

Original Research Article

**BIOCHEMICAL RESPONSE AND HISTOPATHOLOGIC ALTERATIONS IN FRESHWATER FISH *PAPYROCRANUS AFER***

**Abstract**

This study investigated the effects of pollutants on the biochemical responses and histopathological alteration of *Papyrocranus afer* from Banegbe River at different locations (upstream, middle-stream, and downstream). The levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in blood samples of the fish and the histopathologic alterations of the gills and liver of the fish. The gills and liver tissue of the fish were collected and processed using routine histologic techniques for fixing and embedding in paraffin and staining of sections with haematoxylin and eosin. Evaluation of the plasma enzymes showed significant increases ( $p < 0.05$ ) in plasma ALT, AST, and ALP activity in all the sampled points of Banegbe River when compared to the control 1 (Orogodo river) with middle-stream having significant ( $p < 0.05$ ) higher concentrations when compared to down-stream and up-stream. The main alterations observed in the liver of the fish at Bangbe River were Kupffer cell hyperplasia and focal necrosis of the liver. While the gills showed alteration of the structure of the epithelium, hyperplasia of the epithelium of the primary lamella, epithelial lifting, vacuolization, alteration of the structure, and occurrence of aneurysms in the secondary lamella, all these alterations were as a result of pollutant in the river. Control 1 showed normal parenchymal cells of the liver and normal gill structure.

**Keywords:** Biomarker, Transaminases, Pollutant, Banegbe river, *Papyrocranus afer*.

**Introduction**

The contamination of freshwater systems, through the discharge of industrial effluent, domestic wastes, oil spills, agricultural pesticides, and other man-made activities into the aquatic environment is a matter of concern (Canli *et al.*, 1998). Around 1500 chemical substances are been listed as pollutants in the freshwater ecosystem. Indiscriminate use of such chemicals can lead to the contamination of our natural water resources such as lakes, reservoirs, rivers, ponds, streams, and other low-lying areas (Dellinger *et al.*, 2011). These chemicals disturb the whole

environment, mainly the aquatic ones, thereby causing their effects indiscriminately on both man and animals, leading to needless mortality of aquatic organisms, in particularly fish (Osman *et al.*, 2010; Suneetha, 2012).

Various organic and inorganic wastes in industrial and domestic effluents discharged indiscriminately into water bodies are responsible for water pollution (Berman and Lal, 1994). Industrial effluents have been shown to be a complex mixture with harmful agents which include toxic by-products (Wagemann *et al.*, 1984). Many effluents have been shown to be hot, of extreme pH value, and normally contain high levels of dissolved salts (Abel, 1989). Metcalf and Eddy, (1995) showed in their research that, the most common means of treated wastewater disposal is discharge and dilution into ambient waters. Most of these effluents contain harmful compounds which have been reported to enter and accumulate in aquatic fauna and flora causing several physiological changes in them (Berman and Lal, 1994). Aquatic animals live in very intimate contact with their environment thus; absorb contaminants from the surrounding contaminated water which ultimately affect their health (Olaifa *et al.*, 2004). The accumulation of these pollutants in the fish tissue not only affects the health of the fish (productivity and reproductive abilities of the organisms) but also the health of the human beings that depend on the organisms as a major source of food (Dickman and Leung, 1998).

Studies have shown that increased contamination of aquatic environments causes severe morphologic and physiologic alterations in aquatic organisms (Mazon *et al.*, 1999). In a polluted environment, particularly where pollutants occur in chronic and sublethal concentrations, changes in the structure and function of aquatic organisms are more frequent than mass mortality.

The biochemical changes in the activities of enzymes in plasma/serum and functional organs are often regarded as the most important indices of the status of the internal environment of the fish (Inyang *et al.*, 2014). Some enzyme analysis are used as a biomarker in assessing the toxicity of xenobiotics in fishes. Biomarkers may be any measurable biochemical, cellular, physiological, or behavioural change in an organism or group that indicates exposure to organic and inorganic pollutants (Depledge, 1994).

Enzymes are necessary for normal cellular metabolism including that of the liver, and the degenerative changes due to the combined pollutant toxicity exhibited in the liver alter the level of a number of its enzymes. These plasma enzymes; aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) are released in acute and chronic liver disorders. While lactate dehydrogenase (LDH) is released from the liver after its cellular damage and failure due to pollutants (Malbrouck *et al.*, 2003). Enzymes are also biomarkers of acute hepatic damage; thus, their bioassay can serve as a diagnostic tool for assessing necrosis of the liver cells (Dobšíková *et al.*, 2009).

These enzymes representing toxicant-induced changes in biological systems can serve as links between environmental contamination and its effects, providing, therefore, vital information on the ecosystem health (Maria *et al.*, 2009), and providing relevant data about possible pathological processes in fish.

Comment [G1]: alterations

Fish plays an important role not only in human diets but also in livestock nutrition. African knife fish is also among the common freshwater fish widely consumed within Ughelli Environs. In aquatic animal species, fish are the inhabitants that cannot escape from the detrimental effects of these pollutants and are therefore very susceptible to physical and chemical changes which may be reflected in their blood components. Fish in the aquatic environment can be exposed to a multi-pollution state and the occurrence of sequential exposures is a vital aspect of ecotoxicological research (Brown *et al.*, 2006).

Studies carried out on various fish species have revealed that pollutants may alter the biochemical parameters both in tissues, and in the blood, and can it also cause morphologic changes. Another way of evaluating the effects of pollutants on fish is to examine their organs for morphologic changes (Poleksic and Mitrovic-Tutundzic, 1994). [In this work](#), the gills and liver of African knife fish were examined. Histopathologic studies have been used to evaluate the effects of contaminants on the health of fish in the environment and to help establish a causal link between exposure to toxic substances and the various biological responses (Schwaiger *et al.*, 1997).

Comment [G2]: delete

The fish liver plays an important role in metabolism and it is the major organ for the accumulation, biotransformation, and excretion of contaminants in fish (Figueiredo-Fernandes *et al.*, 2006).

The gills of fish are sensitive organ which is easily damaged by numerous contaminants, even at low concentrations (Karlsson, 1983). Since the gills have a large surface area in contact with the external environment, and perform various important roles (respiration, excretion, and osmoregulation) they are particularly sensitive to chemical and physical changes of the ecosystem, thereby being the target organ in fish for contaminants carried by water (Mazon *et al.*, 1999). The histopathologic changes in the structure of these gill organs can lead to respiratory disturbances and electrolyte imbalance in the fish (Cerqueira and Fernandes, 2002).

The Banegbe River serving as a source of fish for human consumption runs through the industrialized areas of Ughelli exposing it to anthropogenic contaminants. Therefore the study was carried out to determine the state of the river by assessing the histopathological alteration on the liver and gills, also enzyme activity (Aspartate Aminotransferase, Alanine Aminotransferase, and Alkaline Phosphatase ) of *Papyrocranus afer* at three sampling points along Banegbe River at Ughelli.

Comment [G3]: our study

## Material and Methods

### Fish samples

The fish samples were harvested from Banegbe River at three different points,

- 1 At the point of discharge of the thermal power plant effluent into the river,
- 2 up-stream from the place of discharge of the power station effluent
- 3 down-stream from the place of discharge of the effluent

A total number of 56 African knife fish (*Papyrocraruns afer*) was collected with the help of professional local fishermen using fishing nets. Four fishes of the same species were collected from the Orogodo River. Also, four were bought from a pond at Ekpan. Live specimens of *P. afer* were transferred into water buckets and brought to the laboratory for further analysis. Fish blood samples were collected for the determination of biochemical

Comment [G4]: little No

Comment [G5]: little No

parameters. The fishes were immediately sacrificed, and the gills and liver were taken for histopathology study.

### **Assay of serum aminotransferase Activities**

The activity of alanine aminotransferase, aspartate aminotransferase (AST) and alkaline phosphatase (ALP) was assayed by the method for Reitman and Frankel (1957) as outlined in the Randox kit.

### **Histological Study**

The histological evaluation was carried out on the liver and gills of fish samples as described by Bancroft *et al.* (1996). For the histological study, the liver and gills from each fish sample were removed after dissection and preserved in 10% formalin. Then representative blocks of gill and liver tissues from each lobe were taken from paraffin embedding using the standard micro technique. Section (5µm) of the gills or liver stained with haematoxylin and eosin will be observed microscopically for histology using 400X magnification.

### **Statistical Analysis**

The data were expressed as mean  $\pm$  SD and test of statistical significance was carried out using a one-way analysis of variance (ANOVA). The data obtained were analyzed using Statistical Product and Service Solutions (SPSS), version 18.  $P < 0.05$  was considered significant.

### **Result**

Biochemical markers; Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and Alkaline phosphatase (ALP) activities of the blood serum content of fish samples.

The serum ALT activity presented as mean  $\pm$  SD, from the result in Table 1 showed a significant decrease ( $p < 0.05$ ) in the enzyme activity of group 1 (control 1) when compared with different locations at Banegbe River and control 2 (group 2). Significant ( $p < 0.05$ ) increase in ALT level in the order of; middle-stream (group 11- group 14) >down-stream (group 7 to group 10) >up-stream (group 3 to group 6) were observed.

**Comment [G6]:** if you search for infected or affected liver and gills or any necrosis you say histopathological study

The AST activity presented as mean± SD for different locations in Banegbe River, Orogodo River, and Ekpan Pond are shown in Table 1 below. A significant ( $p<0.05$ ) decrease was observed in control 1 when compared with different locations at Banegbe River and control 2. The significant increase in AST activities was in the order of; middle-stream (group11- group14) <down-stream (group 7 to group 10) < up-stream (group 3 to group 6) at Banegbe River.

A significant decrease ( $p<0.05$ ) in the serum ALP level was observed across the groups from upstream (group 3 to group 6) to downstream (group 7 to group 10) and then middle-stream (group 11- group 14). However, a significant ( $p<0.05$ ) decrease in the serum ALP level of control 1 (group 1) was observed when compared with the second control (group 2)

**Table 1. Liver Marker Enzyme of Serum (IU/L).**

Groups	ALT	ALP	AST
Group 1	23.00±1.41	17.82±2.86	64.00±1.63
Group 2	60.00±17.45	26.07±2.74	136.75±11.26
Group 3	23.00±3.16	20.53±1.55	98.25±12.12
Group 4	41.00±15.47	20.30±1.72	81.25±23.48
Group 5	44.00±9.93	22.84±2.13	97.25±24.10
Group 6	48.00±7.79	22.92±2.79	106.50±15.25
Group 7	49.84±7.62	23.86±2.12	88.00±13.95
Group 8	50.00±8.83	24.04±0.99	117.00±10.89
Group 9	50.00±11.19	24.43±1.30	119.25±26.31
Group 10	58.00±14.02	25.11±1.06	98.75±26.70
Group 11	49.00±11.83	24.96±1.93	133.00±7.53
Group 12	60.00±2.45	23.07±1.94	126.75±5.68
Group 13	62.00±14.46	31.28±5.01	143.75±20.34
Group 14	72.25±10.75	31.81±4.37	159.75±18.15

Results are expressed in Means ± SD (n = 3)

Group 1 = Control 1;

Group 2 = Control 2

Group 3 = Upstream 1;

Group 4 = Upstream 2

Group 5 = Upstream 3;

Group 6 = Upstream 4

Group 7 = Downstream 1;

Group 8 = Downstream 2

Group 9 = Downstream 3;

Group 10 = Downstream 4

Group 11 = Middle-stream 1

Group 12 = Middle-stream 2

Group 13 = Middle-stream 3

Group 14 = Middle-stream 4

### Photomicrograph of the fish gill.

**Figure 1A:** From Orogodo River (control 1) showed normal gill structure with primary lamellae, secondary lamellae, and central venous sinus, the elastic cartilage of the filament (black arrow) and efferent branchial arterioles (red arrow) were intact.

**Figure 1B:** Fish from Ekpan pond (control 2) showed degeneration of the secondary lamella with clear deformed, clubbed secondary lamella (black arrow) and epithelial lifting's, were also discovered.

**Figure 2A:** From Orogodo River (control 1) showed normal gill structure with primary lamellae, secondary lamellae, and central venous sinus, the elastic cartilage of the filament (black arrow) and efferent branchial arterioles (red arrow) were intact.

Comment [G7]: the same as fig 1 A

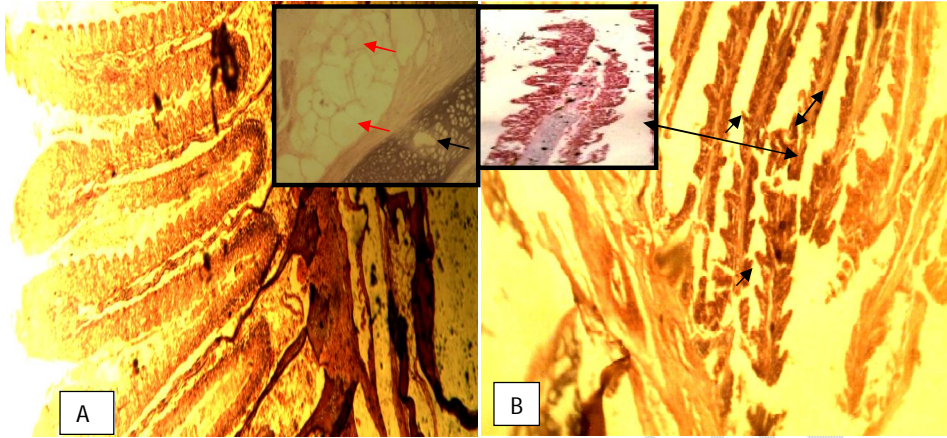
**Figure 2C:** Fish from upstream below showed occlusion of some of the efferent branchial arterioles (arrow) in the gill. The central venous sinus maintained a clear appearance and the interstitium of the primary lamella is also expanded with minor oedema.

**Figure 2D:** Fish from the middle stream showed multiple hemorrhages that is, engorgement in the gill structure due to increased blood flow.

Comment [G8]: the gills showed

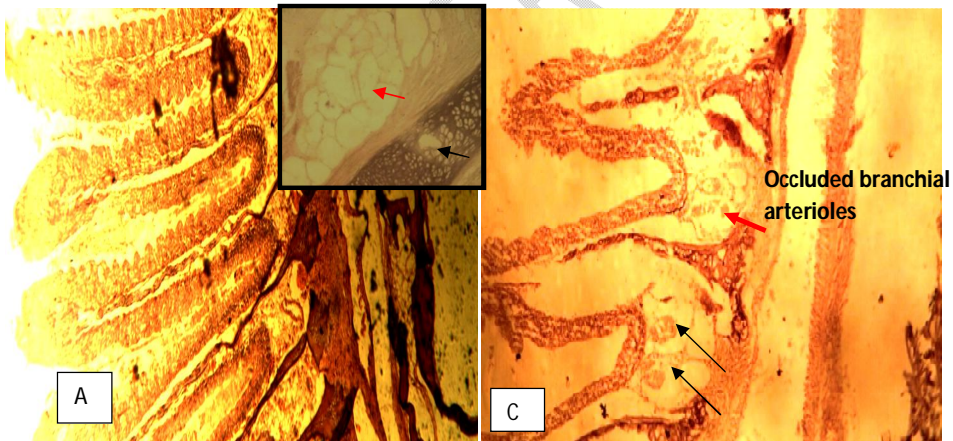
**Figure 2E:** Fish from down-stream showed distorted secondary lamellae (black arrow) and oedema (star).

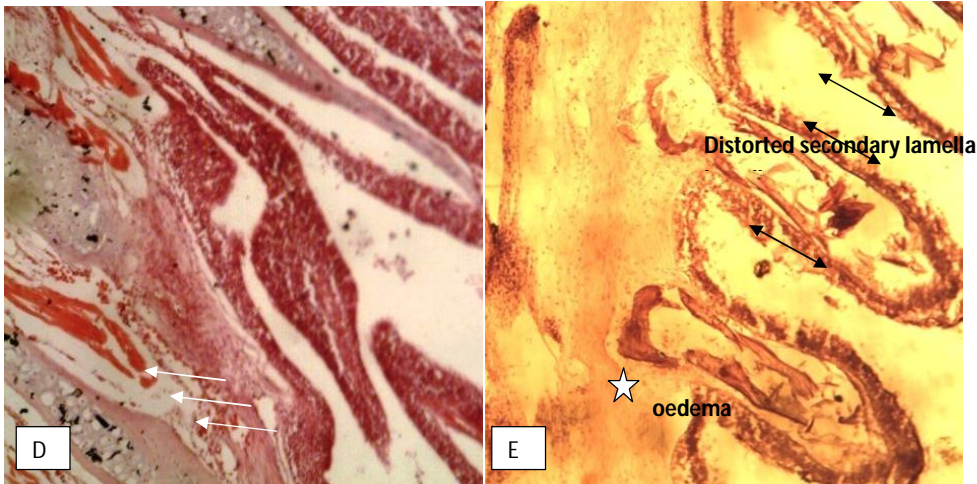
Comment [G9]: the gills showed



**Figure 1. Photomicrograph of gill structure of the Orogodo River (control one) and Ekpan pond (control two)**

**Where A = Orogodo River and B = Ekpan pond**





**Figure 2: A Photomicrograph of gill structure of fish samples from different location at Banegbe River and Orogodo River**

Photomicrograph of fish; A = Control (Orogodo river) C = Up-stream

D = Middle-stream E = Down-stream. H and E. mag. 400X

Comment [G10]: ( )

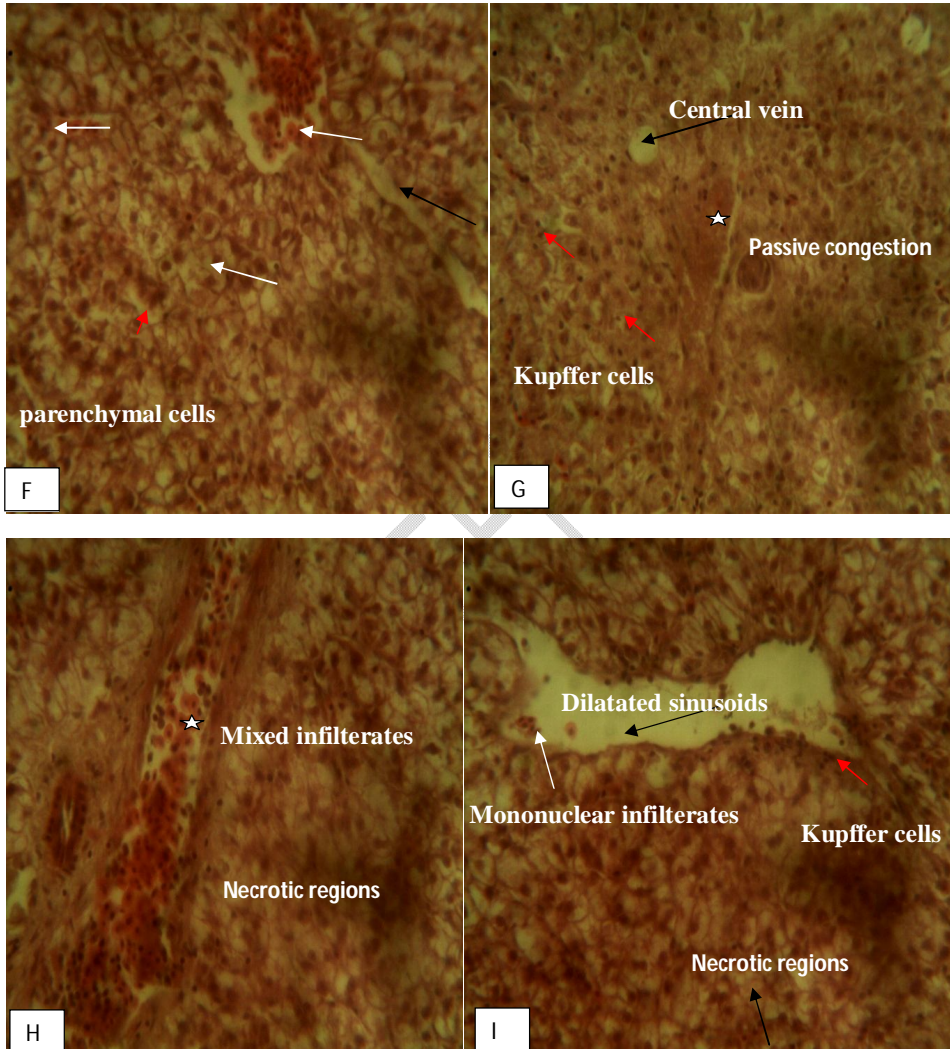
### Photomicrograph of fish liver section.

**Figure 3F:** A photomicrograph of the liver from control showed normal parenchymal cells (red arrow), intact sinusoids (black arrow), and a central vein infiltrated with mononuclear cells (white arrow). Also, the mononuclear cells are found within the hepatocyte chords.

**Figure 3G:** Fish from upstream showed, central vein (black arrow), Passive blood congestion (star), hepatocyte cytoplasmic vacuolation (white arrow), and Kupffer cell hyperplasia (red arrow).

**Figure 3H:** Fish from the middle stream showed necrosis of the liver and infiltration of mixed infiltrates in the enlarged sinusoid.

**Figure 3I:** Fish from downstream showed, enlarged sinusoids with little influx of mononuclear cells. Focal necrosis of the liver and cytoplasmic vacuolation was also observed.



**Figure 3: Photomicrograph of liver tissue of fish samples from different location at Banegbe River and Orogodo River**

Photomicrograph of liver; F = Control (Orogodo river) G = Up-stream

H = middle-stream I = Downstream. H and E. mag. 400X

Comment [G11]:

## DISCUSSION

Several soluble enzymes of blood serum have been considered relevant stress indicators. Therefore, activities of serum ALT, AST, and ALP have been commonly used in the diagnosis of fish diseases as well as in the detection of tissue damage caused by environmental pollution and indicate stress-based tissue impairment (Palanivelu *et al.*, 2005).

In this present study, it was observed that pollutants from industrial effluents channeled into Banegbe River caused a significant elevation in the blood serum of ALT, AST, and ALP activities of African knife fish when compared to control in group 1 (Orogodo River) from another river.

Our result is similar to the finding of Essien *et al.* (2015) who reported the same trend of a significant increase in ALT, AST, and ALP in the blood serum of Tilapia from Okirika River, Port Harcourt in down-stream and up-stream when compared to the control and their presence in the blood may give information on tissue injury or organ dysfunction.

Elevation in the activities of plasma AST, ALT, and ALP in *Tilapia spp.* from Lake Qarun reflects hepatic and myocardial impairment, leading to extensive liberation of the enzymes into the blood circulation (Mohamad and Gad, 2009).

Al-Attar, (2006) also reported that the elevation of serum AST, ALT, and ALP may be due to liver dysfunction. In addition, the increase of serum AST, ALT, and ALP may be attributed to hepatocellular damage or cellular degradation, perhaps in the liver, heart, or muscle.

AST and ALT belong to the plasma non-functional enzymes which are normally localized within the cells of the liver, heart, gills, kidneys, muscle, and other organs. It is also considered to be important in assessing the state of the liver and some other organs (Verma, Rani, and Delela, 1981). Their presence in blood plasma may give information on tissue injury or organ dysfunction. Monitoring of liver enzyme leakage into the blood has proved to be a very important tool in liver toxic studies (Salah El-Deen and Rogeps, 1993). Increased release of ALT into the blood is indicative of damage to the integrity of hepatocyte membranes and the elevated AST activities are due to mitochondrial disruption as a consequence of xenobiotics (Qiu *et al.*, 2009).

Our present results are also in agreement with the findings of (Jee *et al.*, 2005) who noticed an increase in activities of serum AST and ALT, in Korean rockfish (*Sebastes schlegeli*) exposed to pollutants. Abumourad *et al.*, (2014) also observed an elevation in the blood levels of AST and ALT and concluded that the increase may be due to the cellular damage in the liver and that high level of these enzymes in serum are usually indicative of disease and necrosis in the liver of the fish.

Also, Hadi *et al.*, (2009) and (Karan *et al.*, 1998) reported an increase in ALT and AST activities of plasma-serum in tilapia and *Sparus aurata* with *Cyprinus carpio* exposed to different concentrations of heavy metals respectively.

Aruljothi, (2013) reported elevation of ALT and AST of freshwater fish exposed to arsenic, and that all these elevations were a result of necrosis of the liver which causes an increase in the permeability of cell membrane resulting in the damage of the liver tissue.

Begum, (2004) has also reported that alanine and aspartate aminotransaminase were enhanced in hepatic and gill tissues. The elevated activities of both the transaminases (AST and ALT) indicate stepped-up transamination where feeding of amino acids into the TCA cycle occurs in order to cope with the energy crisis during cypermethrin stress

Therefore, in this study, the increase in enzymes activities could be due to the leakage of these enzymes from the liver cytosol into the bloodstream as a result of liver damage by contaminant, which also gives an indication of the hepatotoxic effect of the toxicants since ALT and AST are intracellular enzymes. Elevated AST and ALT activities are also indicative of higher utilization of aspartate and alanine as a substrate for gluconeogenesis in fish liver cells that suggest a heavy drain on metabolites during stress to provide intermediates for the Krebs cycle. Mobilization of these amino acids as substrates to gluconeogenesis probably occurred in African knife fish due to the exposure to these pollutants in the Banegbe River.

ALP enzyme is a sensitive biomarker to metallic salts since it is a membrane-bound enzyme related to the transport of various metabolites (Lakshmi *et. al.*, 1991). Alkaline phosphatase is involved in the synthesis of nuclear protein, nucleic acid, and phospholipids. These enzymes are associated with the transmembrane transport mechanism, ion transport, maintenance of ionic strength, and cell growth in the organ (Moog, 1946). The increased activity of ALP in fish has been linked to the increased catabolic tissue breakdown in Melano macrophage centers (Agius and Cushman, 1986). Ochmanski and Barabasz (2000) reported that the increase in the activity of ALP in blood might be due to the necrosis of the liver, kidney, and lung.

Our present results suggest that an increased level of ALP in the order of middle-stream < down-stream < up-stream before, might be due to the toxic effect of the contaminant in the Banegbe river. The increased activities can be attributed to the destruction of cell membranes and lysosomes which in turn leads to hepatic damage. The increased level of ALP activity suggested that there is the involvement of lysosomes in pollutant toxicity. Because tissue distribution of alkaline phosphatase is virtually ubiquitous, especially within cell membranes, and would easily leak out of the cell in pollutants-induced tissue damage. This result is also in agreement with the finding of (Sabae and Mohamed, 2015) that reported elevation in ALT and ALP in *Tillapia* spp from Lake Qarun.

There was an elevation in the value of ALT, AST, and ALP in the second control (fish from Ekpan pond which is group 2) from our present result when compared to the first control (Orogodo river fish). This may be attributed to contamination in the pond since Ekpan pond is usually dug close to the bank of the Warri River. There is a flow of the river into it with different species of fish alongside. The river water might have been polluted with different pollutants from industrial effluents sewage, and other sources.

### **Histopathology of the gill**

Gill surfaces are the first target of aquatic pollutants (Spicer and Weber, 1991). The gills are important organs, which participate in many important roles in the fish, such as respiration, osmoregulation acid-base balance, and nitrogenous waste excretion (Heath, 1987).

According to the present study, the histological study of the gills shows a normal gill structure with primary lamella (PL), secondary lamellae (SL), and central venous sinus (CVS), the elastic cartilage of the filament and efferent branchial arterioles were intact in the fish from Orogodo

river (Fig.1, Plate A). The Fish from Banegbe at different points and Ekpan pond water (Fig. 1 Plate B, C, D, and E) resulted in several forms of histopathological changes such as degeneration of the secondary lamella with clear deformed, clubbed secondary lamella, epithelial liftings fusion of some secondary gill lamellae, hemorrhaging in the primary lamella, oedema in the gill, deformed tips of the secondary lamellae and Vacuolations observed around the muscle layer of the gill. Other observations on the fish include epithelial lifting, interstitial oedema, and blood congestion in the vascular axis of primary filaments.

This result is similar to the findings of (Jalaludeen *et al.*, 2012) that reported lesions in the epithelial layer, hypertrophy in mucous cells, and vacuolation in gill membrane in gills of *Tilapia mossambicus* exposed to cadmium sulphate for 10 days. While for 20 days, oedema and destruction of either epithelial cells or a few lamellae were curled off the secondary lamellae which leads to congestion and hemorrhage of gills. Coutinho and Gokhale, (2000) also found epithelial lifting in the gills of carps (*Cyprinus carpio*) and tilapias (*Oreochromis mossambicus*) exposed to the effluents of a wastewater treatment plant.

It is possible that the damage to the gills could be a direct result of the salts, heavy metals, pesticides, sewage PAHs, and PCBs which are conveyed to the water (Van Den Berg, 1993). Fishes are directly exposed to poisons occurring in the external environment which often cause pathology in them (Mallatt, 1985). The gills are among the most vulnerable structures of the fish because of their external location and are in intimate contact with the external environment (water) and particularly sensitive to changes in the quality of the water are considered the primary target of the contaminants. (Camargo and Martinez, 2007). So, they are liable to damage by any irritant materials whether dissolved or suspended in the water (Roberts,1978). The presence of pollutants from industrial effluents and other sources could be the reason for the pathology changes in the gills of the fish. The alteration in blood vessels, from serve stress as a result of exposure to contaminant causes increased blood flow inside the lamellae, damaging pillar cells that results in blood congestion. (Rosety-Rodriquez *et al.*, 2002). These alterations were common in the fish from the Banegbe River and Ekpan Pond water.

Carmargo and Martinez, (2007) reported dilation of the marginal channel, hyperplasia of the epithelial cells, lifting of the lamellar epithelium fusion of some secondary lamellae, blood congestion, hypertrophy of epithelial cells and lamellar disorganization, lamellar aneurysms and hemorrhages with rupture of the lamellar epithelium of neotropical fish caged in an urban

stream. These alterations in the gill are also in line with the findings of this present work. The hemorrhage in the gills is a result of the effect of rupture of the gill epithelium and could be interpreted as a reflation of the direct action of toxic agents on the tissue (Timmink, 1983). Hyperplasia, edema, fusion of lamella, and lamella aneurysm were seen when Caspian kutum was exposed to Linear Alkylbenzene Sulfonate (Naeemi *et al.*, 2013)

Alterations like epithelial lifting, hyperplasia, and hypertrophy of the epithelial cells, besides partial fusion of some secondary lamellae are examples of defense mechanisms, since, in general, these result in the increase of the distance between the external environment and the blood and thus serve as a barrier to the entrance of contaminants (Fernandes and Mazon, 2003) that could impair blood-water exchange by reducing the surface area of the secondary lamellae that is in contact with the water (Kelly, 2007). Such alterations are non-specific and may be induced by different types of contaminants (Mallatt, 1985).

#### **Histopathology of the liver.**

The organ most associated with the detoxification and biotransformation process is the liver, and due to its function, position, and blood supply (Van der Oost *et al.*, 2003), From this present result, there were no histopathological changes observed in the liver of the control fish from Orogodo river, it shows normal parenchymal cells, intact sinusoids, and central vein infiltrated with mononuclear cells. Also, the mononuclear cells are found within the hepatocyte chords. The main alterations found in the liver of African knife fish from Upstream of Banegbe River before the effluent discharge point are passive blood congestion, hepatocyte cytoplasmic vacuolation, and Kupffer cell hyperplasia.

Fish from the middle stream of Banegbe River shows the following pathological changes. Necrosis of the liver and infiltration of mixed infiltrates in the enlarged sinusoid caused clogging of the sinusoid which blocks the blood from the hepatic artery and interbiliary portal vein which will have to pass through the sinusoid to get to the central vein. The clogged sinusoids can as well impeded the function of Kupffer cells and then allowing spent erythrocyte to build up in the blood instead of being removed.

Fish from downstream showed enlarged sinusoids with little influx of mononuclear cells. The Kupffer cells are also aligned in the sinusoid which are phagocytic in nature and participate in

the removal of spent erythrocytes and other particulate debris from circulation. The function of the Kupffer cell was not impeded because the sinusoid is not clogged up, although, they are enlarged. Cytoplasmic vacuolation was also observed which is the cells adaptively altered to resist further degeneration and it can reflect a cellular adaptation beneficial to the host.

Ekpan pond fish showed Necrosis of the liver and Congestion of the sinusoid. Congestion is a blood circulation disturbance due to the increasing volume of blood in the blood capillary. Vacuolar degeneration is known as an acute swelling of the organ (Rejeki *et al.*, 2008). The pathologic changes in the liver of fish from different points at Banegbe River and Ekpan Pond were a result of an accumulation of pollutants in the fish.

Camargo and Martinez (2007) observed irregular-shaped nuclei, nuclear hypertrophy, nuclear vacuolation, and the presence of eosinophilic granules in the cytoplasm and bile stagnation. Naemi *et al.*, (2013) reported Congestion and dilation of the sinusoid, hepatocyte, and vacuolar degeneration were detected in *Caspian kutum* exposed to sublethal concentrations of Linear Alkylbenzene Sulfonate. And that the tissue damage of the fish exposed to Linear Alkylbenzene Sulfonate detergent may be due to the accumulation of the detergent in them. Congestion of sinusoid, shrinkage of hepatocytes, slight atrophy, and vacuolar degeneration have also been reported in the liver of fishes after chronic exposure to Linear Alkylbenzene Sulfonate (Hampel *et al.*, 2008; Rejeki *et al.*, 2008).

Similar to our results is the liver of *Tilapia zillii* and *Solea vulgaris* from Lake Quarun, Egypt, which showed degenerative, necrotic, inflammatory, hyperemic, and thrombosed alterations (Mohamed, 2009). Also, El-Naggar *et al.*, 2009, showed that bioaccumulation of some heavy metals (Fe, Cu, Zn, Mg, Pb, and Cd) in the liver of *Oreochromis niloticus* in the Nile caused similar changes in the hepatopancreas like ours and the above mentioned

Sabae *et al.*, (2015) reported vacuolar degeneration of the hepatocytes with focal areas of necrosis, hemorrhage, aggregations of inflammatory cells as well as haemosiderin between the hepatocytes, dilation with intravascular haemolysis in hepatic and hepatoportal blood vessels, coagulative necrosis, nuclear pycnosis and focal areas of fibrosis in *Tilapia* spp. from Lake Qarun as a result of pollutants.

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