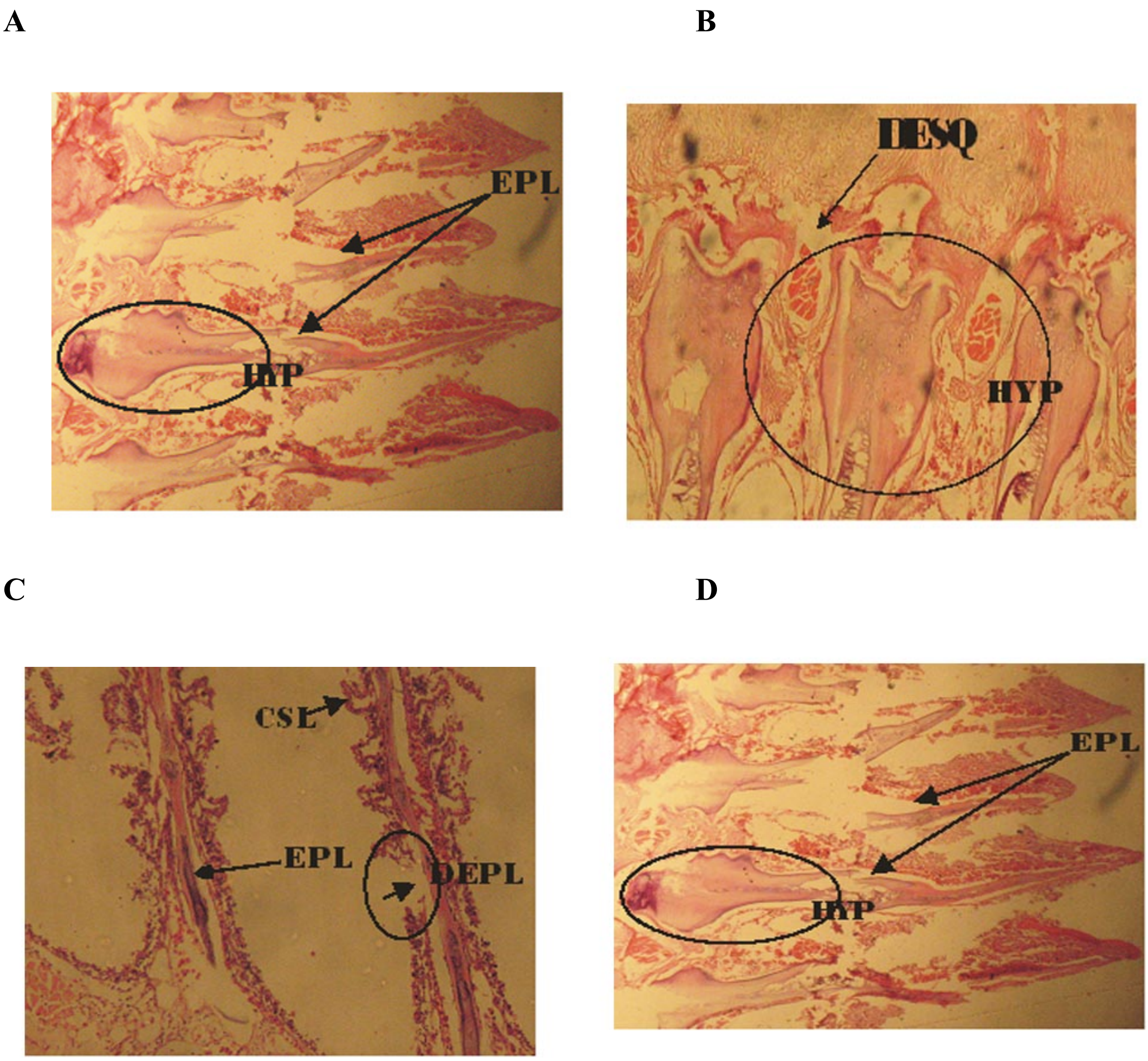

Plate 2: (A), Photomicrograph of gill tissue of *Heterobranchus. longifilis* from the polluted portion of Asa River showing with severe epithelial lifting of secondary lamellae and hypertrophy. **(B),** Gill tissue of *H. longifilis* from THE polluted portion of the river showing severe hypertrophy; an indication of necrosis. **(C),** Gill tissue of *H. longifilis* from the river showing a pronounced curling of secondary lamellae (CSL), epithelial lifting with total disappearance of primary and secondary epithelial (DEPL). **(D),** Gill tissue of *H. longifilis* from theriver revealing severe epithelial lifting of secondary lamellae and hypertrophy (HYP) (X400 mg).

Fish gill functions for respiration, ion regulation, acid – base balance and nitrogenous wastes excretion and constitute over 50 percent of the total surface area of the animal that make it sensitive to chemical changes in water. Several histological alterations were recorded in the gill tissues of the two fishes examined and this may be attributed either directly or indirectly to the impact of the river water pollution as fish gills are very sensitive to changes in the composition of their environment especially the water borne toxicants. Consequently, injury to the gill epithelium is a common response observed in the fish exposed to varieties of contaminants.

Abnormal changes recorded in the gill tissue of the two investigated fish species, may be an indication of the reaction of contaminants and this is in conformity to the findings of (Olurin, *et al*., 2006 and Ogundiran *et al.,* 2009) who have reported related effects for other species that were exposed to different pollutants. Cellular hypertrophy, hyperplasia of the epithelium cell, fusion of secondary epithelium, lifting of lamellae epithelium and congestion of blood vessels were noted aberrations in this study. This is in consonant with the submission of Camargo and Martinez (2007) who reported same scenario in *P. lineatus* being caged in Brazilian Cambe stream polluted by the industrial, domestic and agricultural wastes. Arellano *et al.,* (2000) also observed a gross epithelial lifting of the secondary lamellae after exposure to heavy metals, such as cadmium and copper which agreed well with the present finding. Lifting of lamellae epithelium and lamellae fusion could be protective as it diminishes the extent of vulnerability of the gills surface area (Van Heerden *et al.,* 2004).
All the histological changes observed in the gills of *C.buthupogon* and *H. longifilis* in this present work may have resulted from hypoxia, respiratory failure problems with ionic and acid base balance, this is in supports of the findings of Wood and Solvio, (1991) and Allen *et al*, (1994). In addition, reactions of fish gill to contaminant include inflammation, hyperplasia, lamellae fusion, over-secretion of mucus, epithelial lifting, flattening and thickening of the secondary lamellae and so on. As a consequence of epithelial lifting, there is an increase in the distance between the water and blood, impairing oxygen uptake and increase the rate of respiration in view of compensating for the low entrance of oxygen (Speare *et al*, 1991; Winkalar *et al.,* 2001; Cerqueira and Fernandes 2002; Oliveira-Ribeiro *et al.*, 2002; Mazon *et al.*, (2002); Thophon *et al.,* (2003); Stentiford *et al.*, 2003; Fernandes and Mazan, 2003 and Ogundiran and Fawole, 2007).

Liver Histology

Liver is vital organ that is most affected by the pollutants in the water due to its role in the detoxification and bio-transformation processes. It also serves as an integrator for biochemical and physiological functions, and caries out key functions in excretion of xenobiotics. Histological alterations recorded in the liver of the studied species includes, varying degrees of hepatocytotic degeneration, congested central vein, vacoulation, Distortion of sinuses, inflammation, heamolysis, vacuolation, dilation and destruction of central vein and cellular necrosis. This finding is in conformity to several other findings wo and focal area of necrosis. All these observed aberrations were in agreement with many studies that examined the effects of different pollutants on fish liver (Ptashynski *et al.,* 2002; Fanta, *et al.,* 2003 and Ogundiran *et al*., 2009).

A **B**

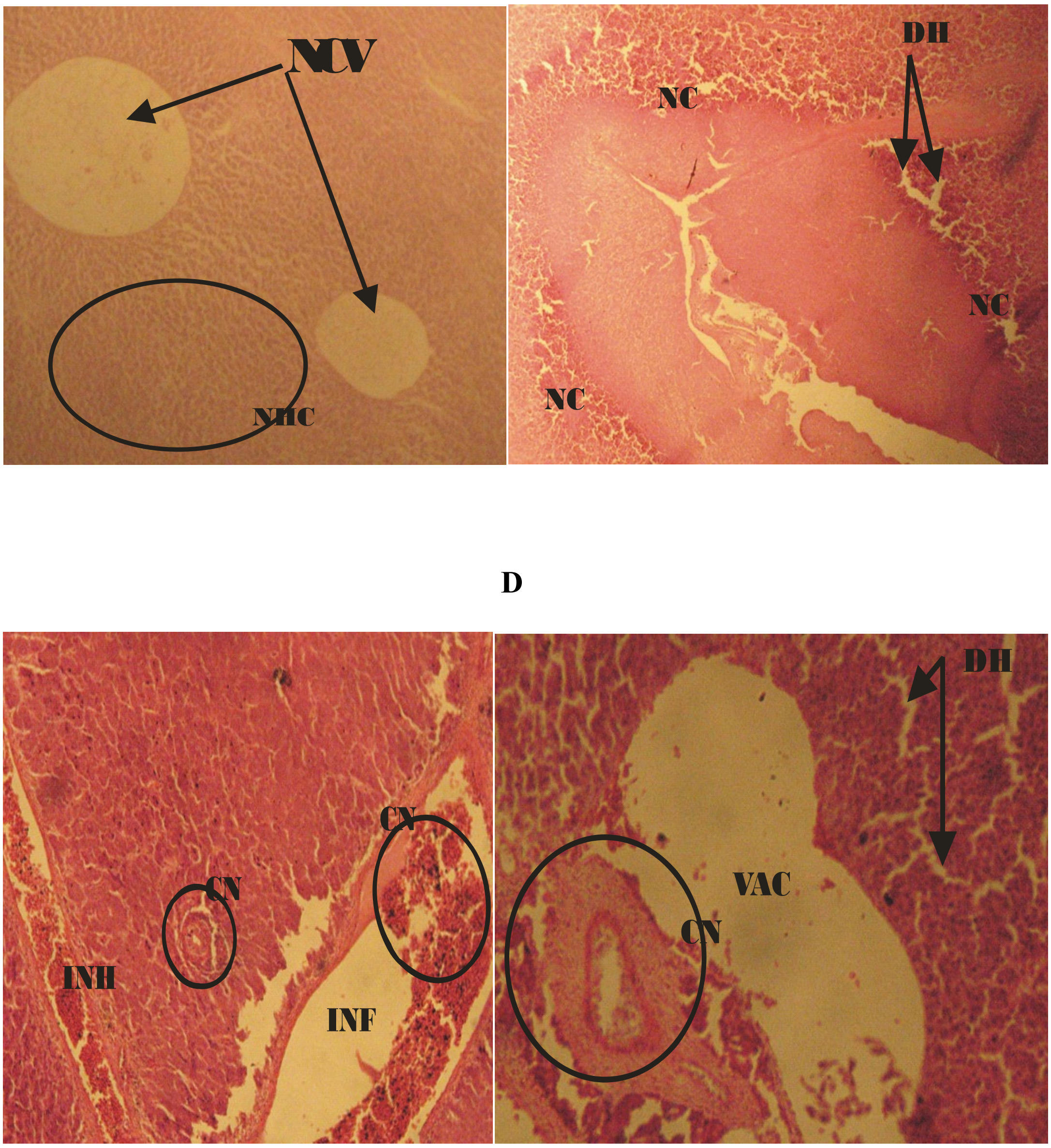

Plate 3: (A), Photomicrograph of a fairly normal liver tissue of *C. Buthupogon* from a polluted portion of Asa River revealing normal Central vein (NCV} and hepatic cells (HC). **(B),** Liver tissue of *C. Buthupogon* showing degenerated hepatocytes (DH), sinusoidal distortion and cellular necrosis (NC). **(C),** Liver tissue of *C. Buthupogon* showing mild congestion of central vein (CN) with partial inflammation in between the hepatocytes and intravascular hemolysis (INH). **(D),** Liver issue of *C. Buthupogon* showing severe hepatic degeneration (DH), vaculation or inflammation in-between hepatocytes with severe congestion of central vein *(CN) (X400 mg)*

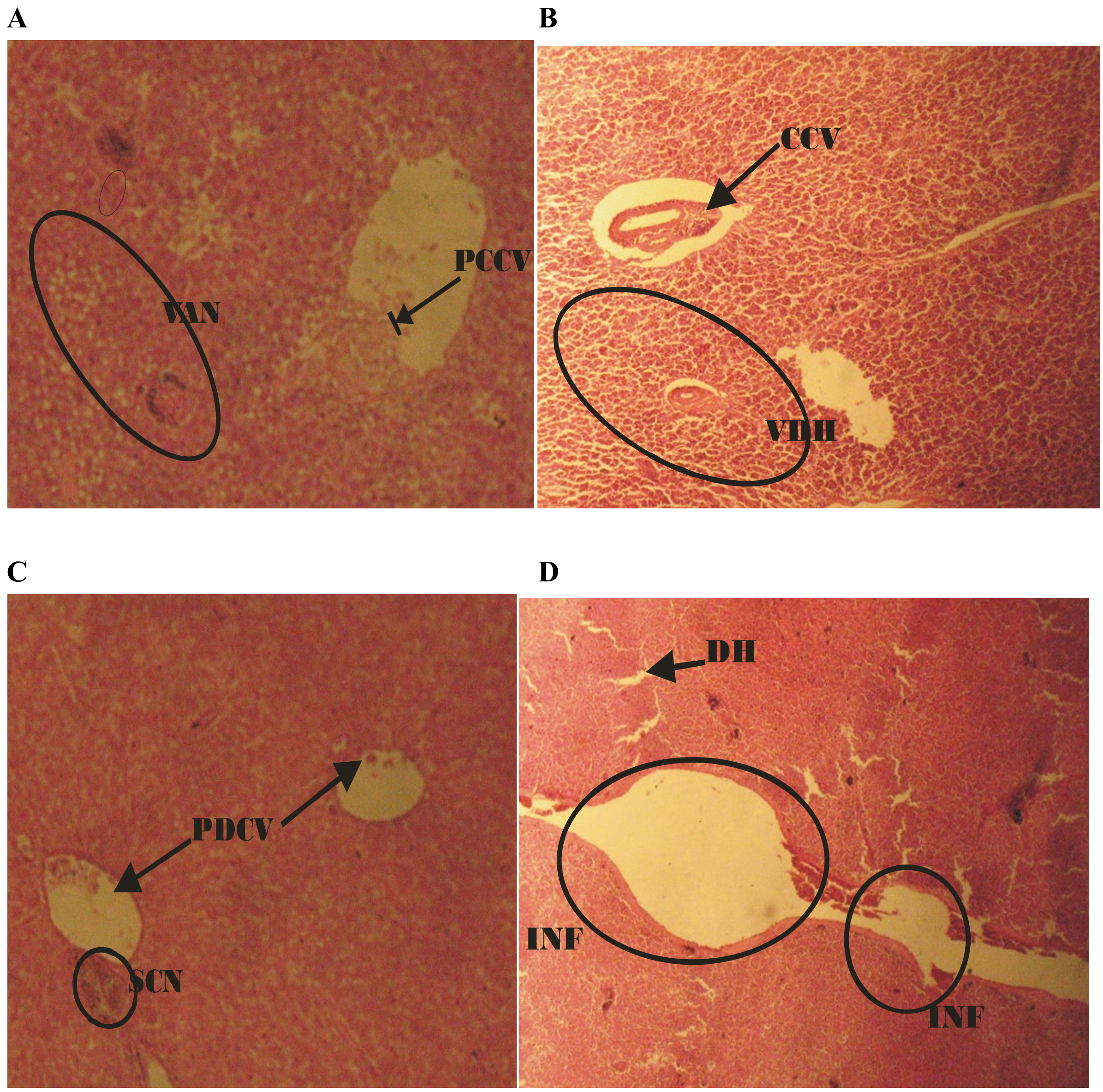

PLATE 4: (A), Photomicrograph of l*iver tissue of H. longifilis from* polluted portion of *Asa River showing partial congestion of central vein (PCCV), vocal area of cellular necrosis (VAN) with sinusoidal distortion.* (**B**), Liver tissue of *H. Longifilis* showing vacular degeneration of hepatocytes (VDH) and congested central vein (VDH)**. (C),** Liver tissue of *H. longifilis* showing partial congestion of central vein (PCCV) and a severely congested area (SCN). **(D)** Liver tissue of *H. longifilis* showing severe hepatic degeneration (DH), vaculation of hepatocytes. (X400 mg).

The recorded histological alterations observed in this study may be due to the additive or cumulative effects of increased metal concentrations in the liver and these agreed significantly with the findings of Authman and Abbas (2007), which submitted that the liver has an important detoxification role of endogenous wastes products as well as externally derived toxins such as heavy metals mixtures. The hepatocytes revealed fatty degeneration with pronounced vacoulation and necrosis and manifestation of hemorrhage. Hemorrhagic lesions were abundant and this may be due to high pollution index or probably because of the inflammation of liver tissue in between hepatocytes. Fatty degeneration of hepatocytes in the liver may also be attributed to an oxygen deficiency as a result of gill degeneration or the vacuolar dilation and intravascular hemolysis observed in the blood vessels (Mohamed, 2001; Mohammed, 2001; Ogundiran, *et al*., 2009 and Ogundiran, *et al*., 2010).

Vacoulation, inflammation and congestion were early stages in the hepatic degeneration, thus, these could be used as histological biomarker of different level of exposure (Stentiford *et al*., 2003; Au, 2004 and Ogundiran *et al.,* 2010). Hepatic necrosis as generally observed in this work, has also been established to be the order of the day in fishes collected from contaminated ecosystem with metals (Olojo *et al.,* 2005). The significantly high values of heavy metals in the liver could be linked to the occurrence of heterogeneous parenchyma in the liver of the two fish species in response to the metal of these fish to the polluted water of Asa River.

Therefore, it is possible to use liver hepatocytotic alterations as a biomarker to assess the impact of heavy metals or other pollutants toxicity on fish health and production. This study however, investigated the non-suitability of fish from polluted sites with respect to anthropogenic discharge. Consequently, this study has been able to establish the fact that, exposure of fish to even low concentration of toxicant in the aquatic phase can induce various toxicological effects and histological degradations which depend on the period of exposure, physiological status of the resident species, volume of water (in terms of seasonal variation) and concentrations of such pollutants. Obtained results showed that the histological alterations in the gill and liver tissues aremost likely caused by increased concentrations of certain pollutants. Accordingto the previous water quality studies of the Asa River, metals concentrations were found at an elevated level. This information verifies that histopathologicalchanges are valuable biomarkers for field evaluation, especiallyin tropical regions that are naturally affected by variety ofenvironmental variations. It should be highlighted thathistopathology is able to assess the initial effects and reactionsto acute exposure to chemical stressors.

Conclusively, *C. buthupogon* and *H. longifilis*in Asa River are indeed responding to some stressors whose exact nature could be anthropogenic. Accordingto the previous water quality studies of the Asa River, concentrations of iron,copper, cobalt, arsenic, lead, chromium and chlorine were at elevated levels and these substances could be responsible for the observed histopathological alterations. Present study represents an additional reason to proceed with a detailed monitoring of this river and the wildlife within it. Therefore, consumption of river foods from Asa River should be discouraged and urgent water monitoring system is required in the river

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