PRINT ISSN 1119-8362
Electronic ISSN 1119-8362

Full-text Available Online at
https://www.ajol.info/index.php/jasem
http://ww.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 25 (6) 1081-1085 June 2021

Toxicity of Brewery Effluents (Burukutu Effluent) to *Clarias gariepinus* and its Effect on some Target Organs

***ARIYOMO, TO; AKINSOROTAN, AM; NWACHUKWU, EN**

Department of Fisheries and Aquaculture, Federal University Oye-Ekiti, Ekiti State, Nigeria
**Corresponding Author Email: tolulope.ariyomo@fuoye.edu.ng; Other Authors Email: ademola.akinsorotan@fuoye.edu.ng; nkechinwachukwu01@gmail.com*

ABSTRACT: A 96-hour bioassay was conducted to determine the toxicity of brewery effluent (Burukutu effluent) to *Clarias gariepinus* juveniles. The fish were exposed to 4 varying concentrations 154, 231, 308, 385 ml/L and a control, 0ml/L. Physiochemical parameters (pH, temperature and dissolved oxygen) of treated water during the study differed significantly from the control ($p<0.05$). The values of RBC, PCV, Hg reduced as the concentration increased ($p<0.05$) while the WBC increased ($p<0.05$) as the concentration increased and the 96h LC_{50} of 646ml/L was obtained. *Clarias gariepinus* juveniles exposed to brewery effluent showed behavioural changes such as erratic swimming, loss of equilibrium, gasping for air, loss of reflex, discolouration, and it led to the death of some fish. The histopathological changes of the gills of fish exposed to brewery effluent for 96 hours at different concentrations showed swollen and shortened lamella, epithelial lifting, lamellar degeneration and loss, swollen lamellae with focal epithelial sloughing. The histology of the liver of the fish exposed to varying concentrations showed pigmented macrophages, vacuolation showing the signs of hepatocyte degeneration, fatty changes and pigmented macrophages. The data obtained from this investigation showed that brewery effluent caused stress-inducing effects on *Clarias gariepinus* during the exposure period.

DOI: https://dx.doi.org/10.4314/jasem.v25i6.29

Dates: Received: 20 March 2021; Revised: 27 April 2021; Accepted: 07 May 2021

Keywords: Bioassay, Beer, *Clarias gariepinus*, histology, haematology

Beer is the world's oldest and most widely consumed alcoholic beverage and the third most popular drink after water and tea (Evans, 2011). Brewing beer involves steeping a starch source, commonly cereal grains in water and fermenting the resulting sweet liquid with yeast (Evans, 2011). Burukutu is an alcoholic beverage drink. It has a characteristic sour taste due to the presence of lactic acid, a pH that ranges between 3.3 and 3.5 and an opaque colour because of suspended solids and yeast. It contains vitamins, iron, manganese, magnesium, potassium and calcium. It is made up of about 26.7g of starch and 5.9g of protein per litre (Kayode et al., 2005).

Burukutu is produced in numerous local alcoholic beverage brewing plants in Nigeria and the effluents generated during production are emptied into the surrounding waters and land. These brewery plants have been known to cause pollution by discharging effluent into receiving streams, groundwater and soil water (Kebede, 2018). The resultant effects include stench, discolouration and a greasy oily nature of such water bodies as well as the aquatic organisms staying in the water. A range of alterations related to physiological abnormalities has also been observed in fish living in water bodies receiving high discharges of effluent from these industries (Adeboyejo et al., 2012). This study, therefore, is focused on evaluating the acute toxicity of the burukutu effluent on some haematological parameters and the histopathology of the gills and liver cells of *Claris gariepinus*.

MATERIALS AND METHODS

Source of Experimental Fish and Effluent: A total of 200 apparently healthy *Clarias gariepinus* juveniles of mean weight and mean total length 12.5g and 11.5cm respectively were acquired from a reputable farm in Ado Ekiti. The fish were acclimatized in the laboratory of the Department of Fisheries and Aquaculture for 14 days. Commercial feed (35 % CP) was used to feed them twice daily. The brewery effluent used was gotten from Odogbo barracks Oyo State.

Experimental Protocol: To determine the LC_{50} value of burukutu effluent on *Clarias gariepinus* juveniles, a range-finding test was carried out using 100 juveniles following the procedure described by Akinsorotan (2015). The animal ethic guidelines of the Federal University, Oye Ekiti was adhered to. The range finding test lasted for 96 hours. Feeding was stopped 24 hours before the definitive test in order to reduce water pollution that could result from faecal

droppings decomposition and also to reduce the stress that could arise from feeding to the fish. Definitive concentrations of 154 ml/L, 231 ml/L, 308 ml/L and 385 ml/L of the effluent were measured and introduced into experimental tanks filled with 13 litres of water. There was a control experiment without the effluent concentration too. The tests were carried out in duplicates.

Water quality analysis: During the definitive test, water temperature, dissolved oxygen (DO), and pH were measured at 24hrs intervals, using standard methods. Mortality was also recorded daily.

Behavioural Observation: The behavioural and morphological responses of the fish were observed after exposure of the fish to the various concentrations of the effluent.

Haematology: Blood samples were collected from fishes across the treatments and control by caudal puncture into heparinised syringes already treated with Ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. Packed cell volume (PCV), haemoglobin concentration (Hb), red blood cells, white blood cells and the counts were estimated using methods described by Svobodova *et al.*, (1991).

Histological Analysis: Gills and liver of *C. gariepinus* were excised upon dissecting the fish. To prevent deterioration, fixation of the excised organs was done in 10% formalin. The fixed organs were dehydrated in 50%, 70%, 90%, and 100% levels of alcohol. The dehydrated organs were cleared in a mixture of alcohol and xylene in an equal ratio for three hours. The specimens were embedded in molten wax and sectioned with the aid of a microtone to thin sections using a microtone and then stained in haematoxylin and eosin. The stained specimens were observed under a light microscope and interpreted accordingly.

Statistical Analysis: Data collected on haematological characters of *C. garipienus* were subjected to the one-way analysis of variance (AN0VA) test. Some of the data collected during the course of the experiment were further subjected to Statistical analysis using the regression routine of SPSS (Statistical Package for Social Sciences) version 16. Data collected on mortality was subjected to Probit transformation method and the LC_{50} value was determined accordingly.

RESULTS AND DISCUSSION

Physicochemical parameters of water with varying concentrations of brewery effluent during 96 hour period: The water parameters monitored were temperature, dissolved oxygen, pH. These parameters differed significantly from the control. Temperature and dissolved oxygen decreased with increasing concentration ($p<0.05$). However, pH increased with increasing concentration ($p<0.05$). The mortality of *C. gariepinus* was observed in all concentrations (154, 231, 308, 385ml/L) of brewery effluent used except in the control. Mortality varied significantly amongst the different concentrations. The LC_{50} obtained using Probit transformation was 646 ml/L (Fig. 1). *Clarias gariepinus* exhibited distress behavioural responses due to the effects of the brewery effluent. The observed behavioural responses were erratic swimming, occasional gasping for breath and frequent surfacing which increased as the concentration increased. The LC_{50} obtained using Probit transformation for fish exposed to brewery effluent was 646 ml/L (Fig. 1).

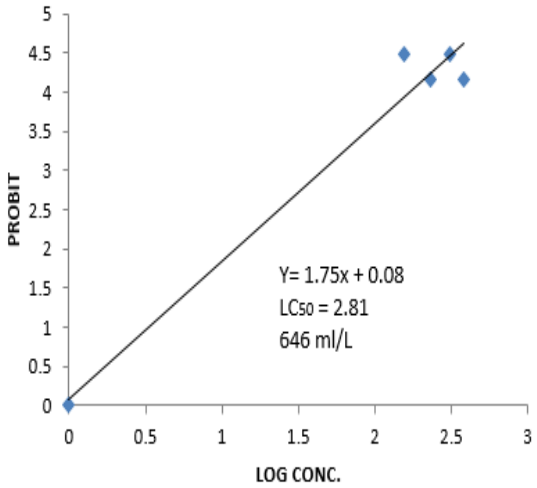

Fig. 1: Mortality (96h – LC_{50}) of *Claris gariepinus* in different concentrations of Buruku effluent

Haematological result of Clarias gariepinus exposed to varying concentrations of brewery effluent: The value of the packed cell volume (PCV) in fish not exposed to brewery effluent was the highest (39.00 ± 0.00). The lowest PCV (29.75 ± 0.35) was recorded in test fish exposed to 385 ml/L of brewery effluents and it was significantly different ($p<0.05$) from other concentrations. The value of Red blood cells (RBC) in the control fish (3.15 ± 0.07) was significantly different ($p<0.05$) from fish treated with 308 ml/L (1.85 ± 0.07) and 385 ml/L (1.60 ± 0.00) of brewery effluent respectively. In the treatments containing brewery effluents, the highest RBC (2.80 ± 0.14) was recorded in fish exposed to 154 ml/L while the lowest (1.60 ± 0.00) was recorded in the fish exposed to 385 ml/L of brewery effluent. Of all the treatments containing brewery effluents, the lowest WBC (7.60 ± 0.14) was recorded in catfish exposed to 154 ml/L of

the effluent, this was however different ($p<0.05$) from WBC recorded in fish exposed to 385 ml/L (9.60 ± 0.14). However, Hb was highest in the control and lowest in 385 ml/L (9.90 ± 0.14). Blood count differentials showed that neutrophils were significantly different ($p<0.05$) in all the fish exposed to the varying concentrations of brewery effluent. The highest neutrophils were recorded in 385 ml/L (92.50 ± 0.70) while the lowest was recorded in the control (65.00 ± 0.00). Lymphocytes equally showed significant differences ($p<0.05$) in blood samples collected from the varying concentrations with the lowest (5.50 ± 1.72) recorded in the 385 ml/L concentration while blood Lymphocytes were highest (20.00 ± 0.00) in *Clarias gariepinus* in the control.

Histopathological changes observed in the gills and liver of C. gariepinus exposed to varying concentrations of brewery effluent: The gills and liver of the fish were examined to assess the histological effect of brewery effluent on them. Examination of gills and livers of fish in different concentrations showed varying degrees of damage to the tissues (Table 1). Examination of the gills of fish in the control revealed that there was no visible change in the gill structure of *C. gariepinus* on gross examination, no visible mucus or lesions. Microscopic examination of the gills of the untreated container revealed a normal parallel arrangement of the gill filament consisting of primary lamellae and other arrays of delicate secondary lamellae with epithelial cells intact. There was slight congestion of the gills, swollen and shortened lamella and epithelial lifting in 154 ml/L concentration. There were swollen lamellae with focal epithelial sloughing in 231 ml/L concentration. However, at higher concentrations, 308 ml/L and 385 ml/L respectively, there was high level of degeneration in the filament, and fragmentation of the lamella, and there was vacuolated of the filaments, there was mucus lining on the gills, particularly in higher concentrations, erosion of the gills and they showed the sign of necrosis (the cells were already dying because of too little oxygen reaching the cells via the blood). Histological studies revealed that microscopic examination of the controlled experiment for *C. gariepinus* showed normal liver appearance, normal hepatocytes morphology including minimal vacuolation, lipid and glycogen storage, and normal arrangement of the hepatic cord. The organs were intact and had no visible gross lesions. The liver of fish in 154 ml/L & 231 ml/L showed slight hydropic degeneration, and that at high concentrations (308 ml/L & 385 ml/L), the liver showed appreciable cellular changes with large space information and vacuoles in the tissues. The cellular arrangement of liver cells was distorted; lesions were also present on the tissues of the liver. The results obtained from the experiments indicate that brewery effluent had a direct impact on the tissues of the gills and livers *Clarias gariepinus*.

Tables 1: Histopathological changes observed in the gills and liver of *C. gariepinus* exposed to varying concentrations of brewery effluent

Conc. (ml/L)	Organs	Histopathological changes observed
0.00	Gills	Normal gill lamella and epithelium
154	Gills	Swollen and shortened lamella, epithelial lifting
231	Gills	Swollen lamellae with focal epithelial sloughing
308	Gills	Swollen lamellae with localized fusion
385	Gills	Signs of lamellae, degeneration and loss
0.00	Liver	Normal portal triad and central vein with mild fatty changes
154	Liver	Slight degeneration, and mild fatty change
231	Liver	Periportal lymphocytic infiltration with fatty changes
308	Liver	Pigmented macrophages, vacuolation and fatty changes.
385	Liver	Hepatocyte degeneration, fatty changes and pigmented macrophages

The values of the physicochemical parameters of the control and the other concentrations indicated that the brewery effluent had a negative impact on water quality. Mortality of the *C. gariepinus* increased with an increase in the various concentrations of the brewery effluent. The mortality of fish could have resulted from changes in the water quality given that mortality increased with an increase in pH. Values of pH increased during the exposure period and layers of mucus were seen on the gills, particularly on the gills of fish in the higher concentrations, this could have caused a reduction in oxygen intake and resulted in the death of fish (Ariyomo et al., 2017). The rapid opercula movement and rapid release of bubbles in fish of higher concentration of effluent could be attributed to stress caused by the reduction in dissolved oxygen level. Enujiugha and Nwanna (2004) reported that reduction of oxygen levels can adversely affect fish life and as such lead to mortality of the fish. Juveniles exposed to different concentrations of brewery effluents moved more rapidly, lost equilibrium in water and began to show sideways swimming. Some attempted to jump out of the contaminated water. The mouth and the gills of fish were gaping. These behaviours are similar to those described by Ogeleka et al., (2009) and Samuel et al., (2008) by fishes used in toxicity studies. The erratic

behaviour of the fish in this study showed respiratory impairment, and this may be a result of the effect of the brewery effluent on the gills, this is in agreement with the opinion in other studies which showed that at increased lethal concentrations, the behavioural responses of the test organisms greatly increased and the organisms later became inactive and this is a normal situation in acute and sub-acute toxicity test (Ayoola, 2008; Ogundiran, et al., 2009). Hyperactivities observed in this study are probably due to the disturbances in the metabolic state resulting in the depletion of energy. It is possible that animals that have higher metabolic activities could require higher level of oxygen and thus would embark on higher respiratory activities (Canli and Kargin, 1995). Lethargies and loss of equilibrium observed in this study may be due to depletion of energy in the body of the exposed animals (Anderson et al., 1988). The reduction in PVC, Hb and RBC of fish exposed to the burukutu effluent may be as a result of haemolysis and shrinkage of RBC by the effluent leading to a decrease in hematocrit value which indicates fish anaemia. This aligns with findings by Mohammod Mostakim et al., (2015) during a 28 days chronic exposure of silver barb, *Barbonymus gonionotus,* to Quinalphos. Increase in the WBC count and neutrophils (but not lymphocytes) with increasing concentration of burukutu effluent recorded in this study could be due to an attempt by the fishes to fight against the antigens (pollutants) which led to the production of more antibodies (WBC) to defend the fish against the toxicant (Masud & Singh, 2013). This indicates that the fish is capable of synthesizing antibodies to defend itself against the entry of a toxicant. In other studies, there was a decrease in neutrophil counts but an increased number of lymphocytes when freshwater fish, *Channa punctatus,* was exposed to monocrotophos (Agrahari et al., 2006), which agrees with the present study.

Varying pathological changes were observed at different concentrations. There was an increase in mucus secretion in the gills and on the body surface, haemorrhage in the gills and discolouration in the eye and on the skin which were visibly obvious in the fish in the higher concentrations. Histology of the gills revealed varying degrees of lamella deformations, gill hyperplasia and lamella fusion with increasing concentration. This is in line with the work of Adeboyejo et al., (2012) in *C. gariepinus* exposed to industrial effluents. The liver of the exposed organisms revealed varying levels of vacuolated cells with increasing concentration, which indicates fatty degeneration of hepatocytes. Furthermore, there were distortions in the arrangements of the livers cell and cellular necrosis, severity also varied with increasing concentration of the effluent. The observed distortions in the arrangements of the livers cell and cellular necrosis may have resulted from the pressure of the detoxification process exerted on the liver to produce new liver cells (Ezemonye and Ogbomida, 2010). It is evident from this study that increasing concentrations of the brewery effluent when present in any water body could lead to abnormal behavioural responses and dysfunction in fish health and general condition. Hence, adequate preventive measures must be taken to ensure the indiscriminate channeling of this effluent into our water bodies having in mind that man is the ultimate target of any negative consequences resulting from such acts.

Acknowledgements: The authors are thankful to the Department of Fisheries and Aquaculture, Federal University, Oye Ekiti where the experiments were conducted. We will also like to appreciate Jeremiah Jimoh for comments on the earlier draft of the manuscript.

REFERENCES

Adeboyejo, OA; Fagbenro, OA; Adeparusi, EO; Clarke, EO (2012). *Acute toxicity of industrial effluents from Agbara environs of Ologe Lagoon on early life stages of African catfish Clarias gariepinus.* In: 27th Annual Conference and Biennial General Meeting of the Fisheries Society of Nigeria (FISON), 25-30 Nov 2012, Bayelsa, Nigeria, pp. 257-264

Agrahari, S; Pandey, KC; Gopal, K (2006). Effect of monocrotophos on erythropoietic activity and hematological parameters of the freshwater fish, *Channa punctatus* (Bloch). *Bull. Environ. Contam. Toxicol.* 76: 607-613

Akinsorotan, AM (2015). Histological Studies of African catfish (*Clarias gariepinus*) exposed to varying concentrations of Dizensate (Glyphosate: N-phosphonomethyl glycine). PhD Thesis. Delta State University, Abraka, Nigeria. 132p

Anderson, T; Forlin, L; Hardig, J; Larsson, A (1988). Physiological disturbances in fish living in coastal water polluted with bleached kraft pulp mill effluents. *Can. J. Fish. Aquat. Sci*. 4: 1525-1536.

Ariyomo, TO; Jegede, T; Adeniran, AF; Omobepade, BP (2017). Toxicity of Cassava Effluents to Catfish, Clarias gariepinus, and the Effect on Some Target Organs. *Ann. Aquac. Res.* 4(2): 1034.

Ayoola, SO (2008). Histopathological Effects of Glyphosate on juvenile African Catfish (*Clarias*

gariepinus). *Am. Eurasian. J. Agric. Environ. Sci.* 4 (3): 362-367.

Canli, M; Kargin, F (1995). A comparative study on heavy metals (Cu, Cr, Pb and Ni) accumulates in the tissue of carp, *Cyprinus carpio*, and Nile fish, *Tilapia* niloticus. *J. Zool.* 19: 165-171.

Enujiugha, VN; Nwanna, LC (2004). Aquatic oil pollution impact indicators. *J. Appl. Sci. Environ. Manage.* 8 (2): 71-75

Evan, E. (2011). The Oxford Companion to Beer. Oxford University Press, 960 pages.

Ezemonye, L; Ogbomida, TE (2010) Histopathological Effects of Gammalin 20 on African Catfish (*Clarias gariepinus*). *Appl. Environ. Soil. Sci.* Article ID 138019, 8

Kayode, APP; Agbidi, A; Linnemenn, AR; Nout, MJR; Hounhouigana, JD (2005). Quality of farmer's varieties of sorghum and derived foods as perceived by consumers in Benin. *Ecol. Food. Nutr*. 44: 271-294.

Kebede, TB (2018). Wastewater treatment in brewery industry, Review. *Int. J. Eng. Dev. Res.* 6(1): 716-722.

Mohammod Mostakim, G; Zahangir, MM; Monir Mishu, M; Rahman, MK; Islam, MS (2015). Alteration of Blood Parameters and Histoarchitecture of Liver and Kidney of Silver Barb after Chronic Exposure to Quinalphos. *J. Toxicol.* 2015:415984

Ogeleka, DF; Ezemonye, LIN; Okieimen, FE (2009). Sublethal effects of industrial chemicals on fish fingerlings (*Tilapia guineensis*). *Afr. J. Biotechnol.* 9 (12): 1839-1843.

Ogundiran, MA; Fawole, OO; Adewoya, SO; Ayandiran, TA (2009). Pathologic lesions in the gills of *Clarias gariepinus* exposed to sublethal concentrations of soap and detergent effluents. *J. Cell Anim. Biol.* 3(5): 78-82.

Masud, S: Singh, IJ (2013). Effects of cypermethrin on some hematological parameters and prediction of their recovery in a freshwater teleost, *Cyprinus carpio*. *Afr. J. Environ. Sci. Technol.* 7: 852-856.

Samuel, OB; Suleiman, OQ; Odiete, WO (2008). Synergistic Evaluation of Oil Dispersant and Oil-Detergent Mixtures Using African Catfish, *Clarias gariepinus*, Fry. *J. Fish. Aquat. Sci.* 3 (5): 280-290.

SPSS Inc. (2008). SPSS Statistics 17.0, SPSS Inc., Chicago IL.

Svobodova, Z; Pravda, D; Palackova, J (1991). Unified methods of haematological examinations of fish. Research Institute of Fish Culture and Hydrobiology, Vodany, (Czechoslovakia) 49Pp.