The organism selected for this study was internationally recommended for ecotoxicological assessments, and have significant importance in aquatic food chains, facility of handling in the laboratory, and sensitivity to potentially toxic compounds (OECD, 1992; OECD, 2002). It has also been suggested by Doudoroff *et al* (1959) that economically important local fish species be used in toxicity assay. *Clarias gariepinus,* family Claridae, is a fish of high commercial value and are very common in Nigerian freshwaters. It is of high nutritive value. On the basis of availability, commercial and ecological importance, *Clarias gariepinus* was chosen for this study.

Collection of Wastewater

Collection of samples involved collection of wastewater samples at the point of discharge into the reservoir before releasing it into the environment from Federal Superphosphate Company. About 100L of the sample were taken in plastic containers and transported to the Department of Biological Sciences, Fisheries & Hydrobiology Laboratory, Ahmadu Bello University Zaria, and it was preserved using Trioxonitrate (V) acid.

Collection and maintenance of fish

Fingerlings of *Clarias gariepinus* were obtained from Miracle Farms ABU Zaria, in cold boxes with water to the Department of Biological Sciences, Fisheries & Hydrobiology Laboratory, Ahmadu Bello University Zaria. They were acclimatized for two weeks in 4 oval/rectangular shaped bath tubs, separately containing water of about 150L. The fish were fed on Coppen's pelleted commercial feed of 2mm in size at 5% biomass of 35% crude protein diet three times daily.

De-chlorination and changing of Test water

Water was renewed partially thrice a week using de-chlorinated tap water. Water was aerated during holding and acclimation. No aeration was carried out during the assay. Each time of renewal (during acclimation), three quarters of the water was siphoned out using a rubber hose of 5mm bore and topped up with de-chlorinated water.

Physico-chemical parameters of dilution water

The physico-chemical parameters of the dilution water (de-chlorinated tap water from Ahmadu Bello University Zaria) were monitored daily. These parameters include, pH, Temperature, Total Dissolved Oxygen and Electrical Conductivity. The pH and Electrical Conductivity were determined using a pH/Hanna multi parameter instrument (model H13952). Temperature was determined by the use of mercury in glass thermometer and Dissolved Oxygen was determined by the modified Winkler Azide Method (Lind, 1979; APHA, 1985; 1990).

Acute toxicity bioassay

The renewal method of bioassay 96h were conducted in the laboratory following methods by Spraque (1973) and APHA (1985) to determine the toxicity of wastewater against *Clarias gariepinus.* The bioassay test was carried out in 12 glass tanks each of size 30.5 X 30.5 by 92.5cm into which approximate quantity of wastewater from Superphosphate Fertilizer Company was taken to give a final volume of 25.0L. The nominal concentrations of wastewater used were 0.0, 5, 25, 50, 75 and 100.

The mixture of the toxicant and dilution water was allowed to stand for 30minutes before the introduction of test fishes.

The fishes were randomly distributed to each glass tank as suggested by Spraque (1973). Each tank contained 10 fish. Each assay was replicated simultaneously and repeated once to determine reproducibility. Aeration of the test water was suspended a few minutes prior to addition of the toxicant and during the test. The toxicant and the test water were renewed after 48hours during each series to test. The fish were starved 24hours prior to the start and during each assay test

Histopathology

Gills and liver obtained from fish that have just died (in case of those concentrations and from fish that survived the acute exposure were removed and fixed in formo-saline (Alan *et al*, 1983). The tissues were washed in running tap water for at least 2 hours to remove traces of formalin. This was followed by dehydration using successive percentages of alcohol (30, 50, 70, 90 and

100%). They were then infiltrated in chloroform and blocked in paraffin wax 58-60^{0}C melting point. Samples were embedded in fresh molten wax using L-shaped embedding moulds. Sections of 8µm thickness were cut and stained in haematoxylin and eosin (H&E). Permanent slides were prepared with these sections and microphotographs taken with a magnification of X400. This were examined and compared with those for control. Data that was obtained from physico-chemical analysis in wastewater was subjected to Analysis of Variance (ANOVA) using Microsoft Excel for Windows 2007 were used to test differences between levels of treatment and to separate means respectively.

III. RESULT AND DISCUSSION

Physico-chemical Analysis of Wastewater Used: Acute Exposure

The values for the physico-chemical parameters for the wastewater used for the research in the test tanks are shown in Table 1. These parameters of the test water vary slightly ($p<0.05$) during the bioassay. Water quality parameters analysed were temperatur e, electrical conductivity, pH and dissolved oxygen.

Table 1: Mean water quality parameters for control and treatment tanks in Acute Bioassay

Water quality parameters	100 (ml/L)	75.00 (ml/L)	50.00 (ml/L)	25.00 (ml/L)	5.00 (ml/L)	0.0 (ml/L)
pH	3.10	3.61	3.80	3.95	4.10	7.15
Temperature (0C)	33.52	32.67	31.18	31.00	30.42	33.10
Dissolved Oxygen (ml/L)	2.20	2.50	2.65	2.70	3.00	5.60
Electrical Conductivity (µS/cm)	1013	724	492	390	250	170

pH increased with increase in concentration and the exposure time, the control was neutral as against that of the tanks with the toxicant which was acidic. Concentrations of the toxicant had effect on the pH value and affected the quality of water and the environment, from this study pH had a range between 4.16-4.82 during the acute study which revealed that the toxicant was acidic as observed from the pH scale which was against the lamella. General observations showed evidence of proliferative lesions in the gill pH scale of 5.5 -9.5 as recommended by WHO(1984)/FEPA(1991) and this acidic nature of the toxicant most have led to the mortality of the fish during the bioassay; Wynne *et al*. (2001) reported that, pH has profound effects on water quality affecting the ability of bacteria which require slightly acidic pH to degrade toxic substances to less harmful forms. The temperature remained fairly constant in all the test tanks. The temperature remained fairly constant in all the test tanks. Exposure period also had an effect on the temperature during the period of study. Parameters such as pH, temperature and DO in the control were significantly higher than those in the test tanks ($p< 0.05$) and this may lead to negative environmental problems, low 2.60-3.50 DO mean values of led to the death of some fishes in the treatment tanks. Alberto *et al.*, (2005), observed that the first responses of fish to environmental hypoxia (low DO levels) were changes in ventilation and cardiovascular functions stimulated by cathecholamines released from andregenic nerves and chromaffin tissues where they are both synthesized and stored also Gabriel *et al*. (2007) reported that the DO level in the control were significantly higher than those in the treatment. Electric conductivity (EC) showed that at higher concentration of the toxicant the value of EC was high; this might be due to high concentration of the ions that were found in the wastewater.

Histopathology for Gills

From the histological sections of the gill are presented in plate II and III, gill of control fish has a normal structure with the filament and lamellae consisting of filaments attached to cartilaginous gill bar with fingerlike projections (secondary lamellae) on each side of the gills (plate II). Sections of gill from different concentration of the wastewater from SFC were the most damaged as observed in plate III. At 100ml/L wastewater there was oedema of gills epithelium and mutilation of the gill rakers. At 5ml/L concentration of wastewater there was less damage to gills. Damage done to the gills was dose-dependent.

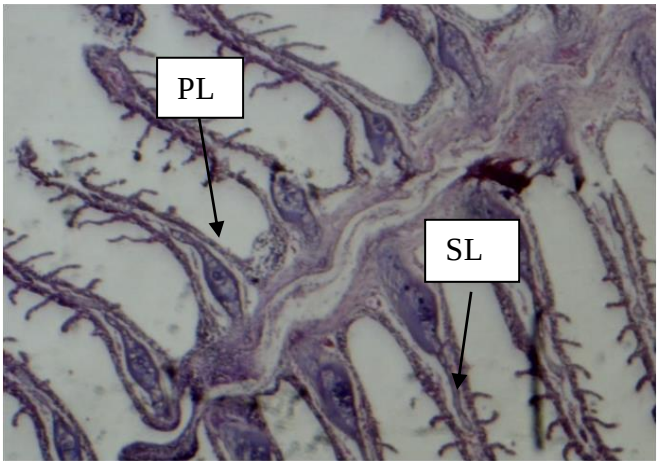

Figure 1 : Plate I: Photomicrograph of Gill cells of control Fish, PL and SL intact (PL= primary lamellae, SL=secondary lamellae). X40

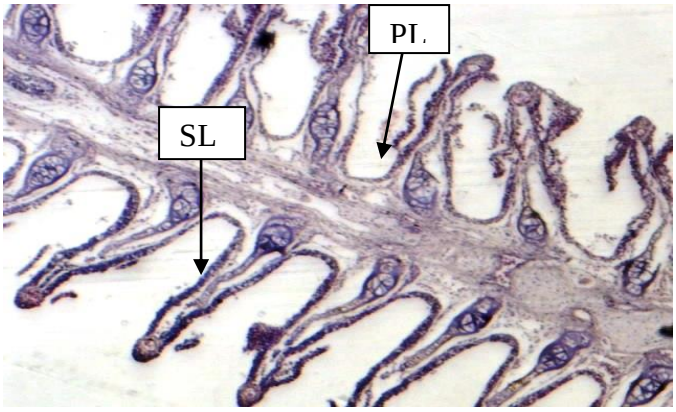

Figure 2 : Plate II: T.S of gill of *Clarias gariepinus* exposed to acute concentration (50ml/L). (PL=primary lamellae), complete filament detachment. X40

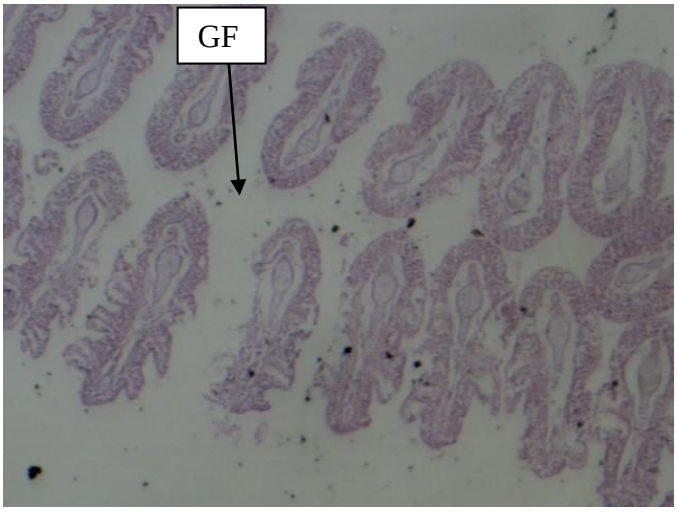

Figure 3 : Plate III Gill filaments of *C. gariepinus* from (100ml/L) after 8weeks of exposure showing Complete erosion of filament(GL= gill filament)X40

The gills of fish exposed to the acute concentration of the toxicant and those of the control fish exposed for 96hrs were compared and there were slite changes observed. Some of the observed changes include marked loss of secondary lamellae, attenuated primary lamellae and loss of epithelial cells as observed in the tank with 100ml/L of toxicant and this was due to high concentration of Pb (0.91 mg/L), effect on the gills of fish might affect the respiratory system of the fish and the liver too was affected which led to the death of fish as was revealed by the histopathological studies when compared with tissues gotten from the control fish, Findings too by Dhanapakiam, *et al*, (1998) on histopathological alterations in the fish gills were been used in biomonitoring the effects of various pollutants in the aquatic environment. Fafioye *et al*.,(2004) reported similar findings on histopathological response of *Clarias gariepinus* to plant extracts under brief exposure.

Other pathological alterations observed in this study (i.e. erosion of gill villi, oedema, matting of gill filaments, necrosis and hyperemia (vascular congestion) agrees with reports by Dhanapalkiam *et al*.(2004), Harper and Wolf, (2009) Pathan *et al*.(2010), their report was that specific reactions to acute localized oxygen deprivation of gill tissues). Au, (2004), Tang and Au, (2004) Nordberg *et al*. (2005), reported that gill lesions such as oedema formation and shortened gill filaments did not only indicate possibilities of impaired respiratory functions but impaired osmo-regulatory functions too.

The reduced availability of oxygen to respiratory surfaces like gills was a direct consequence of hypoxic conditions traceable to anthropogenic impacts on water quality of aquatic systems (Adeogun *et al.* 2011, Adeogun and Chukwuka, 2012).

Sub- lethal studies on the fish showed loss of epithelial cells along with marked attenuation of primary and secondary lamellae at the highest concentration of 19ml/L and slight attenuation of primary and secondary lamellae at the lowest concentration of 5ml/L.

Similarly, Dhanapalkiam *et al*. (2004) reported that the gill lesions in the carp, *Labeo rohita* exposed to sub-lethal concentrations of tannery effluent and reported severe damages to gill architecture including swelling of primary and secondary epithelial cells. Peebua *et al*. (2008) also reported histopathological alteration in the gill of *Oreochromis niloticus* exposed to alachlor and observed gill alterations ranging from oedema of

theepithelial system to hypertrophy and hyperplasia of epithelial cells. Pathan *et al*. (2010) also reported pathological changes and lesions ranging from epithelial hypertrophy, swelling in pillar and mucous cells to hemorrhage in gill lamella of a freshwater fish *Rasbora daniconius* exposed to paper mill effluent which was high in organic content.

Histopathology for Liver

Plate IV shows the features of liver sections of control fish. Observations showed hepatocytes and other cells systematically arranged. The epithelium of the veins is lined with various epithelial cells. Plate V that is fatty degeneration of the liver. In fish exposed to 75ml/L of wastewater, necrotic hepatocytes were observed. Oedema of the liver was dose dependent.

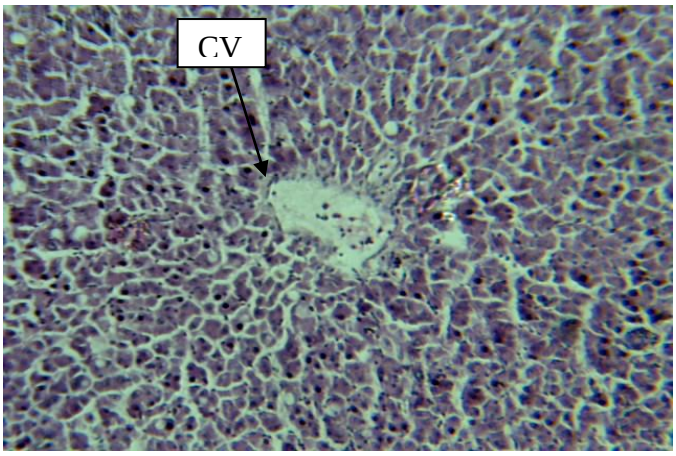

Figure 4 : Plate IV: T.S of the cells of control fish showing with homogenous cytoplasm. (CV= central vein, PC parenchyma cells) X100

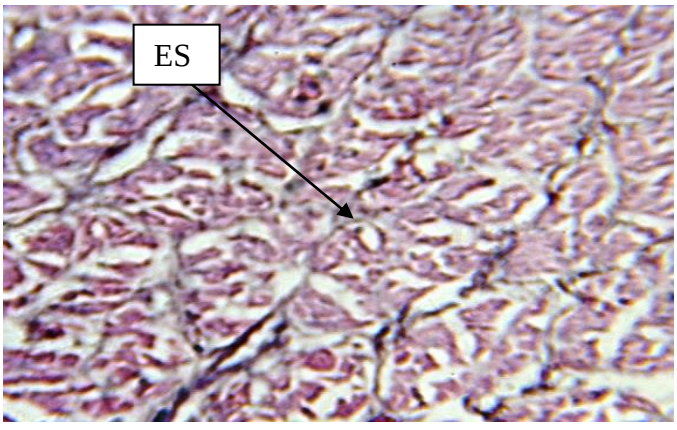

Figure 4 : Plate V: T.S of the liver cells of *Clarias gariepinus*, extensive areas of necrosis exposed to 50ml/L (ES=sinusoids) X100

The acute study of liver exposed to the toxicant revealed necrosis and marked fatty changes at 50ml/L. Liver statuses was also observed which was as a result of glycogen accumulation as a result of breakdown of liver cell. Necrosis and mild statuses was observed in fish exposed to 9ml/L concentration of the toxicant, other observations made revealed mild fatty change and focal peri portal inflammation. Cengis *et al. (*2001) reported some histopathological alteration in the liver of mosquito fish *(Gambusia affins)* such as sinusoid enlargement, haemorrhage, pikonosis, vacuolization of cell cytoplasm, infiltration of mononuclear lymphocytes, hyperthrophy and congestion after exposure to sub-lethal concentrations of endosulfan.

IV. CONCLUSION

The acute and sub-lethal histopathological degeneration observed include necrosis, attenuated primary lamellae, loss of epithelial and mucus cells, marked loss of secondary lamellae, oedema, haemorrhage, vascular congestion, hyperplasia and hypertrophy in a variety of anatomical sites including gills. The liver showed mild fatty change, moderate steotosis and mild inflammatory infiltrate.

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