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Histological and Hepatic Enzymes Response of *Oreochromis niloticus* and *Clarias anguillaris* to Pollution in Asa River, Ilorin

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Abstract

Fish can be considered as a good organism for bio-indicator in aquatic habitats as they accumulate contaminant from the water bodies through food. Aquatic ecosystem pollutants response in fishes can lead to biochemical changes and histological alterations. This present the need in assessing ecological and public health effect of toxicity in Asa river on the selected fish samples that are commercially important in the environment. Two stations of Asa river were selected where water and fish were collected for physicochemical parameters of the water, enzymatic and histological investigations in the laboratory in April, 2019. The heavy metals of the water in Unity area of Asa river was higher than that of the standard limit and Asa reservoir. There was an increase in the ALT, AST and ALP biochemical analysis carried out on *Oreochromis niloticus* and *Clarias anguillaris* with reduction in total protein level in Unity station. The histopathology of the gills of both *Clarias anguillaris* and *Oreochromis niloticus* found in Unity station of Asa river revealed thinner and loner secondary lamella in degenerating secondary lamella and mildly distorted cartilage with epithelial lining in *Clarias anguillaris*. Hence, proper management of the river is necessary to restore the ecology and make the water fit for use.

Keywords: *Asa River, Public Health, Histological, Management*

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I. INTRODUCTION

Freshwaters serve as resources for agricultural activities, household and industrial applications (Anifowoshe *et al.*, 2018). They are unavoidable in many sectors in the society and civilization needed in the kitchen for cooking, recreation and fishing activities (Orubu, 2006). The increase in population, industries, urbanization, improper environmental law awareness, non-implementation of environmental regulations, effluent discharge both from industries and households bring pressure on water resources and leads to aquatic pollution (Liyanage and Yamada, 2017). Most importantly, discharge of wastewater into the water bodies brings regression of water quality and causes serious problems to threaten the aquatic life resulting into negative effects on human systems and fish life activities (Bastami *et al.*, 2015; Khalek *et al.*, 2016). Among the problems of our priceless environment, pollution perks up the most interest; this is because the impact of pollution on a living organism is the most direct (Emre and Balogun, 2014).

Over the world, pollution is a challenge in the water environment (Aiman *et al.*, 2016). Aquatic ecosystems were faced anthropogenic activities challenges as a result of a toxicant burden especially the heavy metals that naturally present in minute concentration, but its activities are toxic in higher concentration (Pandey *et al.*, 2008; Ondarza *et al.*, 2012; Pereira *et al.*, 2013; Adeniyi *et al.*, 2017). The pollutant of different forms can also have long persistence effects and accumulate in water and sediments having effects in the aquatic bodies' system equilibrium (Galadima *et al.*, 2011; Monroy *et al.*, 2014; Khalek *et al.*, 2016). Aquatic organisms, such as fish, are used as a bioindicator in aquatic habitats due to the accumulation of pollutants in the water and indirectly via the food web (Findik and Cice, 2011). Due to protein content and other nutrient value, these fishes were consumed

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by humans but vulnerable as they cannot escape the pollution impact (Mahboob *et al.*, 2014).

Response to industrial, sewage and agricultural pollutants in fishes can lead to histological changes in the muscle, gills, kidney and liver (Anifowoshe *et al.*, 2018; Oladipo *et al.*, 2018a). They can be used to know the sample of projected health effects of the entire population in the environment. The fish gills, liver, kidney and muscles resulted in many pathological alterations in various species as exposed to different toxicants (Benli *et al.*, 2008; Osman *et al.*, 2009; Syasina, 2011).

Fishes *Oreochromis niloticus* and *Clarias anguillaris* are important and economic freshwater fish species in Asa river, Ilorin, Nigeria. There widespread have been reported in different reservoir and river within the state, attributing it to their feeding ability on different food items, growing to a large size, high fecundity rate and ability to withstand a wide range of physicochemical conditions (Araoye, 2008; Öner *et al.*, 2008; Mustapha, 2010; Authman *et al.*, 2013; Oladipo *et al.*, 2018b). This present research aims to assess the ecological and environmental health aspects of toxicity in Asa river by assessing its water quality parameters, as well as enzymatic and histopathological investigations of the selected fish samples, which that, are commercially important to human in the environment.

II. Materials and Methods

A. Sampling Stations

The study was carried out on two different stations of Asa River. The first station is located at Unity where industrial and domestic waste discharge on the river and Aliara/Temioda town where the Asa dam reservoir lies that serves as the comparative control for the study (Figure 1).

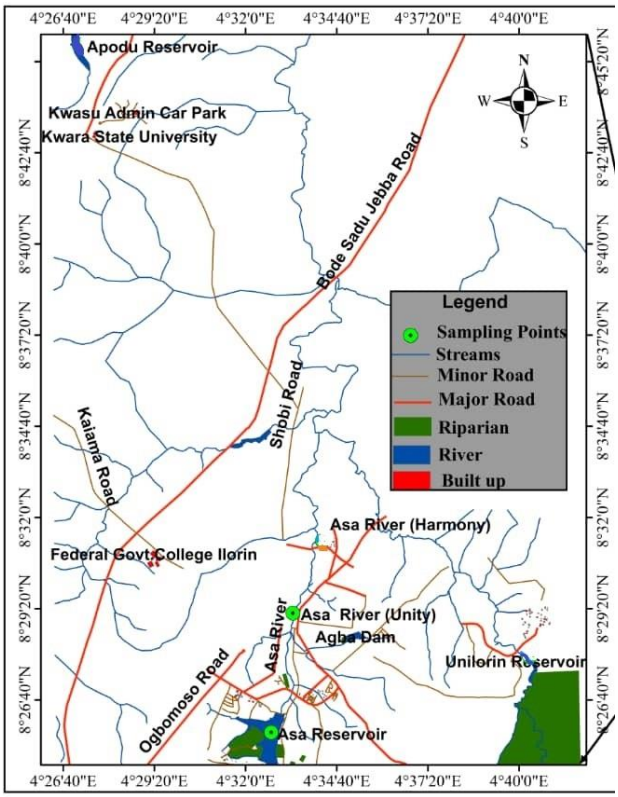

Figure 1. A geographical map of the Asa River segment showing the study area and sampling points.

B. Sample Collection and Physicochemical Analysis

Water samples were collected from two stations and analyzed for the physicochemical parameters. The pH, electrical conductivity (EC) water temperature, and total dissolved solids (TDS) were calculated *in situ* on the water surface with the aid of Hanna portable pH/ EC/ TDS/ Temperature combined waterproof tester model HI 98129. The dissolved oxygen (DO), chemical oxygen demand (COD)biochemical oxygen demand (BOD) and some heavy metals such as lead(Pb), chromium (Cr), cadmium (Cr), manganese (Mn), and arsenic, were measured using Hanna multi-parameter for laboratories model (HI 83200) in hydrobiology and fisheries unit laboratory in the department of Zoology, University of Ilorin, Ilorin Nigeria.

Collection of the fish samples was done in the early hours (6:00-8:00am) in April 2019 with the help of a fisherman using basket and gill net. Fishes were transported to the laboratory using rectangular transparent plastics with a perforated covering to aid aeration and prevent escape.

Juvenile fish samples of *O. niloticus* and *C. anguillaris* were collected, selected for biochemical and histopathological analysis. The blood sample was collected through the caudal vein of the fish and stored in sample (EDTA) bottles. The analysis of biochemical enzymes, Alkaline phosphate (ALP); Aspartate aminotransferase (AST); Alanine aminotransferase and Total protein activities were assayed using the respective reagent kits (Reitman and Frankel, 1957).

Different tissues of gills and liver of the two fish species were also collected through dissection from both sampling point. The standard method of Gobinath and Ramanibai, (2014) was used with little modification. Briefly, the tissue samples collected after dissection were fixed in buffered formal saline for a day (24 hours) and rinsed vigorously with distilled water and process for paraffin tissue embedding using the method of Drury and Wellington (1980). The sections obtained from studied organs stained using hematoxylin and eosin (H & E) using the standard procedure of Bancroft and Gamble (2008) and observed using an Olympus light microscope.

C. Statistical Analysis

The experimental values were presented as mean ± standard deviation (SD). The Statistical Package for Social Science (SPSS) version 21.0 was applied to evaluate the differences between the test species and location. The multi-group comparisons of the mean were assessed out by one-way analysis of variance (ANOVA) test and the level of statistical

significance were estimated at $p<0.05$ using the Duncan multiple range test (DMRT).

III. Results

pH for Aliara and Unity is lesser than the standard, COD and BOD for unity is higher than that of Aliara and the standard. While that of Aliara is equal to the standard. The amount of heavy metals (iron, magnesium, zinc, copper, lead, manganese, chromium, arsenic and cadmium) found in Asa river (Unity) is way higher than that of the standard limit and the reservoir. The control site had the same level of heavy metals as the standard (as depicted in Table 1).

Table 1: Physical and Chemical Properties of the Sampling Stations.

Parameters	Asa Reservoir	Asa River (Unity)	Who
pH	5.75	5.65	6.5-8.5
TDS mg/dl	250	425	500
Conductivity	120	855	-
Temperature	31	31	25
DO mg/dl	4.1	5.94	-
BOD mg/dl	0.6	4.35	-
COD mg/dl	2128	5296	-
Chromium ppm	0.097	3.865	0.05
Lead ppm	0.45	2.699	0.05
Cadmium ppm	0.325	2.385	0.05
Manganese ppm	0.028	0.029	-
Magnesium ppm	0.000	4.635	-
Arsenic ppm	0.000	0.263	-
Copper ppm	0.250	1.283	1.0
Iron ppm	0.381	2.781	0.3
Zinc ppm	0.000	3.040	5.0

WHO: World Health Organization; NESREA: National Environmental Standards and Regulations Enforcement Agency; USEPA: US Environmental Protection Agency.

The Biochemical parameters (ALT, AST, ALP and total protein) from selected *O. niloticus* and *C. anguillaris* fish were analyzed and were shown in Table 2. Biochemical analysis carried out on *O. niloticus* and *C. anguillaris*show significant increase in Alanine aminotransferase (ALT), Alkaline phosphate (ALP) and Aspartate aminotransferase (AST) enzymes and significant reduction in total protein level (Hypoproteinemia) of fish species found in both Aliara and Unity stations. There was an increase in the value of the enzymes analysed for biochemical analysis in *O. Niloticus* than *C. anguillaris*.

Table 2. Biochemical Analysis of O. niloticus and C. anguillaris from the Sampling Stations.

Site	Species	ALP (U/I)	Total protein (mg/dl)	AST (U/I)	ALT (U/I)
Asa Reservoir	*O. niloticus*	24.84 ± 0.02^{c}	18.658 ± 0.15^{b}	110.6 ± 1.09^{a}	36.18 ± 0.14^{a}
	C. anguillaris	15.18 ± 0.03^{a}	17.917 ± 0.41^{b}	117.8 ± 2.69^{b}	34.32 ± 0.42^{a}
Asa River (Unity)	*O. niloticus*	44.16 ± 0.04^{d}	17.252 ± 1.08^{a}	134.2 ± 0.28^{c}	52.02 ± 1.42^{b}
	C. anguillaris	23.46 ± 0.01^{b}	15.846 ± 0.67^{a}	122.2 ± 1.41^{b}	35.48 ± 0.16^{a}

Means within the same column with different superscripts differ significantly ($P < 0.05$).

The representative photomicrograph of the gills of *O. niloticus* and *C. anguillaris* from Asa reservoir is shown in Figure 2a and 2b indicating the secondary lamella (SL), blood vessels (BV) and supporting cartilages (CA) surrounding the blood vessels and holding the secondary lamella in place with no signs of pathological alterations. In Figure 3a, the photomicrograph represents the gills of *O. niloticus* showing the (SL), BV and supporting CA surrounding the blood vessels and holding the secondary lamella in place. There are few signs of pathological alterations and the secondary lamella appears thinner and longer. The gills of *C. anguillaris* photomicrograph in Figure 3b indicate the (SL), (BV) and supporting (CA) surrounding the blood vessels and holding the secondary lamella in place. They appear to be some pathological alteration in the histomorphology of the secondary lamella as they appear to be degenerating, distorted cartilage with epithelial lining.

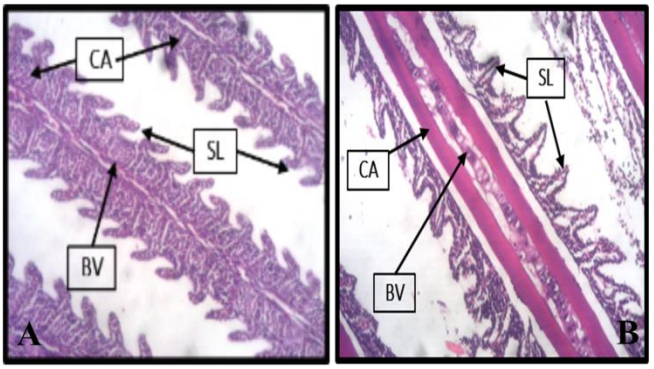

Figure 2. Histopathological Analysis of the Gills of *Oreochromis niloticus* (2a) and *Clarias anguillaris* (2b) in Aliara. Secondary Lamella (SL), Blood Vessels (BV) and Cartilage (CA).

Figure 4a, shows the photomicrograph of the liver of *O. niloticus* at high power magnification. Compactly crammed hepatocytes with intensively stained nuclei are seen evenly distributed in the liver tissue. There is no histopathological alteration.

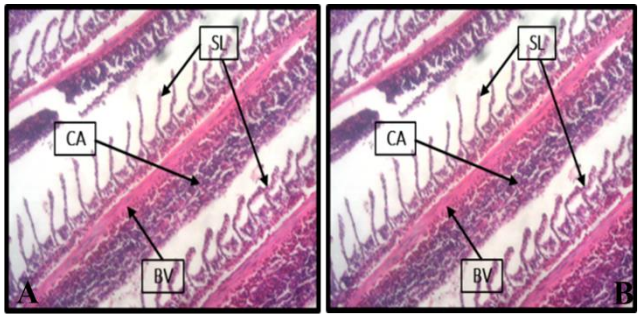

Figure 3. Histopathological Analysis of the Gills of *O. niloticus*(3a) and *C. anguillaris* (3b) in Unity. Secondary Lamella (SL), Blood Vessels (BV) and Cartilage (CA).

Figure 4brepresents the photomicrograph of the liver of *C. anguillaris* revealing the hepatocytes at the higher power magnification. Densely packed hepatocytes with intensively stained nuclei are seen evenly distributed in the liver tissue. There is no histopathological alteration. Whereas Figure 5a

represents the photomicrograph of the liver of *O. niloticus* showing the densely packed hepatocytes with intensively stained nuclei being seen poorly distributed in the liver at high power magnification. There is a histopathological alteration as sinusoid dilation and distension, oedema and vacuolation while in *C. anguillaris*, photomicrograph of the liver in Figure 5b shows the densely packed hepatocytes with intensively stained nuclei are seen evenly distributed in the liver tissue at high power magnification. There is similar histopathological alteration as observed in Figure 5a; sinusoid dilation and distension, oedema and vacuolation

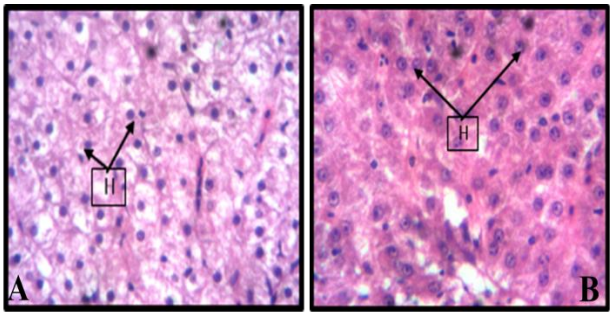

Figure 4. Histopathological Analysis of the Liver *O. niloticus* (4a) and *C. anguillaris* (4b)in Aliara. Hepatocytes (H).

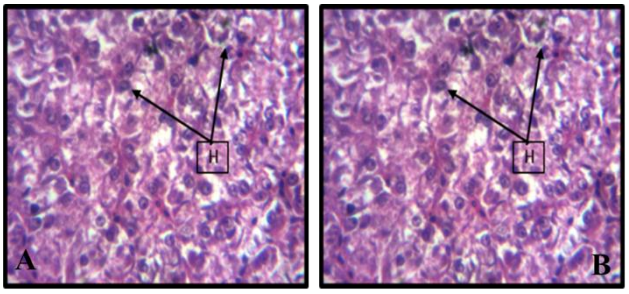

Figure 5. Histopathological Analysis of the Liver *O. niloticus*(5a)and *C. anguillaris* (5b)in Unity

IV. Discussion

Water contamination is caused directly or indirectly by human anthropogenic activities (Karadede *et al.*, 2004). These contaminants pollute the water bodies and thus pose a threat to the life of aquatic organisms as well as human via drinking (Akpan and Ajayi, 2016).

The source of heavy metals at the Asa river study sites is mainly of exogenous origin (domestics, industrial and agricultural wastes) (Eletta *et al.*, 2005). Since water plays a germane role in maintaining human health, the observations and level of physicochemical parameter above the standard limit might provoke the anomalies in the ecology and fauna present in the river (Mustapha, 2010). This may be due to the inappropriate release of industrial and household wastes continuously into the water body to elevate the aforementioned physicochemical parameters and therefore cause a detrimental long-time health effect in people and animals making use of the water (Almeida *et al.*, 2002).

In the histopathology analysis, the liver of fish found in Asa reservoir exhibits a normal structure when compared with fish found in Asa river (Unity), which shows some lesions such as oedema and vacuolation. *Oreochromis* shows a mild effect in the liver tissue compared to *Clarias*. The gill, which is one of the vital organs, participates in many crucial roles such as respiration, osmoregulation (salt and water balancing), and excretion in the fish. Due to its sensitivity to changes in water quality, it is therefore considered as the primary target of contaminant because of its close contact with the external (Kranz *et al.*, 1990). The gills represent the target organ for the toxicity of dissolved metals because they are the main site for entry for the surrounding element, the gill has a large surface and tiny epithelium making metals easily penetrate through it (Genten *et al.*, 2009). Alteration in the histopathology of the gills of both scaled *O. niloticus* and *C. anguillaris* found in Unity station of Asa river revealed thinner and loner secondary lamella in degenerating secondary lamella and mildly distorted cartilage with epithelial lining in *C. anguillaris* as reported by authors being an effect of pollution on histology of vital organs in fishes (Al-Marjan and Abdullahi, 2016; Hamdamin *et al.*, 2017; Oladipo *et al*., 2018a). This might be as a result of the presence of scales acting as a protective sheath to the fish and preventing toxicants from invading the fish body leaving only the gills for penetration and causing more effect on it which is consistent with the findings of Perkins *et al.* (1972).

The reduction in total protein at Unity as compared to Aliara is in line with the observations of El-Sheekh *et al.* (2011) who also reported a low protein level in fishes from a polluted environment. According to Fontana *et al.* (1998), the decrease may be due to the damage of protein-synthesizing subcellular structures and inhibition of the hepatic synthesis of blood protein. In this study, an elevation in ALT, ALP and AST enzymes was recorded in fishes found in Unity station than Aliara station which corroborates the study of Chen *et al.* (2004), who suggested that the increase indicates impairment of the liver, hepatocellular damage or cellular degradation. Hepatic cellular damage also leads to the leakage of these enzymes, which consequently led to their leakage or rupture into the blood circulation (Mouse *et al.*, 2008). These findings also agree with the study of Fatma and Nahed (2000); Yamawaki *et al.* (1986) and Shalaby (2007).

The concentration of aquatic pollutant may be measured through water, sediment or organism present, since the accumulation of the constituent can induce abnormalities in the histology of the fish (Ademoroti 1996). The present result justified this effect of pollution in the liver and gill of the studied fish is in accordance with the studies that fish living inside polluted waters have higher frequencies of abnormalities and the abnormalities vary based on the kind of contaminants presents and the species of fish (Adekunle *et al.*, 2008; Ogundiran *et al.*, 2009).

It was reported that lower concentrations of heavy metals are usually accumulated in pelagic fish compared to the benthic fish (De Jonge *et al.*, 2015). This has resulted in settling of

the weighted metals in the water to settle down in the benthic region to accumulate more in the bottom dweller fishes than pelagic fishes, and closely related species can respond to the same given toxicants in different ways (Adelekan *et al.*, 2011). This effect can be biomagnified in humans consuming those fish via food chains (Almeida *et al.*, 2002).

V. Conclusion

The results of the physicochemical parameters and the effect of pollutants on *O. niloticus* and *C. anguillaris* from the Asa river using biochemical and the histopathology of gills and liver of the fishes reveal that the unity section of Asa river is polluted. This is due to domestic waste and industrial effluents discharge. Hence, proper management of the river is necessary to restore the ecology and make the water fit for use.

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Conflict of Interest

We declare no conflict of interest.

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