Journal of Environment and Earth Science
ISSN 2224-3216 (Paper) ISSN 2225-0948 (Online)
Vol.5, No.10, 2015

Assessment of the Toxic Effect of Mixed Effluents from Trans-Amadi Industrial Layout on Tilapia (Oreochromis Niloticus) in Okrika River, Port Harcourt, Rivers State, Nigeria

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Abstract
The toxic effect of the mixed effluent (industrial, domestic and municipal) discharged into Okrika River on Tilapia (*Oreochromis niloticus)* was assessed. Tilapia samples were collected at about 500 meters from point of entry of mixed effluent into the River (downstream) and about 1.5 kilometers from the point of entry of mixed effluent into the River (upstream) while Tilapia from a fish pond affiliated to Rivers State Sustainable Development Authority (RSSDA) was used as control. Malondialdehyde (MDA) concentration and glutathione S-transferase (GST) activity were assayed in the liver homogenate. Alanine amino transferase, ALT; Alanine aspartate transferase, AST and Alkaline phosphatase, ALP were investigated in the fish blood serum. Histopathologic section of the liver was also examined. Results showed that liver MDA concentration increased significantly ($p<0.05$) in downstream samples (2.45 ± 0.77 to 6.09 ± 1.57nm/mg tissue) with no significant change in upstream liver MDA. Also, the Hepatic GST was significantly increased in downstream (5.59 ± 1.09 to 16.80 ± 0.71 IU/L) as well as significant decrease upstream (5.59 ± 1.09 to 3.65 ± 1.48 IU/L) in comparison with the control. ALT, AST, ALP activities in the exposed fish serum showed marked increases downstream (ALT: 99.8 ± 3.5 IU/L; AST: 277.02 ± 39.8 IU/L; ALP: 40.38 ± 11.4 IU/L) at $P<0.05$ when compared to the control (ALT: 77.8 ± 14.3 IU/L; AST: 150.8 ± 50.7 IU/L; ALP: 15.34 ± 5.6 IU/L). Histology of the liver showed vacuolar degeneration, focal areas of necrosis and aggregation of inflammatory cells between the hepatocytes. This study elucidates negative biochemical changes on the metabolism of the fish due to the presence of mixed effluent in the River.
Keywords: Toxicity, Histopatology, Tilapia, Effluent and Glutathione S-transferase

INTRODUCTION

Mixed effluents are adverse by-products of economic development and technological advancement. Inappropriate disposition may become hazardous to health and environment. Effluents from industries can generate both inorganic and organic waste mixed with waste waters from the production processes, which leads to change in both biological and chemical parameters of the receiving water bodies (Gomez et al., 2008).

Okrika River forms the coastal axis of the Trans-Amadi Industrial Layout and is highly habited along its coastline by people, companies, jetties, etc. It is subjected to varieties of xenobiotics, originating from industrial effluent, drainage/domestic runoffs and sewage from the surrounding city, and marine (boat) traffic.

Aquatic food is constantly relied upon as source of protein and economic power through sales to the public. It therefore becomes imperative to highlight the integrity of the aquatic life and water quality following the enormous anthropogenic activities in and around the river.

Nile *tilapia* is one of the most important commercially cultured *tilapia* species indigenous to Nigeria (Ayoola, 2008). Tilapia is distinguished by its adaptation to living in fresh, brackish and nearly saline water, and can survive in partially polluted water (Zyadah, 1997). It is less sensitive to most toxic substances than most aquatic species. Any toxicant that affects tilapia would most likely be toxic to other aquatic organisms (Murungi & Robinson, 1987). This research focuses on the effects of aquatic pollution from discharged mixed effluent in Okrika river on Tilapia (*Oreochromis niloticus)* using selected biochemical biomarkers and histopathologic assay of the liver.

MATERIALS AND METHODS

EXPERIMENTAL ANIMALS

A total of fifteen adult Tilapia (*Oreochromis spp.)* fish of both sexes with a mean average weight of 88.24 ± 26.41g were used for this study. The fishes were caught alive with hook from Okrika river and the Rivers State Sustainable Development Agency (RSSDA) assisted fish farm by random sampling and transported to the laboratory in a well aerated big plastic container with water.

EXPERIMENTAL DESIGN

The Tilapia fishes were assigned into three groups consisting of five (5) fishes each.

Control group: Tilapia fishes obtained from the RSSDA-assisted fish farm
Downstream group: Tilapia fishes obtained from about 500 meters from point of entry of mixed effluent into the Okrika River
Upstream group: Tilapia fishes obtained from about 1.5 kilometers from the point of entry of mixed effluent into the Okrika River.

SAMPLE PREPARATION

The fishes where sacrificed immediately they arrived at the laboratory with a blow on the head and weighed. A sterile scalpel was used to make a neat slit on the dorsal portion from the heart to the gill and allowed to bleed; whole blood was collected into lithium heparinized bottle for enzyme/biochemical assay. The fishes were then properly dissected and the liver was quickly removed with the aid of sterile forceps, washed in ice-cold saline to remove the adhering body fluid and then divided into three portions. The first and largest lobe was put in ice cold normal saline for MDA assay, the second for antioxidant enzyme (Glutathione-S-Transferase, GST), assay while the third was fixed in 10% formalin for preparation of histopathological studies. The fish gills were lastly removed after dissection, washed in ice-cold saline, divided into two parts and put in ice cold normal saline and 10% formalin respectively.

BIOCHEMICAL ASSAY

The biochemical parameters analyzed was carried out using commercial kits from Randox laboratories Ltd (Northern Ireland). Liver malondialdehyde concentration was assayed by the method of Hunter et al. (1963) modified by Gulteridge and Wilkins (1980), Glutathione-S-Tranferase activity was measured using the method of Awasthi *et. al.,* (1975), Serum Aspartate transaminase (AST) and Alanine transaminase (ALP) activity were assayed according to the method of Reitman and Frankel (1957) and Alkaline phosphatase (ALP) by the colorimetric end point method using the substrate developed by Roy (1970).

HISTOLOGICAL EXAMINATION

The method of Baker and Silverton (1985) was adopted in the preparation of slices on previously fixed tissues (liver and gill) for histological examinations. Following the decalcification, dehydration, impregnation, embedding and section cutting, the tissues were stained using Mayer's acid alum haematoxylin and eosin and mounted in natural balsam. The slides were then examined microscopically for histological changes.

STATISTICAL ANALYSIS

All data are presented as means ± SD of five determinations. The one way ANOVA was used to analyse the data. The results were considered significant at P values of less than 0.05 ($P<0.05$).

RESULTS

The results for assessment of the toxic effect of mixed effluents from Trans-Amadi industrial layout on tilapia in Okrika River are shown below:

Biochemical studies on Tilapia Exposed to Mixed Effluent in Okrika River.
MDA concentration in liver

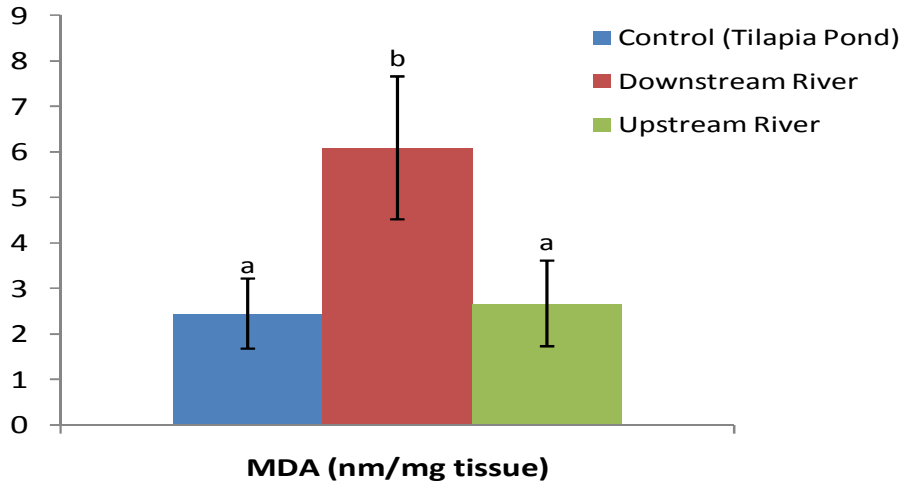

Figure 1: Effect of mixed effluent discharged in Okrika River on liver MDA of Tilapia fishes.
Liver MDA significantly ($p<0.05$) increased in downstream when compared with the value obtained in

the control while liver MDA in upstream showed no significant difference ($p>0.05$) from that obtained in control (Figure 1).

Hepatic glutathione-S-transferase activity

A significant ($p<0.05$) increase in the concentration of hepatic GST of downstream tilapia was observed when compared with the control, while hepatic GST in *Tilapia* samples upstream was significantly ($p<0.05$) lower than that observed in the control samples (Figure 3).

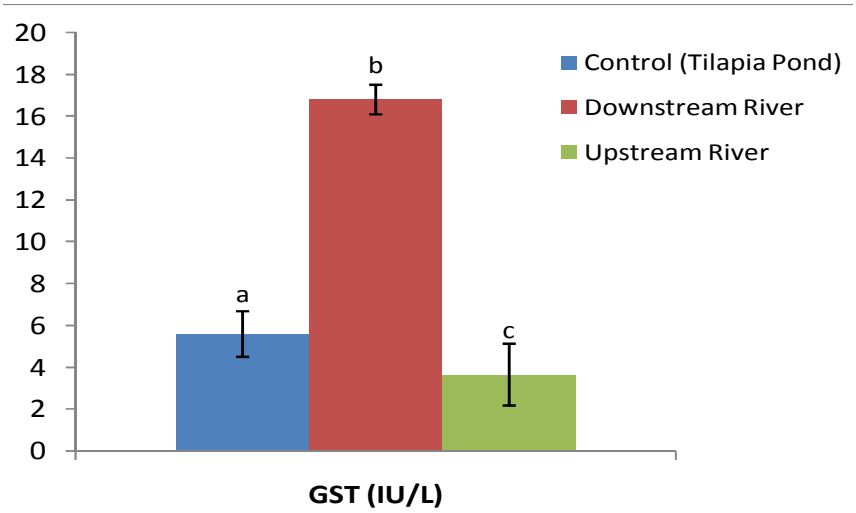

Figure 3: Effect of mixed effluent discharged in Okrika River on hepatic glutathione-s-transferase activity of Tilapia fishes

Serum ALT, AST and ALP

A significant ($p<0.05$) increase in serum ALT was observed in Tilapia samples collected downstream but not significantly ($p>0.05$) different in upstream samples when compared with the control samples. Serum AST was significantly ($p<0.05$) increased in downstream samples when compared with control and upstream samples. Serum ALP significantly ($p<0.05$) increased in downstream and upstream samples as compared with the control Tilapia samples (figure 4).

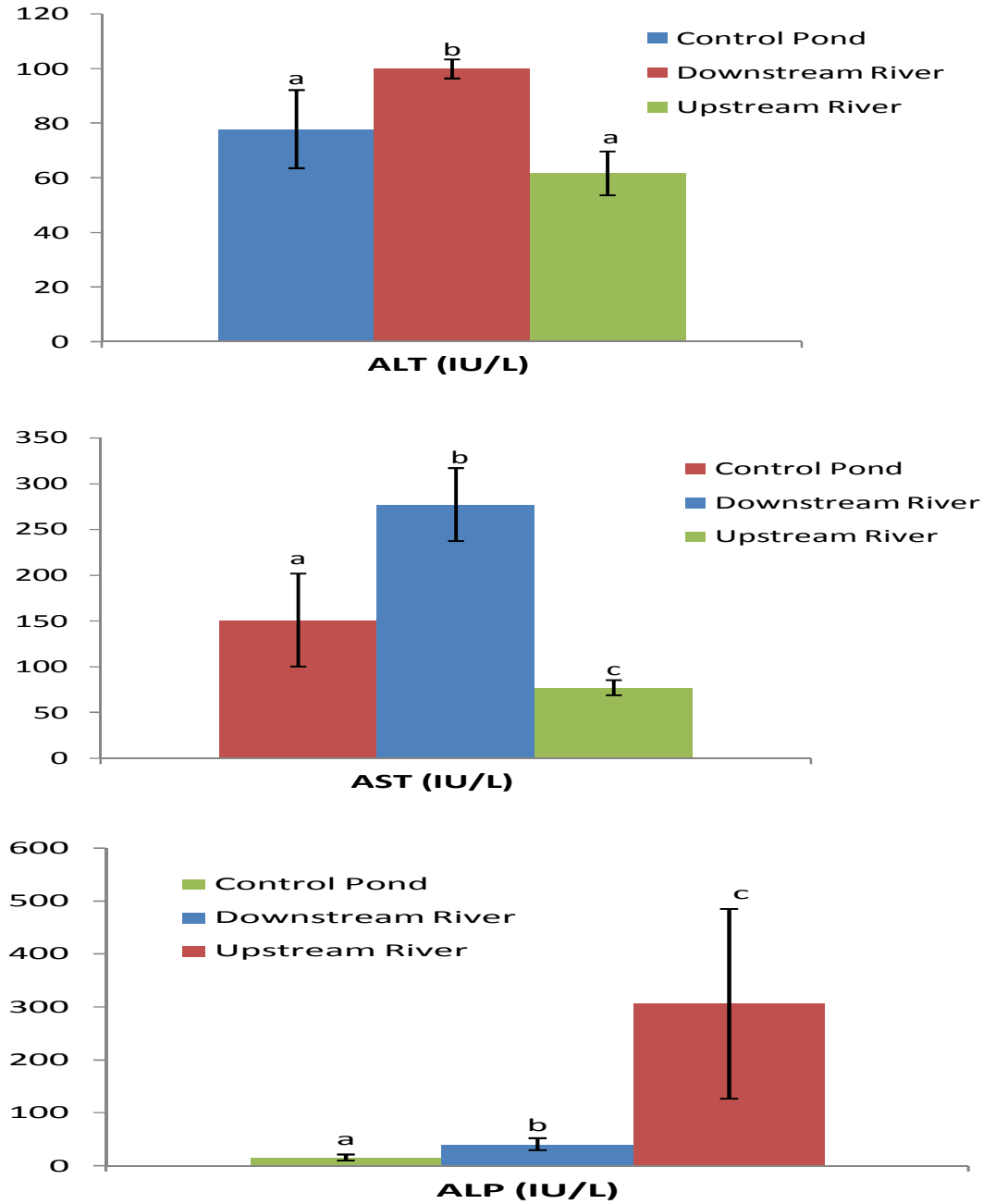

Figure 4: Effect of mixed effluent discharged in Okrika River on serum ALT, AST and ALP of Tilapia fishes

Histopathological examination

Liver histopathology

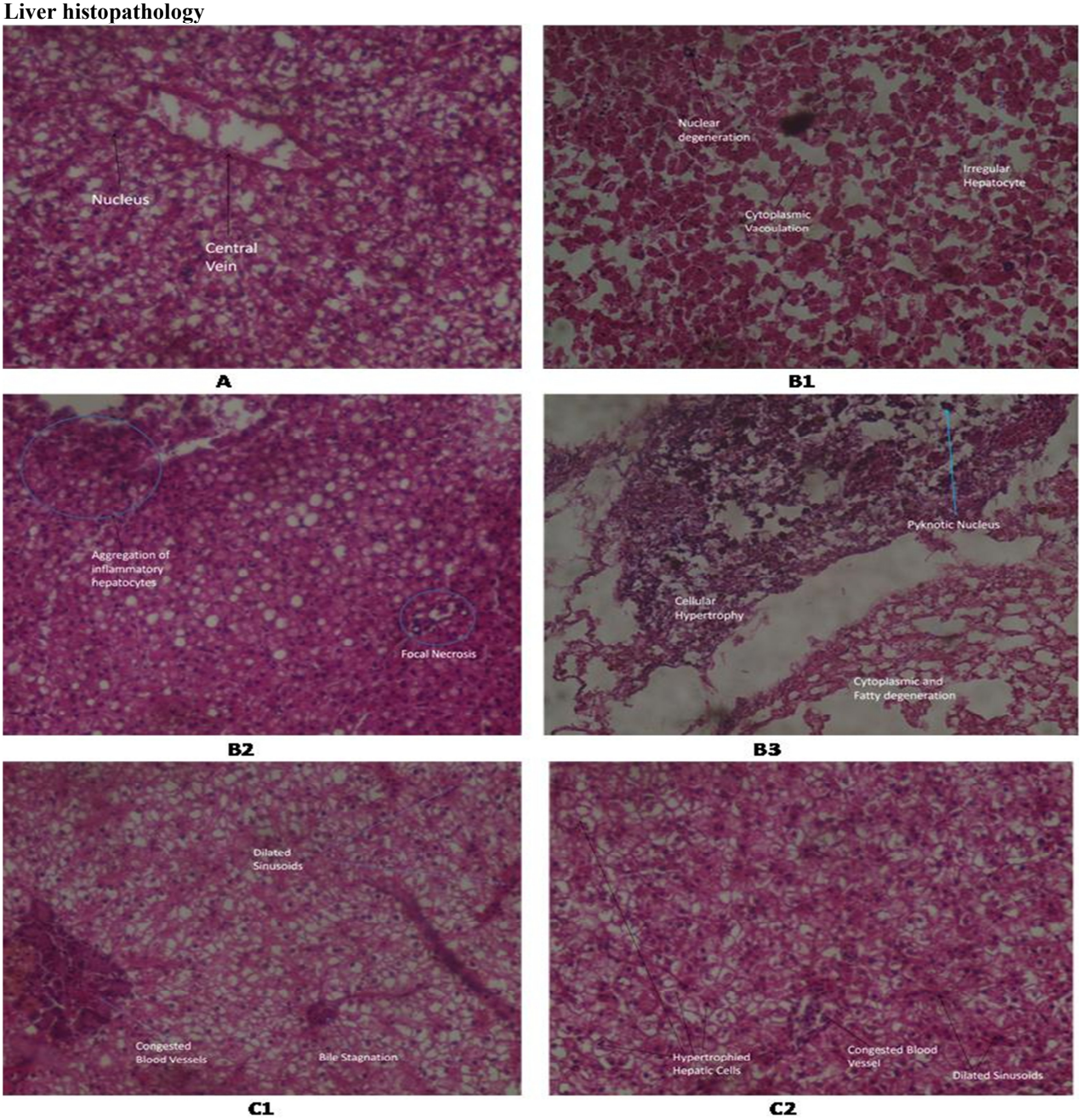

Figure 5: Effect of mixed effluent on liver histopathology of tilapia fishes

Plate A: Normal histological structure of liver (control) showing central vein, nuclei and cytoplasm. **Plate B1**: tilapia liver downstream showing vacuolar and nuclear degeneration, disorientation of hepatic cells, and severe fatty droplets. **Plate B2**: Tilapia liver downstream showing vacuolar degeneration, intracytoplasmic fat accumulation and aggregation of inflammatory cells between hepatocytes. **Plate B3**: Downstream liver showing severe widespread necrosis of lipid vacuoles with pyknotic nuclei and alteration in the structural orientation of hepatocytes. **Plate C1**: Tilapia liver upstream showing mild inflammation of liver cells (lobular) and intrahepatic obstruction (bile staining). **Plate C2**: tilapia liver upstream showing mild fatty change with hepatic cell atrophy and disorientation of the liver parenchyma structure.

Plate A shows the normal histological structure of the liver. The most common lesions in the liver of tilapia (Plates B-C) were alterations in the structural orientation of hepatic cells, vacuolar degeneration in the hepatocytes, focal areas of necrosis and aggregation of inflammatory cells between the hepatocytes. In addition, pyknotic nuclei, fatty change with bilirubin pigment and intrahepatic obstruction, dilation and congestion in blood sinusoids and intravascular haemolysis in hepatic blood vessels were evident in tilapia liver downstream

(Plates B1-B2). Moreover, focal areas of lipid vacuoles necrosis, intracytoplamic fat accumulation and fibrosis were also observed (Plate B3), though these lesions were mild upstream (Plates C1-C2) (Figure 5).

DISCUSSION

Human destructive influence on the aquatic environment is in the form of sub-lethal pollution, which results in chronic stress conditions that have negative effect on aquatic life (Mason, 1991). The result of the present study showed significant elevation in the concentration of liver MDA in Tilapia samples collected from downstream when compared with control. Conversely, there was no significant change in the level of lipid peroxidation in samples collected upstream, which, apparently may be due to "dilution" of the xenobiotics present in the mixed effluent as it spreads across the river through tidal movement. It is known that activities of antioxidant enzymes (biotransformation of xenobiotics) in mammals and other aquatic organisms may be increased by conditions of enhanced oxy-radical generation [Winston & Di Guilio, 1991; Cheung *et. al.*, 2001] and electrophilic intermediates derived from parent chemicals. Glutathione-s-transferase (GST) is one of these enzymes [McLoughlin *et al.*, 2000]. The increased activities of GST are known to serve as protective response to eliminate reactive free radicals. GST catalyze the conjugation of various electrophilic compounds with the tripeptide glutathione, the resulting in conjugates being water-soluble and, thus, more easily excreted. Since GST is involved in detoxification and excretion of xenobiotics and their metabolites, its increased activity in liver of *Tilapia* exposed to mixed effluent may indicate development of a defensive mechanism to pollutants downstream.

It has been reported that membrane lipid peroxidation results in the loss of polyunsaturated fatty acids, decreased membrane fluidity and severe structural changes leading to leakage of enzymes into blood stream (Van Ginkel and Sevanian, 1994). In this study, AST and ALT activities increased significantly in the blood serum of *Tilapia* from downstream. AST and ALT belong to the serum non-functional enzymes which are normally localized within the liver and other organs. Their presence in blood serum may give information on tissue injury or organ dysfunction (Wells *et. al.,* 1986). Monitoring of liver enzymes leakage into the blood has proven to be a very useful tool in liver toxicity studies (Saleh El-Deen & Rogeps 1993). Increased serum transaminases may therefore reflect the presences of hepatic toxicity which has led to extensive liberation of the enzymes into the blood circulation (Daabees *et. al.,* 1992).

Serum ALP activity increased significantly as well as progressively in *Tilapia* caught downstream and upstream respectively. This trend was also reported by Hadi *et al.,* (2009) in fresh water fishes. 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). The increased activity of ALP in *Tilapia* is linked to the increased catabolic tissue breakdown in melanomacrophage centers (Agius & Coushman, 1986). Ochmanski & Barabasz (2000) reported that the increase in the activity of ALP in blood might also be due to the necrosis of liver, kidney and lung. In the present study, the drastic increases in serum AST, ALT and ALP levels may also be due to the hepatocellular degeneration and necrosis of *Tilapia* liver cells due to exposure to the mixed effluent toxicants. This is confirmed by the presence of hepatocellular degeneration and necrosis observed in the histopathologic slides of liver sections examined. Also, *Tilapia* from Okrika River manifests histopathological changes in liver both upstream and downstream. It is possible that the pathological alterations in the tissues of the fish samples could be a direct result of the heavy metals, pesticides, salts and sewage, which enter the river with drainage water (Mohamed & Gad, 2008; Ali *et. al.,* 2008; Ali & Fishar, 2005; Mansour and Sidky, 2003; Gupta & Abd El-Hamid, 2003; Sabae & Rabeh, 2000) including industrial and domestic effluents from the zone. In this study, the liver of downstream *Tilapia* showed vacuolar degeneration in the hepatocytes, focal necrosis and extensive degeneration of cytoplasm with pyknosis of nuclei. These changes may be attributed to direct toxic effects of pollutants on hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals. The vacuolization of hepatocytes might indicate an imbalance between the rate of synthesis of substances in the paranchymal cells and the rate of their release in the circulation system (Gingerich, 1982). In the upstream samples, there were mild necrotic changes involving vacuolar degeneration and aggregation of inflammatory cells indicating that pollutants in the river are dose-dependent. It has been reported that toxicants at lower levels given for a prolonged time causes severe damage to the bronchial system of fish than to short term treatment (Ramesh, 1994). In conclusion, the presence of mixed effluent discharged from Trans-Amadi industrial layout into Okrika River showed negative biochemical changes on the metabolism of the fish.

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