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TOXIC EFFECT OF BREWERY EFFLUENT ON FINGERLINGS OF *CLARIAS GARIEPINUS*

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ABSTRACT

Background. The indiscriminate discharge of waste-water (effluent) as influenced by industrialization and development create significant pollution problems to normal flora and fauna of the ecosystem. Toxicity of brewery effluent remain unknown, particularly its implications on histopathology of fish under continous exposure. The study unravels the effects of brewery effluent on fingerlings of *Clarias gariepinus* under laboratory conditions.

Materials and Methods. Fingerlings of *Clarias gariepinus* (of mixed sex) (5.20±0.42g) were exposed to various concentrations of brewery effluent in five treatment (in duplicate) 15, 20, 25, 30, 35% and 0.00% for control The Toxicity and histopathology of the gills and liver of fish exposed were observed under laboratory conditions.

Result. Acute concentrations had adverse effects on mortality and oxygen consumption. The 96-h LC_{50} of brewery effluent was 23.99ml/l with upper and lower confidence limits of 29.478ml/l and 18.324ml/l respectively. Symptoms of toxicity include restlessness, air gulping, loss of equilibrium, exhaustion and death. Exposure of the *Clarias gariepinus* fingerlings to various lethal concentrations of the brewery effluent were implicated in the histopathological examinations of gills which showed mutilations of gill filament, Oedema, fusion of central venous sinus, swelling, necrosis of various degrees and total lamellar hyperplasia. The liver showed evidence of necrosis and vacuolation of liver cells whereas control group showed both normal gill and liver section.

Conclusion. The indiscriminate or deliberate discharge of industrial effluent (brewery effluent) directly into the aquatic environment without any form of treatment is a common sight in many industrial cities of Nigeria today; therefore, creating awareness for environmental protection through sustainable production processes is of utmost significant.

Keywords: *Toxicity; brewery effluent; fingerlings; Clarias gariepinus.*

INTRODUCTION

Rapid industrialization in Nigeria and the world over has resulted in the substantial increase in the liquid waste (spent wash or effluent), which is traditionally discharged into open land, lakes, streams, ponds or into nearby natural water, causing a number of environmental problems including threat to plants and animal lives and also creating problems such as surface water logging and ground water contamination[1].

The adverse input of diverse industrial wastes has aggravated the problem of contamination, and sewage and industrial disposal has greatly enhanced the addition of heavy metals into the aquatic ecosystem. These pollutants caused the alteration of the natural condition of aquatic medium that brings about behavioural changes as well as morphological imbalance of aquatic organisms[2]. It is worthy to note that it is through industrial revolution and technological advancement that the progress of a nation may be achieved but these activities brings along with it growing problems of waste management and disposal which pose serious threats not only to aquatic organisms but human health risks[3, 4, 5] hence, industrial process and environmental pollution could be complimentary rather than mutually exclusive[6].

Further report (Anonymous)[7] revealed that contamination of lakes, streams, rivers and other water bodies resulting from discharge of toxic liquid waste is one of the problems that have not yet been overcome in both industrialized and developing countries. Often times this waste water (Effluent) are discharged directly without any form of treatment into aquatic ecosystem causing change in the physico-chemical properties of this effluent receiving water. For this reason, surviving

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fishes and other aquatic lives are faced with physiological problems associated with oxygen depletion[8],[9]. Gills represent major site for respiration; they are always in contact with water, which makes them not only important targets for water pollutants but also as efficient indicators of water quality. Essentially, any change in water quality is rapidly reflected in fish gill structure and function, since gills are continuously exposed to ambient water. They are the primary sites of gas exchange, acid-base regulation and ion transfer[10].

Studies of effects of industrial effluent on aquatic organisms, including fish as reported[11] are generally concerned with findings on fish survival. [12] Unsanitary habits of depositing industrial wastes water into the aquatic systems have devastating effects and as such fish and other aquatic life exposed to this effluent receiving Streams, Rivers, ponds and lakes are posing respiratory problems to aquatic life. The respiratory disorder includes gill mutilations, Oedma, fusion of central venous sinus, sloughing of the secondary lamella and haemorrhage as main damages observed on the fish gills in polluted water environment[13], [14]. The liver of fish can be considered a target organ to pollutants, alterations in its structure can be significant in the evaluation of fish health[15], and exhibit the effects of a variety of environmental pollutants[16].

The objective of this paper is to provide information on toxic effect of brewery effluent to fingerlings of *Clarias gariepinus* under laboratory condition. This is vital to aqua-culturist, fish biologist and ecologist for predicting possible effects of effluent in the field.

MATERIALS AND METHODS

Collection and acclimation of experimental fish

This study was conducted at the University of Jos Fisheries Research Unit. Fingerlings (Mixed Sex) of *Clarias gariepinus*, mean weight ± SE (5.20±0.42g), were collected from Renajj integrated fish farm, Jos Plateau State Nigeria. They were transported to University of Jos Fisheries Research Laboratory in well aerated oxygen bags. The fish were held in glass tanks and acclimated to laboratory conditions for a period of two (2) weeks during which the fish was fed on commercial diet (Coppen feeds).

The Test Media

The brewery effluent was collected in 40-litre plastic containers from the discharge point at Jos International Brewery (JIB) factory after which an initial trial experiment was set up and monitored for three consecutive days using the biological test animal, *Clarias gariepinus* to verify their viability in brewery effluent to reduce the concentrations to be used and approximate the range producing the various responses[17].

Experimental Design

The five concentrations were 15, 20, 25, 30 and 35%. The control was an experimental medium with no brewery effluent in it (0.00%). The toxicity of test was conducted by placing randomly ten (10) fish *Clarias gariepinus* in each of the twelve circular plastic tanks 60x30x30cm) capacity were used, as each concentrations were in duplicates. The test solutions (brewery effluent) containing various percentages of the effluent by volume were prepared using de-chlorinated tap water as reported[17]. Feeding ceased 48 hrs before exposure to acute toxicity bioassay media and test solution was renewed every 24 hrs and dead fish removed to avoid additional pollution. The physico-chemical analysis of the test water was performed using standard laboratory methods as reported[18].

Histopathological examinations of the fish gills and liver after exposure period were done using reported methods[19]. The estimation of 96 hrs LC_{50}, upper and lower confidence limits as recommended[20] was used as methods for acute toxicity test. The results from the study were subjected to statistical analysis using analysis of variance (ANOVA) to test the level of significance.

RESULTS

The results of physico-chemical parameters of the test media are presented in Table 1. No mortality was recorded in the control group. The mean values of physico-chemical parameters indicate a significant reduction in the values of dissolve oxygen content compared to the control. PH and alkalinity increased as concentration increases however dissolve oxygen and free carbon dioxide values opposes this trend; there were no significant difference ($p>0.05$) between the values of temperature and the control. The mean mortality values are presented in Table 2. The logarithmic-probability curves of the mean mortality rates are presented in Figure 1.

***Table 1**. Mean water quality parameters measured during 96-h exposure of brewery effluent to fingerlings Clarias gariepinus.*

Parameters	Effluent Concentrations (%)					
	15	20	25	30	35	0.00
Temperature (°C)	21.75±0.43	21.75±0.43	21.75±0.46	21.75±0.43	21.75±0.43	21.75±0.43
Dissolved Oxygen (mg/L	4.83±0.19	4.50±0.14	4.28±0.14	4.13±0.13	3.59±0.13	6.69±0.37
pH	6.95±0.17	6.74±0.15	6.88±0.17	7.00±0.22	7.21±0.33	6.54±0.09
FCO2 (mg/L)	7.33±0.57	7.63±0.60	6.50±0.46	5.33±1.19	4.50±1.72	4.36±0.13
Alkalinity (mg/L)	64.50 ±1.50	77.00±1.77	88.83±2.19	100.33±2.96	113.13±2.91	26.50±1.80

*FCO_2 Free carbondioxie

The mortality rate of the fish increased with increase in the concentrations of the brewery effluent. The symptoms of toxicity revealed that the effects of brewery effluent altered the behavior of the fish before death. The symptoms ranges from restlessness, irritated swimming, loss of balance, air gulping, exhaustion and death. The toxic action of the brewery effluent appeared

to be a combination of mucus precipitation and osmoregulatory stress leading to death from suffocation. The traces of blood observed around the gill cover suggest that the fish might have suffered from gill haemorhage. The mean value of 96-h LC_{50} of brewery effluent was calculated to be 23.99ml/L with upper and lower confidence limits of 29.478ml/L and 18.324ml/L respectively.

***Table 2**: Mean mortality rates of Clarias gariepinus exposed to various acute concentrations of brewery effluent*

Conc. (ml/l)	Log Conc. (ml/l)	Mortality rates (h) 24	48	72	96	Mean mortality (%)	Mean probit mortality
35	1.5441	3.5	2.0	1.5	0.5	75	5.6745
30	1.4771	3.5	2.0	1.0	0.0	65	5.3853
25	1.3979	1.5	2.0	1.0	1.0	55	5.1257
20	1.3010	1.0	1.0	1.5	0.0	35	4.6147
15	1.1761	0.5	0.5	0.5	0.0	15	3.9636
0.0	0.0000	0.0	0.0	0.0	0.0	00	0.0000

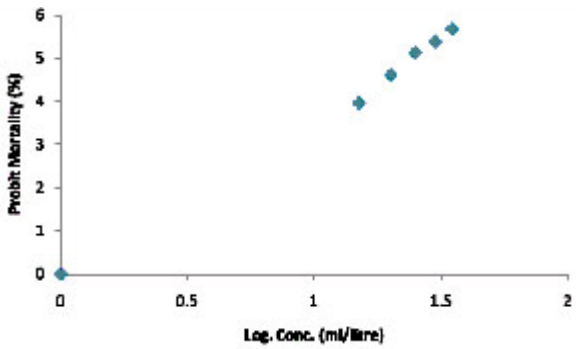


***Fig.1:** Linear relationship between probit mortality against log_{10} Concentration of brewery effluent on Clarias gariepinus fingerlings for 96 h under laboratory conditions.*

Clarias gariepinus fingerlings exposed to various concentrations of brewery effluent showed both severed liver and gill histopathological alterations. Histopathological studies revealed that in control fish, primary lamellae appeared normal and mucus free with well-defined secondary lamellae branched from them (Fig. 2). In general the common modifications was the displacement of the epithelial layer of the secondary lamellae but across all experimental stocks the observed pathological lesions in Figs. 3 and 4 were mutilation i.e total breakage of the secondary lamellae from the basement cells an indication of cellular necrosis and cellular infiltration of the secondary lamellae were prominent. Apart from swelling at the tip of secondary lamellae, degenerative changes of epithelial cells and fused lamellar filaments as noticed in Fig. 4-6 show a complete damage of epithelial cells of both primary and secondary lamellae, an indication of lamellar hyperplasia. Congestion of blood space is evident in Fig 6 however, both shows sign of epithelial thickening of both the lamellae and the gill raker. Lastly, the club shaped deformation at the tip of the secondary lamellae is an indication of Oedematous condition as noticed in Figs. 5 and 6.

Fig. 2 **Fig. 3**

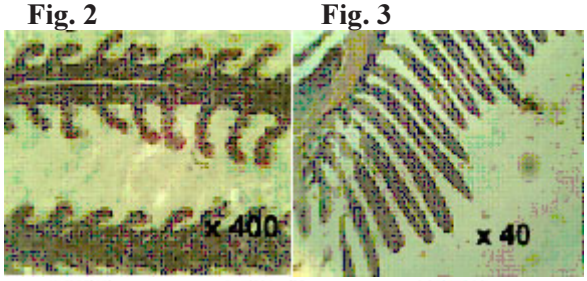
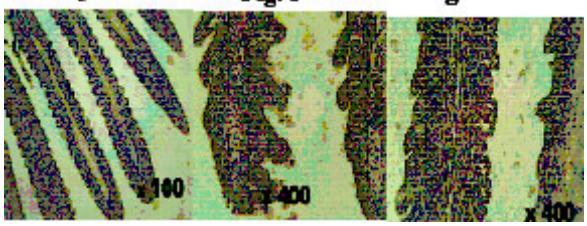


Fig. 4 **Fig. 5** **Fig. 6**



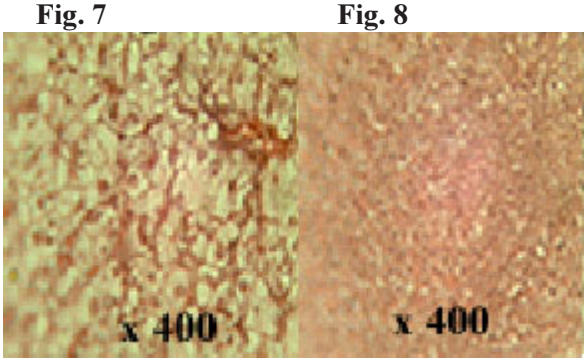
Histopathological section of the gill of Clarias gariepinus exposed to acute concentration of brewery effluent (bf) under laboratory conditions for 96 h;

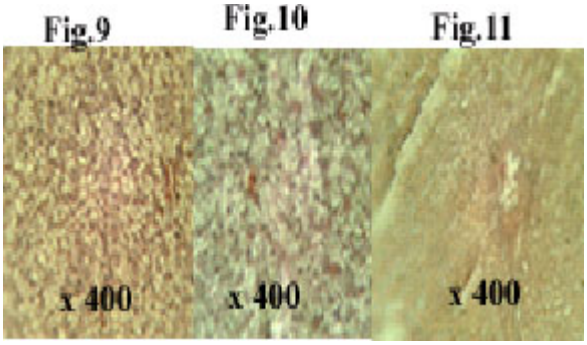
Fig. 2 is the control showing normal gill section with a clear central venous sinus (**CVS**);

Fig. 3-6. Exposure to **20%, 25%, 30% and 35%** respectively, brewery effluent concentration showing various histological anomalies which ranges from Lamellar hyperplasia **(LH)**, Mutilation of secondary lamellae (**MSL**), Congestion of blood space **(CBS)**, infiltration of the secondary lamellae **(IL)**, epithelial thickening of both the lamellae and gill raker, club shaped deformation at the tip of the secondary lamella which is an indication of Oedematous condition **(LO)**,

The control experiment, Fig.7 showed a typical network of hepatocyte with normal staining patterns of the cell. Figs. 8 and 9 revealed varied degree of infiltration of hepatocytes but more pronounced in Fig. 9, in addition necrosis was evident coupled with severe congestion of central vein. Occurrence of cellular hypertrophy and hepatic infiltration of the hepatocytes became more evident in Figs. 10 and 11. Though hepatic necrosis and vacuolation is more pronounced in Fig. 11 coupled with Oedematous condition in both cases.

Fig. 7 **Fig. 8**





Histopathological section of the liver of Clarias gariepinus exposed to acute concentration of brewery effluent (bf) under laboratory conditions for 96 h;

Fig. 7 is the control showing normal liver section of *Clarias gariepinus* showing hepatocytes.

Fig. 8-11. Exposure to **20%, 25%, 30% and 35%** respectively, brewery effluent concentration showing various histological anomalies which includes a pronounced infiltration of hepatocytes (**IH**), cellular hypertrophy (**CH**), hepatic necrosis (**HN**), vacuolation (**V**) and Oedema (**O**).

DISCUSSION

In our study of fingerlings of *Claris gariepinus* exposed to various concentration of brewery effluent showed high sensitivity to the effluent under laboratory conditions. The mean value of 96-h LC_{50} of brewery effluent was 23.99ml/L with upper and lower confidence limits of 29.478ml/L and 18.324ml/L respectively. The mortality increased with increasing concentrations of brewery effluent, a reflection of what[21] reported as regards all categories of toxicant; a threshold is reached at which there is no drastic survival of organisms and that organisms lie within a tolerable zone only below the threshold, but above the tolerance zone are the zone of resistance.

Different LC_{50} values have been reported for different fish species exposed to various types of toxicants hence this variation in the LC_{50} could be attributed to the size, age of fish and nature of the effluent as earlier reported by[14,13,11,8,22,23]. The irregular respiration evident in the exposed fish could have been caused by increased activities of the mucus cells resulting to precipitation and accumulation on the gill epithelia which resulted in osmoregulatory stress causing abnormal behaviour that eventually lead to death from suffocation as documented[24,25]. The traces of blood observed on the dead fish suggest that the fish suffers from gills haemorrhage as described[26]. In this study, the amount of dissolved oxygen (DO) available to exposed fish is less than that of the control group hence exposed fish consumed significantly less oxygen compared to the control. The susceptibility of *Clarias gariepinus* fingerlings exposed to brewery effluent resulted in the degeneration of the gill lamellae which lead to the disruption of the entire respiratory process with far-reaching consequences for oxygen up-take by the fish as reported by[27,5]. The condition observed led to a decrease in the entire respiratory process (gaseous exchange) between the gill and its environment. The whole essence of toxicological research is to evaluate the environmental risk or impact toxicants may have on the ecosystem which provide basis for protection and maintenance of ecosystem exposed to contamination. Fish generally, are excellent bio-indicator of the aquatic environment[13]. It may be noted that they are extremely sensitive to pollution.

Histological investigation of the liver tissues showed a normal structural organization of the parenchymatous cell appearance of the hepatocytes in the control fishes. However in this present study, the histological abnormalities observed were oedema, cellular infiltration, congestion of central vein, vacuolation and cellular necrosis, all of which will compromise the fish performance. This is in agreement with ealier data[28] who reported that teleost accumulated lead (Pb) both directly from diet and indirectly from the aqueous medium through an active food chain by the surface lamellae. The most generally encountered type of degenerative changes was congestion, vacuolization of hepatocyte, cellular infiltration and focal necrosis.

The liver of the exposed organisms revealed slight vacuolated cells which is an indication of fatty degeneration of hepatocytes. Cellular necrosis as observed in this work probably resulted from excessive work required by the fish to get rid of the toxicants from its body during the process of detoxification; a situation that conforms to earlier report[29]. Therefore, necrosis became evident as the concentration increases and this may be due to the inability of fishes to regenerate new liver cells. It was also observed that the histopathological changes in the liver caused metabolic problems. The histological changes observed in this study indicate that the fish were responding to the effects of the contaminants as such these information confirm that histopathological alterations are good biomarkers for both field and laboratory assessment as documented earlier [30, 31].

CONCLUSION

This study has been able to establish the fact that, exposure of *Clarias gariepinus* fingerlings to brewery effluent can induce various toxicological effects and histological degradation. In view of the toxicity effect of this effluent, it can be inferred that, indiscriminate discharge of brewery effluents can induce damages to the tissue and organ, which might make all the living organisms in polluted environment vulnerable to diseases, and eventually leads to death. Therefore, with rapid industrial revolution and the global need for humanity, brewery effluent remains not only a major pollution threats to aquatic ecosystem but also pose a risk to human health. It is imperative therefore to employ

a sustainable production process where industrial development and environmental protection could be complementary rather than mutually exclusive as such a balance is struck between economic development and environmental protection through an excellent pollution management programme.

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