

## The Toxic Effects of Brewery Effluent on *Clarias gariepinus*

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### ABSTRACT:

The analytical study on the effects of brewery effluent on *Clarias gariepinus* was executed at Michael Okpara University of Agriculture, Umudike. The test organism was obtained from a fish pond in the College of Natural Resources and Environmental Management. The pond water was tested to establish the physicochemical properties. The test organisms were subsequently acclimatized in a fourteen day period before the introduction of the effluent in incremental order into the aquaria. The brewery effluent was introduced into the aquaria for the toxicity test in the order of: 0.5 litre, 1.0 litre, 1.5 litres, 2.0 litres, 2.5 litres and 0.00 litre (control). The set up was monitored for 92 hours. The gills from isolated from control and experimental fishes (set ups) were subjected to histopathological examination. The physicochemical parameters of the effluent recorded were: temperature with mean of 29.53°C, Dissolved oxygen (DO) with mean of 4.13mg/l, pH with mean of 7.63 and electrical conductivity with mean of 67.3pS/cm. Photomicrograph shows that there was poorly developed secondary lamella with epithelial hyperplasia and mild congestion of venous sinuses, conspicuous pillar cells and mucous cells in the 0.5l effluent aquaria as compared to the control. It therefore becomes imperative to strictly enforce environmental regulations to forestall the adverse consequences of unregulated effluent discharge.

Keywords: Toxicity, epithelial, hyperplasia, Sinuses, photomicrograph, conductivity

### Introduction

Brewery plant has been known to cause pollution by discharging effluents into receiving streams, groundwater, and soil. Water consumption for breweries generally ranges from 4-8 cubic metres per cubic meter of beer produced. Production steps include malt production, wort production and beer production. Untreated effluent typically contains suspended solids in range 10 - 60mg/l, BOD in range 1000 - 1500mg/l, COD in range 1800 - 3000mg/l and Nitrogen in range 30 - 100mg/l.

Effluents from industrial process steps are variable, for example, bottle washing produces large volume of effluent that, however, contains a minor part of total organics discharged from brewery. Effluent from fermentation and filtering are high in organics and BOD and low in volume, accounting for about 3% of total waste volume but 97% of BOD. Brewery effluents contain organic material such as spent grains, waste yeast, spent hops and grit. Effluent pH averages about seven for combined effluent but can fluctuate from 3 - 12 depending on the use of acid or alkaline cleaning agent.

The effluents adjourn to the receiving stream when discharged and not treated thereby causing damage to the ecosystem. The chemicals and heavy metals present in these effluents produce harmful effects on animals and plants (Nagarajan and Shasikumar, 2002). Industrial effluent contributes to lot of water pollution that forms a threat to aquatic plants and animals (Ramona et al; 2001). A greater part of the pollutants exhibit biomagnifications and bioaccumulation capabilities with a broad spectrum of impacts and stresses on aquatic organisms (Censi et al; 2006). The study is aimed at investigating the effect of brewery effluent on the life of *Clarias gariepinus* (catfish).

### Materials and Method

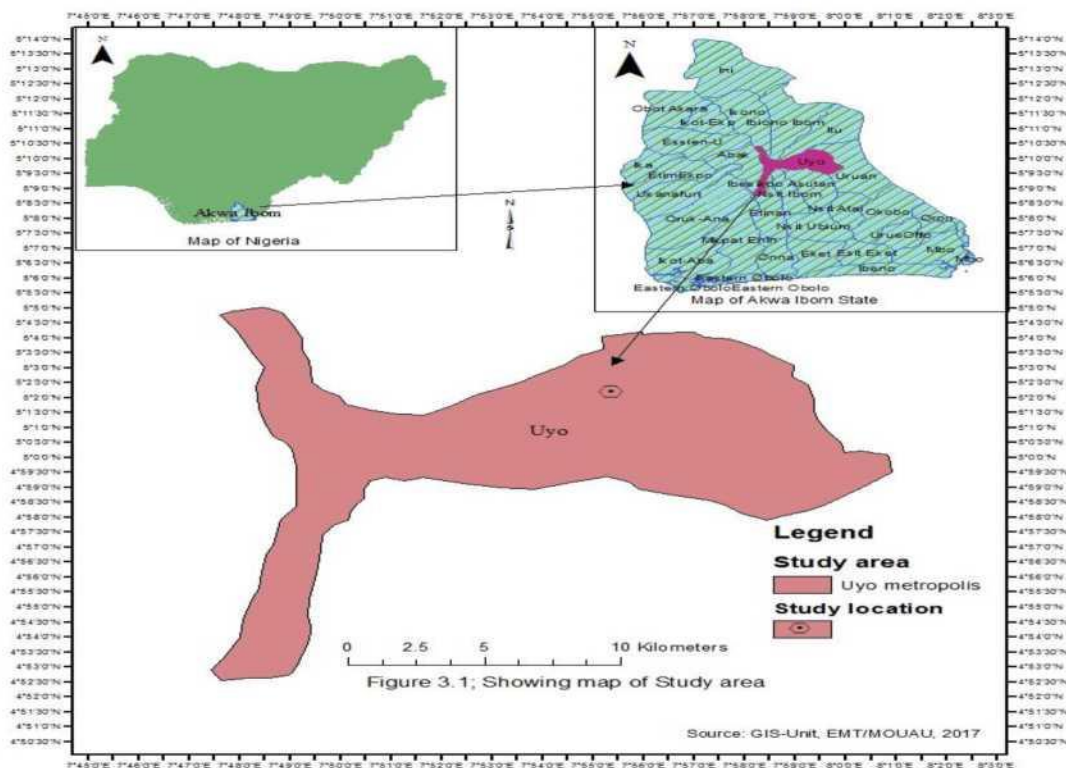
#### Study Area

“Champion Brewery plc” is located in Uyo city. Akwa Ibom State is located in the coastal area of southern part of Nigeria. It lies between latitude 4°30' and 5° 30' N and longitude 7°30' and 8° 15'E. The brewery is located at the industrial layout, Aka Offot Uyo, Akwa Ibom State, with latitude of 5.05°N and longitude 7.9333°E.

Figure 1: Map of Akwa Ibom State showing Champion Brewery, Uyo.

### Sample Collection

The effluent was collected from Champion Brewery Pic. Akwa Ibom State. The water sample was



### Procedure

This experiment was a continuous static bioassay. The test containers were covered with net to prevent the fish from jumping out of the aquaria. The acclimatization period for the fish was 14 days. After the acclimatization, the test organisms were distributed into the plastic aquaria. Each of the aquaria contained 20 litres of the water and ten (10) of the experimental fish. The brewery effluent was introduced into the aquaria for the toxicity test at 0.5, 1.0, 1.5, 2.0, 2.5 and 0 litre (control). The number of test fish in each test aquaria was counted after 24 hours until 92 hours. Animal showing no movement when probed was considered death.

### Materials

(i) Brewery effluent, (ii) *Clarias gariepinus* (catfish), (iii) thermometer for temperature, (iv) A Toledo 320 pH meter use for pH measurement, (v) A mettle Toledo MC 226 for conductivity measurement and (vi) plastic aquaria.

collected from fishery pond in Michael Okpara University of Agriculture, Umudike where the fishes were collected. The entire water sample collected was transported under cooled condition using ice cubes (in coolers) and analysed in less than 24 hours. *Clarias gariepinus* (catfish) which was used for this experiment were also collected from the fish pond.

### Histological Analysis

For histopathological examination, fish organs like gills were collected from control and experimental fishes through trans-spinal dissection, gently rinsed with physiological saline solution (0.9% NaCl) to remove blood and adhering debris and immerse in fixative composed of glacial acetic acid, formaldehyde and ethanol (1:3:7). The fixed tissues were passed through ascending grades of alcohol series 70%, 95%, 95%, 100%, 100%, 100% for dehydration and finally cleared in xylene. Tissues were then infiltrated with molten paraffin wax in a hot air dry oven at 58°C and finally embedded in paraffin wax. After embedding, the blocks were trimmed, sectioned at 5 microns on a rotary microtome (Leitz Rotary type). The sections were

stained with haematoxyline and eosin. The stained slides were covered with slipped DPX mountant. The slides were observed under a microscope attached with digital camera (Olympus E420 camera) and photographed

### Results

Table i.summary of the physicochemical parameters of the brewery effluent during the toxicity test for *Clarias gariepinus*.

Concentration %	Temperature (°C)	Dissolve oxygen (mg/l)	pH	BOD5	Conductivity (pS/cm)
0.	25.0	2.0	7.6	1.8	6.00
0.5mg	25.0	3.01	7.7	2.0	80.2
1.0mg	25.2	3.01	7.6	2.0	80.2
1.5mg	25.5	5.4	7.6	1.9	80.2
2.0mg	25.8	5.4	7.8	2.2	80.2
2.5mg	25.8	5.8	7.8	2.4	80.2

Table i represents the physicochemical parameters of the effluent recorded which are: temperature with mean of 29.53°C, DO with mean of 4.13mg/l, pH with mean of 7.63 and electrical conductivity with mean of 67.3pS/cm. **Table 2.** Mortality rate of catfish that were exposed to the brewery effluent.

Concentration (ml)	Number exposed	of 24 hours	48 hours	72 hours	96 hours	Total mortality
Control (0)	10	0	0	0	0	0
0.5	10	0	0	0	0	0
1.0	10	0	0	1	1	2
1.5	10	0	1	1	1	3
2.0	10	0	2	0	0	2
2.5	10	1	0	2	1	4

Table 2 indicates the number of mortality of the fish that were introduced in each concentration of the effluent used in a particular hour that was observed.

**Table 3: Percentage mortality value observed during the exposure of catfish (*Clarias gariepinus*).**

Concentration (ml)	Number exposed	24 hours	48 hours	72 hours	96 hours
Control (0)	10	0%	0%	0%	0%
0.5	10	0%	0%	0%	0%
1.0	10	0%	0%	10%	10%
1.5	10	0%	10%	10%	10%
2.0	10	0%	20%	0%	0%
2.5	10	10%	0%	20%	10%

**Table 4: Weight and length of catfish that was used.**

Concentration (ml)	Weight of the fish	Length of the fish.
0.5	6.88g.	10.00cm
	6.44g.	10.5cm
	6.50g.	10.5cm
1.0	7.12g	10.6cm
	7.52g	7.1cm
	7.54g	8.0cm
1.5	7.17g	10.00cm
	8.40	10.09cm
	8.44g	
2.0	8.06g	10.7cm
	9.54g	11.2cm
	9.44g	11.0cm
2.5	7.54g	10.3cm
	6.94g	10.00cm
	9.03g	11.00cm

Table indicates the weight and length of the fish (*Clarias gariepinus*) with total mean of 14.156g and 20.84cm.

**Table 5: Behavioural responses of *Clarias gariepinus* to different concentration of brewery effluent.**  
 (+) indicates an increase in responses while ( - ) indicates no response.

**The test of hypotheses shows as follows:**

Chi squared ( $\chi^2$ ) calculated = 9.31384 is  $>$   $\chi^2$  tabulated = 3.141 we therefore reject the null hypothesis ( $H_0$ ) and accept the alternate ( $H_1$ ) hypothesis which states;

- There is significant effect of brewery effluent on gills of *Clarias gariepinus*.
- There is a significant effect that the brewery effluent had reduced the oxygen level of the water in which the fish depend on.
- There is a significant effect of the effluent that led to their death.

Plate 1: Control (0ml)

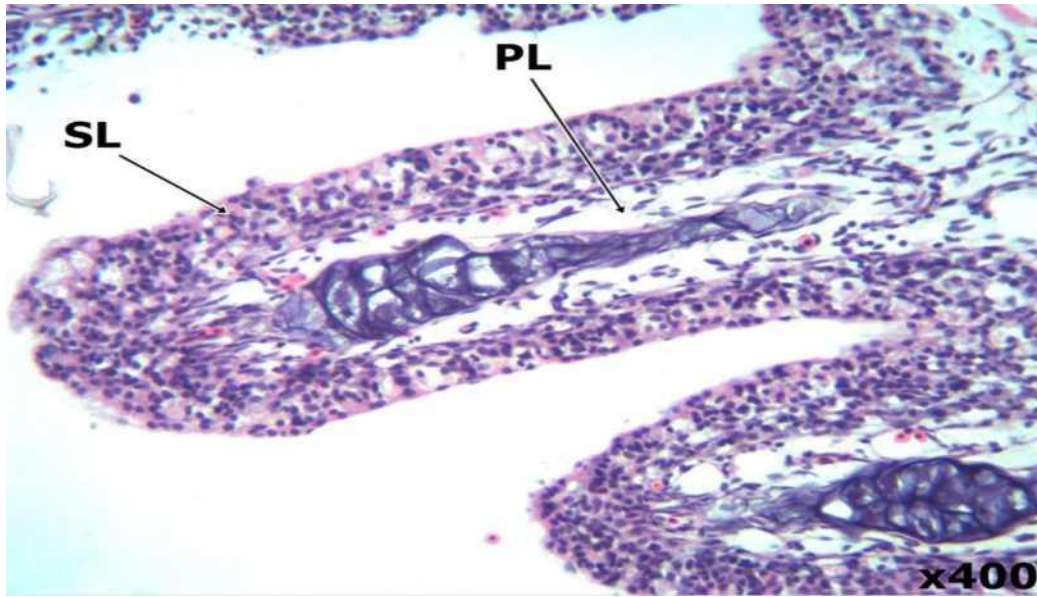


Plate 1: Photomicrographs, it shows fish gills primary lamella with prominent cartilaginous support afferent and efferent arterioles as well as intact epithelial covering.

Plate 2 (0.5ml)

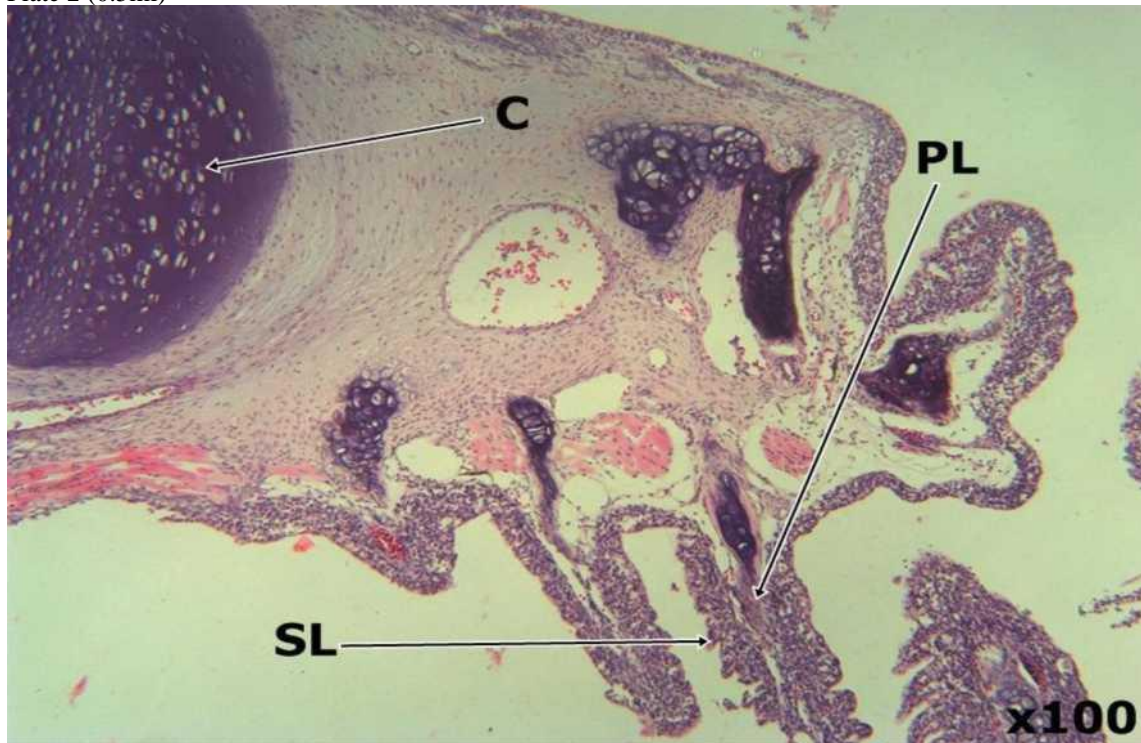


Plate 2: There is poorly developed secondary lamella with epithelial hyperplasia and mild congestion of venous sinuses, conspicuous pillar cells and mucous cells compared to the control.

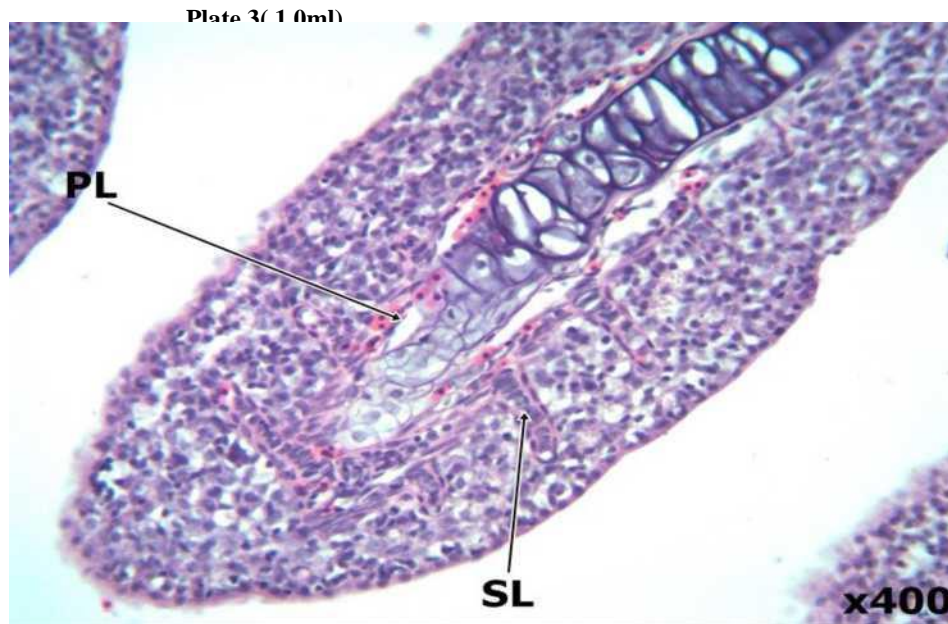


Plate 3: There is poorly developed secondary lamella with epithelial hyperplasia and mild congestion of venous sinuses, conspicuous pillar cells and mucous cells compared to the control.

**Plate 3( 1.0ml)**

Plate 4 (1.5ml)

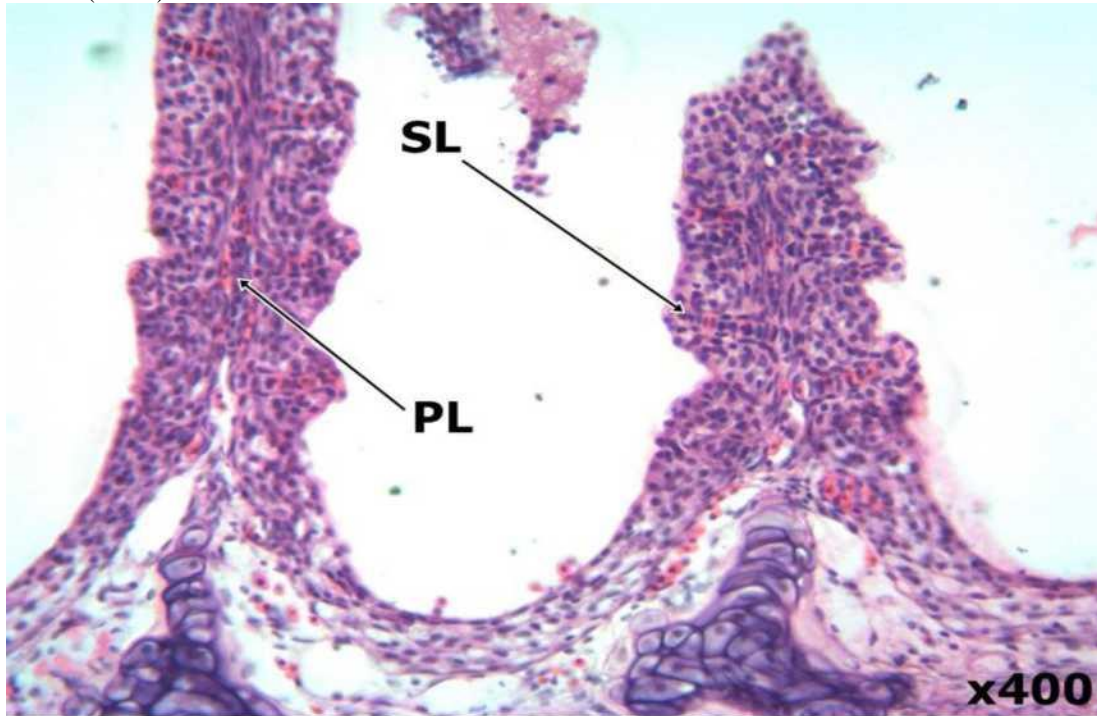


Plate 4: There is well-developed secondary lamella with mild epithelial hyperplasia and marked congestion of venous sinuses as well as moderate population of pillar cells and mucous cells.

**Plate 5 (2.0ml)**



Plate 5: Had a poorly developed secondary lamella with epithelial and hyperplasia and mild congestion of venous sinuses, conspicuous pillar cells and mucous cells Plate 6 (2.5)

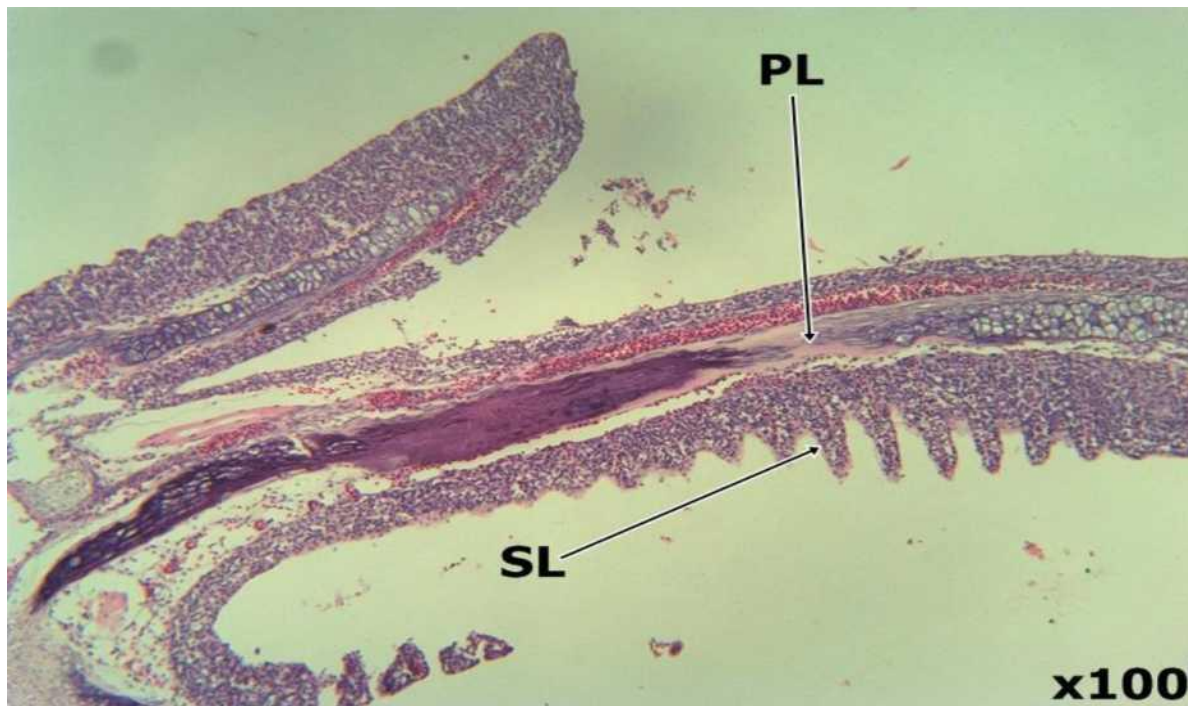


Plate 6: There was moderately prominent secondary lamella with epithelial hyperplasia and mild congestion of venous sinuses conspicuous pillar cells and mucous cells.

### Discussion

From the above result, table 1 which is the physicochemical parameters of the effluent recorded include: Temperature which was within the range of 25.0 -25.8 with the mean of 29.53. The thermometer was inserted into the effluent water until it got

stabilized. The dissolved oxygen content was within the range of 2.0 - 5.8 with the mean of 4.13, the pH of the effluent was within the range of 7.6 - 7.8 with the mean of 7.63, the electrical conductivity of the effluent was within the range of 6.0 - 80.2 with the mean of 67.3. The impact of high organic pollutants

on receiving waters includes creating high competition for oxygen within the ecosystem. (Osinbanjo and Adie, 2007).

From table 2, it shows the value of mortality observed during the exposure of *Clarias gariepinus* to brewery effluent. Within 24 hours of exposure, there was no mortality observed. At 48 hours mortality was observed in 1.5L and 2.0L and no mortality was observed in 0.5L, 1.0L and 2.5L. At 72 hours, mortality was observed in 1.0L, 1.5L, and 2.5L but in 0.5L, and 2.0L mortality was not observed. At 96 hours, mortality was only observed in 1.0L, 1.5L and 2.5L but no mortality in 0.5 and 2.0L.

Table 3 shows the percentage mortality values observed during the exposure of *Clarias gariepinus* to brewery effluent. At the end of the analysis the following percentages were observed and recorded; 0ml (0%), 0.5ml (0%), 1.0 (20%), 1.5 (30%), 2.0 (20%), 2.5 (40%) respectively.

In table 4, the weight of the fish was within the range 6.88 - 9.03g with the mean of 14.156g and the length was at the range of 10.00 - 11.2cm with mean of 20.84cm.

Table 5 shows their behavioural observation during exposure period to the effluent. The control showed no abnormal behaviour but the treatment which is the one the effluent was introduced showed some abnormal behaviour like, changes in skin texture at 48 hours, frequent surfacing, erratic movement and change in behaviour which was observed during 24 - 96 hours of exposure. The behaviour of the intoxicated fish was similar to that described by other authors. Ogeleka et al (2009) observed that *C.gariepinus* swims erratically prior to death. Samuel (2008) recorded coughing, hyperventilation followed by sporadic ventilation, twisting, loss of equilibrium, spiral swimming, convulsion and death.

After the exposure period was done, the gills of the fish was removed and taken to the laboratory for histology analysis to examine the effect it had on the fish.

In the control (a), photomicrographs, it shows fish gills primary lamella with prominent cartilaginous support afferent and efferent arterioles as well as intact epithelial covering. The secondary lamella is poorly developed and consist of epithelial cells mucous cells, pillar cells surround lacunae. The venous sinuses were filled with red blood cells.

In the treatment 1 (plate 1) when compared to the control, there is poorly developed secondary lamella with epithelial hyperplasia and mild congestion of venous sinuses, conspicuous pillar cells and mucous cells.

In treatment 2 (plate 2), there is poorly developed secondary lamella with epithelial hyperplasia and mild congestion of venous sinuses, conspicuous pillar cells and mucous cells compared to

the control.

In treatment 3 (plate 3), there is well developed secondary lamella with mild epithelial hyperplasia and marked congestion of venous sinuses as well as moderate population of pillar cells and mucous cells.

Treatment 4 (plate 4) had a poorly developed secondary lamella with epithelial and hyperplasia and mild congestion of venous sinuses, conspicuous pillar cells and mucous cells

In treatment 5 (plate 5), compared to the control, there was moderately prominent secondary lamella with epithelial hyperplasia and mild congestion of venous sinuses conspicuous pillar cells and mucous cells. Gills were studied histologically and some lamella deformation were observed. Gills hyperplasia and lamella fusion were also reported. Mallatt (1985) reported that the pathological changes of fish gills are induced by elevated heavy metals and low oxygen content in water. Wedemeyer (1996) reported that fishes are more susceptible to stress than many other animal because of their intimate dependence upon their surrounding environment.

These reaction to the effluent were most pronounced in tanks containing the highest concentration (2.5 l). This conformed with the submission of Donalson and Dye (1975), who are of the opinion that fish exposed to low concentration of toxicant do not reach the stage of exhaustion, rather they quickly become adapted to the stressor.

Histopathology is widely accepted as a useful method for the assessment of injury in fish to the adverse short term and chronic effect of industrial effluents. Brewery effluent toxicity test was carried out to determine the actual impact of effluent on organisms. Several studies reported acute effect of brewery effluent on various other fish species. example *O. niloticus* by Sarotherodon spp by Wong (1989). It's indicated that *Clarias gariepinus* found in effluent - contaminated area are sensitive to toxic substances by Adewuyi et al (2010). The impact of high organic pollutants on receiving waters includes creating high competition for oxygen within the ecosystem. (Osinbanjo and Adie, 2007).

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