Acute Toxicity of Kogi State Specialist Hospital Effluent on *Clarias gariepinus* (Catfish)

Rosemary Patrick and Umar Victor
Department of Biological sciences, Federal University Lokoja, Nigeria
patrickrosemary58@yahoo.com

Abstract
This study is to investigate the acute toxicity of hospital effluent on *Clarias gariepinus* gills, flesh and liver as biomarkers of environmental quality in toxicity testing of hospital effluent. The acute toxicity test of hospital effluent was carried out in April 2017 on the histopathology of the gill, liver and flesh of 150 juvenile of (African catfish) *C. gariepinus* with mean weight of 7.8 ± 0.2g and standard length range of 12.5 ± 0.1. Physico-Chemical parameters of the control fell within the permissible limit of standard organizations (WHO and FEPA), while the Physico-Chemical parameter of the effluent was above maximum permissible limit of WHO and FEPA which make the aquatic environment increasingly polluted. Continuous increase in pollution from health care establishment will lead to an explosive loss of aquatic lives. A range finding test was conducted prior to the experiment. The fish were later exposed to triplicates of 0%, 20%, 40%, 80% and 100% concentrations of the effluent for 4 days (96 hours) in a static non- renewal bioassay procedure before biopsy. The median lethal concentration (LC50) value was 13.47%. Backward swimming, loss of equilibrium, and sudden death were observed in the exposed fish and these varied greatly with increase in concentration of the toxicant. This showed that mortality increases with increase in concentration. The gill histopathological changes observed were destruction of the gill arch. Histopathological examination of the liver showed vacoulation of hepatocyte, cellular infiltration and cellular necrosis (i.e. cell death). Occurrence of the gill, liver and flesh anomalies in the test fish show their suitability for use in toxicity testing of hospital effluent.

Keywords: Hospital effluent, Acute toxicity, histopathology, *Clarias gariepinus*.

Introduction

Anthropogenic activities around the water bodies play a major role in the aquatic environment, their activities leads to pollution, bioaccumulation, eutrophication of the water body which results into reduction of oxygen content and hence affect the physico chemical parameters of river body. However, changes in ecological balance of the aquatic environment can alter the population of aquatic species such as fish (Emmanuel *et al*., 2005). Hospital effluent is highly toxic and contains several components which has adverse effects on fish, invertebrates, amphibians and other aquatic organisms. This effluent comes from the administrative and housekeeping functions of health care establishments and may also include waste generated during maintenance of health care processess (Maithreepala *et al*., 2014). Most frequent contaminants in hospital waste water are viruses and pathogens from health care wastes which varies between and within countries and this variation can be attributed to the size of establishments, proportion of in and out patients, waste aggregation options , amount of waste discharged(Visvanathan and Adhikan, 2006). The disposal of this effluent on rivers or water bodies have become a critical issue as they possess potential health risks and damage to the environment i.e. the aquatic environment as well as the health risk of the population(Mithila *et al*.,2013). Furthermore, pathological, chemical, and pharmaceutical wastes can lead to outburst of diseases if left untreated. However the hospital effluents from Kogi state specialist hospital drainage are emptied into the river at Ganaja Bridge.

Clarias gariepinus (Burcell 1822) a species of catfish of the family 'clariidae', has its origin from Africa and its termed African cat fish. It is said to be rich in protein, omega 3 fatty acid and above all a nutritious meal to humans. It has the ability to withstand all weather conditions and adapt to any kind of aquatic environment either fresh or salt water. It is however suitable for this study and will be used to analyze the acute toxicity of hospital effluent. The components of this effluents in the water body has result into increase in acute toxicity level and hence a distortion of the aquatic ecosystems (Germer and Sinar, 2010).

Materials and Methods

Study Area

Kogi state which is a middle belt region of Nigeria is located at longitude 6.74 degrees east and latitude 7 degrees north and south. It is often referred to as the confluence city. However, Kogi state specialist hospital is located at the main heart of the township and has its drainage channeled to the river. Waste or effluent from this industry runs through this drainage and empties it at the river which makes the river increasingly polluted. The study area lies between the drainage.

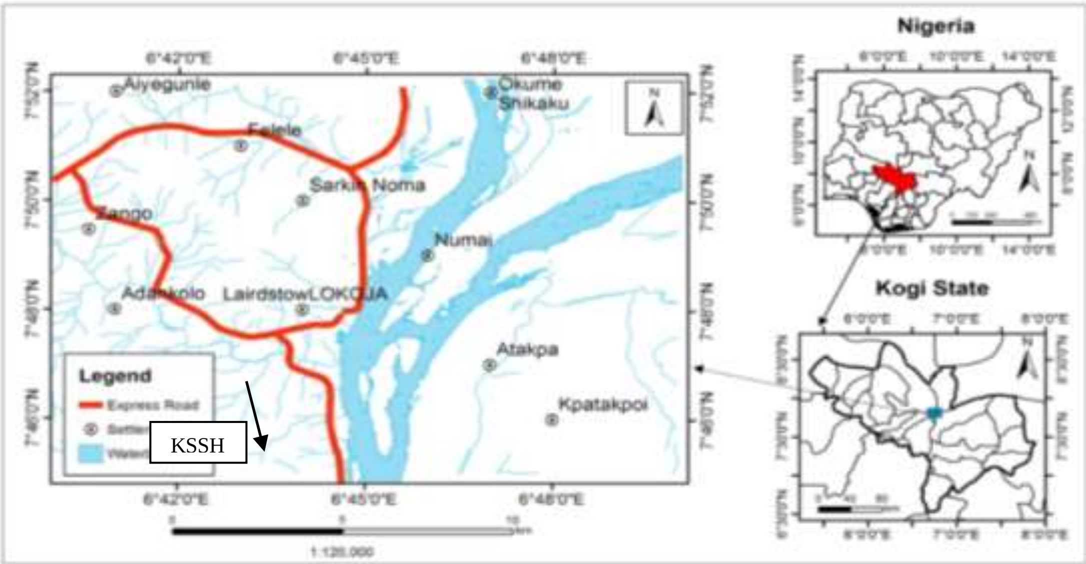

Figure 1: Map of study Area

Fresh Water Sampling

The fresh water was collected from the university tap water. The water was transported to the laboratory using plastic kegs and was poured into a big bowl and was aerated for 24 hours to dechlorinate. Thereafter, the physicochemical analysis such as pH, temperature, total dissolved solid, conductivity was carried out using HANNA pH/EC/Temp meter model 210, while dissolved oxygen and biochemical oxygen demand was titrated.

Fish Sampling

150 juveniles of *Clarias gariepinus* with mean weight 7.8 ± 0.2g and standard length range of 12.5 ± 0.1 cm, procured from UPWA farm in Lokoja Nigeria, was used for this toxicity assay. The test organisms was acclimatized to laboratory conditions for two weeks, in plastic aquaria to avoid overcrowding. During acclimatization, the test organisms were fed once daily with commercial feed pellets. The remnants containing unconsumed feeds and faecal particles were removed from the aquaria and the water was replenished every 24 hours.

Effluent Sampling

The test solution (hospital effluent) used in the toxicity test was collected from the discharge point of Kogi State Specialist Hospital and was transported with plastic kegs to the laboratory. The health organization performs various clinical examinations, laboratory diagnosis, operations, and mortuary. The physico-chemical parameter of the hospital effluent was analyzed.

Examination of Physical-Chemical Parameters.

Water Temperature
This was measured using a portable HANNA pH/EC/Temp metre model 210. The metre was turned on and then the meter probe was inserted into the water to read the temperature of the water, result displayed on the meter was recorded.

Water pH
The water pH was measured by using a portable HANNA pH/EC/Temp meter model 210. The meter was turned on and inserted into the water to read and the result displayed was recorded.

Conductivity
The conductivity of the water was measured using a portable HANNA pH/EC/Temp meter model 210, which was turned on and inserted into the water and the result displayed was recorded. This was measured in µS/cm.

Dissolved Oxygen
300ml of the water sample was collected2ml of manganous sulphate solution was added followed by the addition of 2ml of Alkali-iodide-azide reagent. The mixture was stopped carefully to exclude air bubbles and mix by inverting the bottle a few times. 2mL of conc. Tetraoxosulphate (VI) acid was added, restoppered and mix by inverting several time until dissolution was completed. 200mL of the treated sample was titrated with sodium thiosulphate (0.002N) to a light yellow colour. Then 1ml of starch (indicator) was added turning the sample dark blue. Titration continued until the disappearance of the blue colour was observed. The volume of the Thiosulphate used is equivalent to the milligram of the dissolved Oxygen per litre. This was determined by the winkler method as described by American Public Health Association (APHA, 1985).

Biochemical Oxygen Demand
Samples for which dissolved oxygen (DO_1) was determined was kept in the dark for 5 days. Then dissolved oxygen (DO_5) was determined by the Azide modification of the Winkler method.

$$BOD\ (mg/L) = DO_1 - DO_5$$

where DO_1 = Dissolved oxygen concentration prior to incubation, DO_5 = dissolved oxygen concentration after 5 days.

Toxicity Testing
A static non -renewal bioassay procedure was adopted in which the test media was introduced in different concentrations. A preliminary investigation (range finding test) was carried out prior to the commencement of the research to determine the definitive concentrations suitable for the toxicity testing. The definitive concentrations used for this toxicity tests was 20%, 40%, 80%, and 100%, as well as the control (0%) all in triplicates. Ten fully acclimatized fish were exposed to each concentration of the effluent. The toxicity test was conducted for 96 hours (4 days). This permitted the monitoring of the behavioral and mortality responses of the test organisms to varying concentrations of the hospital effluent.

Histhopathological Examination
After 4 days, fish from different concentration of the effluent as well as the control was dissected and the target organs which are: flesh, gill and liver was sampled differently, placed in a cassette, labeled and was fixed in 10% formalin for 24 hours, then it was dehydrated in different grades of alcohol (70%, 90% and 95% for 1 hour interval and 100% alcohol for 2 hours two times respectively). The tissue was cleared in xylene and infiltrated in paraffin wax in a vacuum oven at a temperature of 58℃, thereafter; it was embedded using the tissue embedding system. The embedded tissue was placed in the refrigerator to give a solid background.

Thereafter, the tissue was trimmed with 9um and sectioned with 2um using the microtome machine to get a thin ribbon like material. The sectioned tissue was placed on a slide and was placed on a slide warmer to melt the wax, then the slide was dewaxed in xylene for 15minutes twice and 5minutes in alcohol two times respectively. Then it was stained with haematoxylin for 20minutes and then into 1% acid alcohol for some seconds. The slide was rinsed with tap water for 8mins and then counter stain with eosin for 1- 2minutes and was introduced into absolute alcohol for some seconds twice. The slide was then oven dry and cover slips was mounted with DPX and then subjected to further examinations.

Statistical Analysis
The determination of lethal concentration was analyzed using a probit analysis (SPSS software). The physico chemical analysis of the effluent as well as the fresh water was represented in a tabular representation. The Histopathological abnormality observed in the cause of this study was shown and explained vividly.

RESULTS

Physico-Chemical Parameters
The result of the physico-chemical parameters of the fresh water and the hospital effluent are represented in the table below. The results were compared with the specifications of the Federal Environmental Protection Agency (FEPA) and World Health Organization (WHO). Other physico- Chemical parameter encountered was Total Dissolved Solid (TDS) using the HANNA probe meter.

According to the table the pH of the control fell within the permissible limit of World Health Organizations (WHO) 2004 and Federal Environmental Protection Agency(FEPA) 1991. The pHvalue in 20% hospital effluent had the average value of 7.29 with the lowest value of 6.11 in 40% hospital effluent indicating acidity. That of 100% and 80% effluent are slightly acidic and neutral. Water temperature varies in the different concentrations as that of the control fell within permissible limit of WHO and FEPA with an average value of 30.4 in 80% concentration and 29.3 in 100% concentration of hospital effluent. Though, the concentrations had increasing and decreasing values of temperature as shown in the table below.

Table 1. Physico-chemical parameters of fresh water and hospital effluent compared with standard organizations

Parameters	0%(control)	20%	40%	80%	100%	WHO (2004)	FEPA (1991)
pH	7.05	7.29	6.11	6.93	6.99	6.5-9.5	6.5-8.5
Temp(℃)	30.4	30.2	29.8	29.3	29.3	< 35	27
Cond(µS)	268	1043	1109	1117	1282	200	200
TDS(mg/L)	183	522	577	589	639	500	500
DO(mg/L)	4.37	1.16	0.19	0.78	0.37	6.0	7.0
BOD(mg/L)	0.51	3.30	2.85	2.72	6.53	10	10

Conductivity (µS) values was seen to be high in 100% concentrations having an average value of 1282 µS and the least value at the control which fell slightly above permissible limit of WHO and FEPA as shown in the table above. TDS value was seen to be low at the control having an average value of 183mg/l which was within permissible limit. That of the hospital effluent was seen to be high especially 100% effluent which had the highest value of 639mg/l as recorded in table 1.

DO values of the control was within the range of permissible limit of WHO and FEPA while there was a decrease in DO level in 20%, 40%, 80% and 100% effluent as seen in table 1 above. Similarly, BOD level was also low in the control and had increase in all other concentrations as seen in table 1 with significant differences in their values.

Behaviourals Changes Observed During Toxicity Testing

The behavioural changes observed at the control group, had no adverse behavioral response or any record of mortality recorded throughout the period of the bioassay. However, the fish exposed to the hospital effluent concentrations showed some signs of dizziness, backward swimming, difficulty in breathing, loss of appetite, falling movement and high mortality rate as seen The stressful and erratic behavior of the fish in this investigation gives a signal to respiratory impairment, and this may be as a result of the effect of the hospital effluent on the gill of the test organisms. However, previous histopathological studies of fish exposed to pollutants have shown that fish gills are efficient indicators of water quality. Fish gills are vulnerable to pollutants in water because of their large surface area and location. However, the gills perform numerous functions, which include respiration, excretion of nitrogenous waste products and acid-base balance. Functional impairments of gills caused by pollutants cause significant damages to the health of the fish, and fish gills are considered to be the most appropriate indicator in detecting water quality.

Table 2: Behavioural Changes Observed During Acute Toxicity Testing

Concentration	24 Hours	48 Hours	72 Hours	96 Hours
0% (control)	No response	No response	No response	No response
20%	Loss of appetite	Difficulty in breathing	Swimming backwards	Mortality
40%	Difficulty in breathing	Loss of appetite	Swimming backwards	Mortality
80%	Loss of appetite	Loss of equilibrium	Swimming backwards	Mortality
100%	Mortality	Swimming backwards	Mortality, loss of equilibrium	Mortality

Fish Mortality

The result of the mortality observed during the experiment is shown in Table 4.3**.**

Table 3: Mortality rate of *Clarias gariepinus* exposed to Hospital effluent

Concentration(%)	Total mortality	Mortality(%)
Control	0	0
20	8	80
40	7	70
80	6	60
100	10	100

LC50 (96 hours) Analysis

LC50 is the effective concentration at which 50 percent(50%) of the organisms dies. This was analysed using a probit analysis(SPSS 21 software). The LC50 at 4 days was 13.47%(Figure 2).

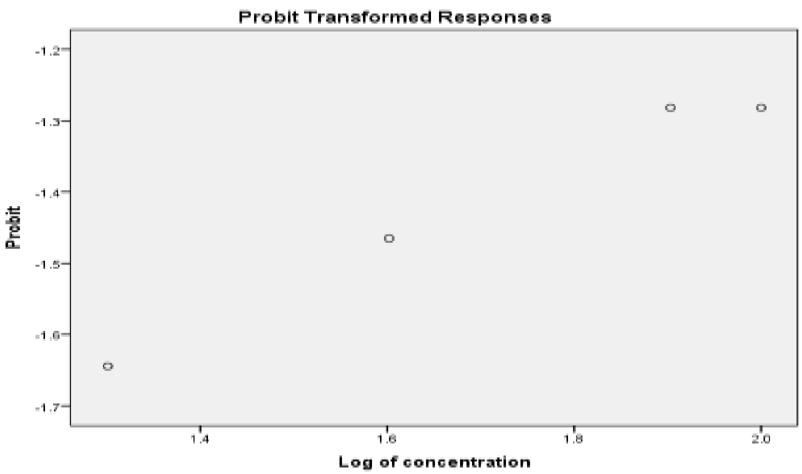

Figure 2: the probit analysis of the LC50 after the acute toxicity test (LC50 =13.47%)

Result of Histology Examination

The result below show the photomicrograph of the histopathological changes observed.

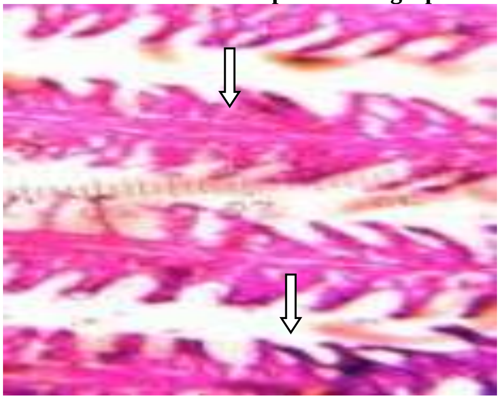

Plate 1: *Clarias gariepinus* gill at the control Showing normal epithelial cells.

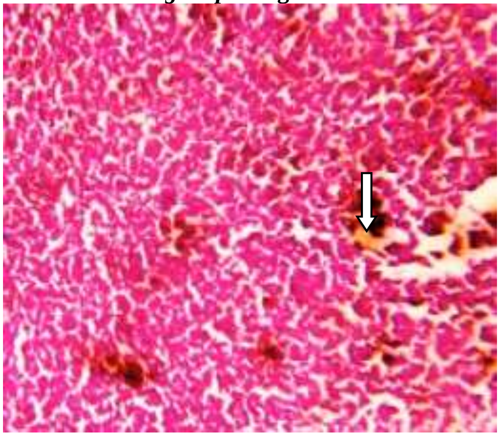

Plate 2: liver of *C. gariepinus* at the control indicates normal hepatic tissues and cells. The arrow shows normal hepatocytes.

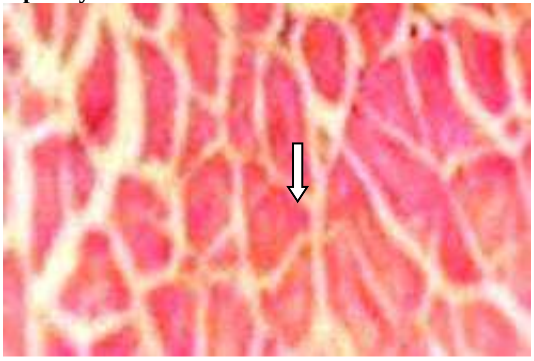

Plate 3: flesh of *Clarias gariepinus* at the control shows normal arrangement of muscles.

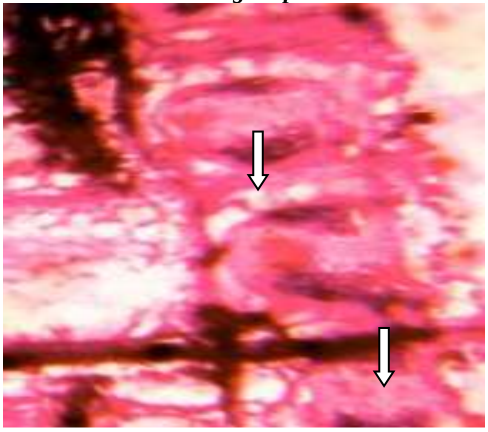

Plate 4: Gill of *Clarias gariepinus* at 20% concentration of hospital effluent. This shows the destruction of the gill arch.

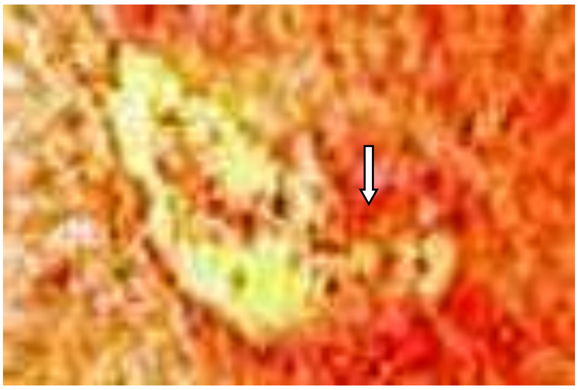

Plate 5: *C. gariepinus* liver at 100% hospital effluent shows widening and thickened wall of the hepatic portal lobules and hepatocytes vacoulation.

Histological Study of Gill: Gills are respiratory organs of fish which is used to obtain oxygen for the purpose of intracellular oxidation and for respiration in water. The gill is the first organ after the skin which is directly exposed to the toxicant. Assessment of gill histology is a good indicator of health status in fish as this conveys dissolved oxygen to the fish.
At the control group, the gill structure observed was normal as shown in plate 1 whereas, in experimental fish, the destruction of the gill lamellae, destruction of the gill cover, gill arch was observed as shown in plate 4.

Histological Study of Liver: The liver synthesizes a variety of organic compounds like uric acid, cholesterol, albumin and other lipids. The liver aids removal of excretory product and detoxify toxic substances. The control group, shows normal hepatocytes and normal hepatic tissues as seen in plate 2 while the experimental group at 96hours shows the hepatocytes enlargement and hepatocytes vacoulation (plate 5).

Histological Study of Skin: The skin is the first organ of fish that is directly exposed to toxicant and the effect is first noticed on the skin. However, the control indicates normal muscular arrangement as seen in plate 3.

Discussion
In present study, the average physico-chemical analysis of the control was seen to be neutral, while that of the effluent at (LC50) 96 hours (experimental group) showed distinct variations among the concentrations. The pH level at 40% concentration indicates acidity which is very harmful for fish. That of the control group fell within permissible limit of WHO and FEPA.
Ibrahim and Abdullahi, (2009) observed that pH values of 6.5 and 9.0 are good for fish production and that water pH affects metabolism and physiological processes of fish. Though the pH of the hospital effluent falls between acidity and slightly alkalinity, especially 40% concentration which was seen to be acidic. This was as a result of accumulation of various wastes in the hospital drainage.

Water temperature of the hospital effluent had variations in all concentrations except that of the control had an average value of 30.4◦C which fell within permissible limit of standard organizations; FEPA (1991) and WHO (2004); this was also similar to the work of Ali *et al*. (2013).The mean temperature for the effluent was between 29.3◦C to 30◦C indicating variations in temperature between the experimental days. According to FEPA(1991) high water temperatures can increase the solubility of toxic substance. However this explains the increased mortality in the test organisms. However, Ane (2013) reported that low and high temperatures can reduce the growth of fish and result into loss of appetite similar to the work of Imsland *et al*.(2006), Bjornsson *et al*.(2001)

Conductivity levels in the control group was within permissible limit of FEPA (1991) and WHO(2004), having an average value of 268µS/cm. While that of the effluent had increase above 200 µS/cm up to 1282µS/cm. This study explains that the increase affects water quality and hence, affects aquatic lives due to its high mortality rate. Similarly, Ugwumba, (2014) reported that conductivity levels increases or decreases due to fluctuations in other physico-chemical parameters. Also, conductivity on fish is important because it directly effects the quality of water used in fisheries. Conductivity measurements is based on ionic composition of the water, many organisms can be hurt or helped by a small imbalance of these ions. Total Dissolved Solid (TDS) level in this study showed variations in the control group but did not exceed the permissible limit. According to WHO (2004) and FEPA (1991), the maximum contamination level is 500mg/L. The effluent group reported above 600mg/L which is very harmful for fish and can hence alter their population. This study also explains that the high TDS in the effluent was due to various wastes deposited in the drainage. According to Akponine and Ugwumba (2014), high TDS level is due to excessive runoff of nutrients into water bodies.

Dissolved oxygen level of the control had an average value of 4.37mg/L which was said to be within the range 3.2 to 5.2mg/L reported by Mustapha (2008) in Moro reservoir in Ilorin and in line with FEPA and WHO standard limits. However, this is very essential to all aquatic lives especially fish. Dissolved Oxygen in the effluent decreased down to an average range of 0.37mg/L in 100% hospital effluents which resulted into high mortality rate during the experimental hours. Dissolved Oxygen in water is an important compound needed for the body activities of fish and other aquatic lives such as the plankton community and other aquatic organisms for photosynthesis. According to WHO (2004) reported that the amount of Dissolved Oxygen in water depends on the source, temperature, chemical and biological processes taking place in a water body. Dissolved Oxygen is hence a measure of the amount of gaseous oxygen in an aqueous solution that plays a vital role in the biology of cultured organisms (Ehiagbonare and Ogundiran, 2010). The low dissolved oxygen observed in the effluent group could be attributed to the decomposition of effluent received from various unit of the hospital, patient waste and so on.

Biological Oxygen Demand (BOD) levels for the control group observed during this study had an average value of 0.51mg/L. Similar to the permissible limit of WHO (2004) and FEPA (1991) which reported BOD concentration range of unpolluted water to <1.0mg/L. The BOD level for different concentration of the hospital effluent increased during the experimental hours. This was due to high accumulation of various waste materials compounded in the drainage. The BOD values of 100% effluent had 6.53mg/L, indicating heavy pollution, with 80% effluent values are surprisingly very low with its average value of 2.72mg/L. Similar to Adakole *et al*. (2002) he gave correlations between heavily polluted waters, based on classification of aquatic bodies. Thus, BOD indicates a potential for reducing the dissolved oxygen content in water and this could further lead to stressful conditions, suffocation, and high mortality rate (APHA, 1992). Mortality rate of the test organisms in different concentrations of the effluent increased during the experiment. While there was no mortality observed in the control at 96hours. The LC50 of *Clarias gariepinus* exposed to hospital waste of 100% concentration had high rate of mortality indicating high level of impurities present in such water as shown in table 2. As a matter of fact excessive run-off of nutrients (eutrophication) is a major cause of high mortality rate of aquatic lives. This is due to the fact that certain pathogens causing diseases and also exposing immune organisms to sometimes unbearable stress are present in these wastes or run off deposited. Thus, the determination of LC50 at 96 hours as seen in figure 2 is the effective concentration at which fifty percent (50%) of the test organisms are killed. This was determined using a probit analysis (SPSS version 21). The LC50 at 4 days was 13.47%. Agboola and Fawole (2014) gave correlations to high mortality rate of *Clarias gariepinus* exposed to pharmaceutical effluent for 21 days. Similarly, Maithreepala *et al*. (2014) reported that waste discharge from hospitals have adverse effect on aquatic lives as well as humans (Adeyemi and Osubor 2009). However, this study brought to lime light the cause of high mortality rate of cat fish (*Clarias gariepinus)* which was due to excessive run off from different department of the hospital or from various units of the health care establishments.

In the present study histological alterations were documented for different tissues namely gills, muscle and liver. Histological observations of the control group had no change and there was no abnormalities recorded. While the histological observations of the test organisms of the effluent showed signs of abnormalities in the organs (gills, flesh and liver) assessed. There was distortion in the gill as shown in the plates above. This was due to the exposure of catfish to hospital effluent. The gill is the first sensitive organ of fish that aids it survival and hence, also tells the nature of the environment. This is because fish gills are vulnerable to pollutants in water because of their large surface area and location. However, the gills perform numerous functions, which include respiration, excretion of nitrogenous waste products and acid-base balance. Functional impairments of gills caused by pollutants cause significant damages to the health of the fish. Fish gills are considered to be the most appropriate indicator of water pollution levels. Also, there are increasing evidence that toxic compounds have the potential to cause the most harm to tissues and organs that are contacted first (Johndevados and Ravichandran,2014) The sections of the liver obtained from the treatment group had disrupted histological organization(plate 5) compared with the control group(plate 2). Some of the deleterious effects seen in the section of the liver obtained from the treatment group include degeneration and disruption of the hepatocytes (liver cells). The hepatocytes frequently contain glycogen and the hepatocytes maintain a steady level of blood glucose (Agboola and Fawole, 2014). A compromise in the integrity of the hepatocytes could lead to improper functioning of the liver. Muscles of the control group showed the normal arrangements of muscle fibers and muscle bundles. However, the effluent groups did not show any marked deformities in the muscles. Histology of the muscles showed the normal architecture as seen in the control. Furthermore, FEPA specifications reported that pollutants residual effects may impair vital organs of fish. Also, the resultant effect could be gradual accumulation of the waste in water which in turn becomes toxic to aquatic organisms and also to human health.

Conclusion

Fish are intimately associated with their aqueous environment, therefore physical and chemical changes in their ecosystems are rapidly reflected as quantifiable physiological measurements. Even though individual concentrations of any drug or toxicant might be low, the combined concentrations from different drugs or toxicants could be fatal to aquatic or human health. The study provides indications of potential adverse environmental impacts of hospital effluent in the receiving environment. It helps to provide and encourage awareness among the general public that hospital effluent is toxic to the environment. This study has shown that gills and liver of *C. gariepinus* are sensitive to, and suitable for toxicological studies of hospital effluent.

Recommendation

Farmers should, therefore, ensure that fish are grown hygienically. The use of polluted water, which has potential risks of transmitting infectious pathogens to aquatic lives, as well as humans, should be minimized or discouraged. Government and other agencies should ensure proper disposal of waste from health establishments, and disallow channeling of drainages into rivers as this is seen to cause harm to aquatic lives, most especially fish, and humans as well.

Further research on how to pre-treat hospital effluent before releasing it to the environment should be encouraged.

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