HISTOLOGY OF GILL AND LIVER OF Clarias gariepinus FINGERLINGS EXPOSED TO TOXIC LEVELS OF DIZENSATE (GLYPHOSATE HERBICIDE).

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ABSTRACT
The toxicity of Dizensate herbicide on the fingerlings was investigated with emphasis on histopathological effects of African catfish Clarias gariepinus juvenile. Static bioassay was conducted to determine the LC_{50} of Dizensate herbicide to African catfish fingerlings. The fishes were exposed to 0, 9.6, 14.4, 19.2, 21.6 and 24.0 mg/l of Dizensate herbicide. Histopathological examinations were performed on the gill and liver of test organisms exposed to Dizensate glyphosate under standard laboratory condition. 144 live and apparently healthy C. gariepinus fingerlings measuring 9.3-10.6cm standard length and weighed between 5.8g and 6.5g were randomly distributed into twelve (40cm x 29cm x 28cm) glass tanks of 60 litres capacity each were filled with 20litres aerated unchlorinated well water at twelve fish/tank for the experiment. The toxicant was introduced at the different concentrations stated above in triplicate per treatment. The lethal concentration (LC_{50}) value of Dizensate herbicide was 18.07mg/l for 96h of exposure. Mean mortality was 0, 17, 58, 75 and 92% in the concentration of 9.6, 14.4, 19.2, 21.6 and 24.0mg/l respectively, while there was no mortality in the control treatment. Toxic reactions exhibited by the fish include erratic movement, air gulping, and loss of reflex, molting, barbell deformation, hemorrhage and excessive mucus secretion in fish exposed to higher concentration of Dizensate glyphosate. Observations on the bioassay test indicated hyper excitability and the eagerness of the test fish to jump out of the pollutant. The study reveal that Dizensate glyphosate is highly toxic to C. gariepinus, therefore it's use directly in water bodies, near fish farms or in areas close to aquatic bodies should be moderated and regulated.

Keywords: Dizensate glyphosate, *toxicity*, African catfish (*Clarias gariepinus*), *histopathological*

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Introduction
Majority of environmental problems of concern today are attributed to the production and eventual release of toxic chemicals which are not only capable of interacting with the environment but also disrupting the ecosystem. Various activities by man of which indiscriminate discharge of herbicides forms agricultural run-off and ended up in aquatic environment affects non target organisms such as fish and other aquatic organism which are of great economic importance to humans. Pollutants in water significantly affect the ability of fish to detect and respond to chemical stimulus. Feeding, Growth, and reproductive performances could also be seriously affected by such polluted habitat. Pollution of aquatic habitat may result in mass fish mortality or their failure to breed in the polluted environment. These chemical affects not only the physiology and survival of aquatic organisms including fish but also can interact with their genetic material which may lead to the mutations and/or carcinogenesis (Goksoyr, 1991).

Toxicity testing of chemicals on animals has been used for a long time to detect the potential hazards posed by chemicals to man. Bioassay technique has been the cornerstone of programmes on environmental health and chemical safety. Aquatic bioassays are necessary in water pollution control to determine whether a

potential toxicant is dangerous to aquatic life and if so, to find the relationship between the toxicant concentration and its effect on aquatic animals (Olaifa et al., 2003). Nowak, 1992 reported that histopathological changes of gills such as hyperplasia and hypertrophy, epithelial lifting, aneurysm and increase in mucus secretion occurred after the exposure of fish to a variety of noxious agents in the water, such as pesticides, phenol and heavy metal. The gill is the first internal organ that has interface with the polluted water body whereas the liver performs a critical role of breaking down of toxic chemicals and hence the most affected organ in the case of pollution.

The likely impact of indiscriminate and uncontrolled use of glyphosate herbicide in sub-Sahara Africa necessitated the need to study the aquatic side-effects of glyphosate herbicide on some vital organs of African catfish. The objectives of this study is to determine the lethal concentration (so as to determine the safe level of the chemical) and the acute toxic effect of glyphosate herbicide with emphasis on the histopathology on *Clarias gariepinus.*

Methodology

According to ASTM (1977), a static bioassay (96–hour) was carried out in the laboratory with the fingerlings of *Clarias gariepinus* (9.3 and 10.6cm standard length and weighing between 5.8g and 6.5g) as the test organisms. This enables us measure parameters to study the toxicity of glyphosate herbicide on the fish, and determine allowable levels of concentrations of glyphosate herbicide within short exposures. The method employed is based on the recommendation for test of acute toxicity of pollutants to fish described by Sprague (1973) as sited by Babatunde et.al (2014)

Experimental fish

Clarias gariepinus is the most commonly cultivated in Nigeria with its ability to withstand stress, attain good weight within short period of time and high commercial value of US$800 (Chika and Nathaniel, 2014). This informed the use of this fish for the study. The fish was obtained from a private hatchery and transported in plastic bowl were used for the experiment. The bioassay experiment was carried out using the laboratory of Federal College of Fisheries and Marine Technology, Victoria Island, Lagos. Histological analysis was done at Histology Unit, Department of Anatomy, College of Medicine, and University of Lagos.

Acclimation of fish

An aquarium of 40cm by 29cm by 28cm, containing non-chlorinated well water were used for the experiment. The fish were allowed to acclimatize for 7 days under laboratory conditions to allow them adapt to experimental conditions (27 ± 2 °C) and to also ensure that the test organism is in good condition of health. The fish were fed during the period of acclimatization and the water was changed every day in order to remove faecal and unconsumed feeds. There was no feeding at the commencement of the experiment.

The determination of the physico–chemical parameters of the water

Data of the physico–chemical parameters of the water used were obtained. Three parameters which included temperature, dissolved oxygen (DO) and the hydrogen ion concentration (pH) were monitored all through the 96-hour period of the experiment. The temperature was measured with a clinical thermometer and the dissolved oxygen of the water was measured with a digital meter (Jenway9071), while the pH was measured using the HANNA HI 9813 GRO CHEK meter.

Procedure

The bioassay was carried out in a rectangular glass tank. Each tank size of 40cm X 29cm X 28cm of 60 liters capacity were filled with 20 litres unchlorinated well water contained twelve fish each. After a range – finding test (the preliminary test), the concentrations prepared for the experiment were 0 mg/L, 9.6 mg/L, 14.4 mg/L, 19.2 mg/L, 21.6 mg/L and 24.0 mg/L, with three replicates. The amount of herbicide which contained the require miligram of Dizensate herbicide was determined from the 480 g/L of Dizensate herbicide formulation. The behavioural pattern of the fish and other external changes in the body of fish were observed accordingly. Dead fish were identified by an absolute lack of movement. They were removed as soon as this was noticed, and disposed. The LC_{50} value of the *Clarias gariepinus* for 96 hrs was calculated using the probit analysis.

Histopathological Studies

At the end of the experiments, one fish per treatment were sampled after 96hour of exposure to glyphosate herbicide for histological analysis. The fish was sacrificed with a blow on the head, using a mallet and was dissected to remove the liver and the gill. Dissection of fish was performed according to the international standard procedures provided in the EMERGE Protocol (Rosseland et al., 2003). All histological were prepared according to a standard procedure for light microscopy analysis (Gautier, 2011). Each of the organs sampled were fixed in 10 % formalin for 3 days after which the tissue was dehydrated in periodic acid Schiff's reagent (PAS) following the method of Hughes and Perry (1976) in graded levels of 50%,70%,90% and 100% alcohol for 3 days, to allow paraffin wax to penetrate the tissue during embedding. The organs were then embedded in molten wax. Tissue was sectioned into a thin section (5-7µm) by means of a rotator microtome and were dehydrated and stained with Harris haematoxyllin-Eosin (H&E) stain as proposed by Bancroft and Cook, (1994) using a microtone and each section was cleared by placing in warm water (38^0C) where it was picked with clean slide and oven dried at 58^0C for 30 minutes to melt the wax. Slides containing sectioned materials/tissue was cleared using xylene and graded levels of (50%, 70%, 90% and 100%) of alcohol for 2 minutes each.

The section was stained in haematoxyline Eosin for ten minutes. The stained slides were observed under a light microscope. At varying X100 magnification, sections were examined and photographed using an Olympus BH2 microscope fitted with photographic attachment (Olympus PM C35 AD4) a camera (OlympusC40 AB -4). The histological lesions were assessed according to the semi-quantitative system proposed by Bernet *et al*. (1999).

Statistical Analysis: Experimental Design

The experiment was laid out in a completely randomized design (CRD) with six treatments for each. The experiment involves the use of fingerlings as test organism and has the following treatment: 0.0mg/l as control and five other treatments (9.6mg/l; 14.4mg/l;19.2mg/l;21.6mg/l and24mg/l). Phase I for the experiment involved the range finding test where fish were exposed to various concentrations of the toxicant until 50% mortality was attained while phase II was a chronic bioassay study that lasted for 96hours. 144 live and apparently healthy *C. gariepinus* fingerlings measuring between 9.3 and 10.6cm standard length and weighing between 5.8g and 6.5g were randomly distributed into twelve (40cm x 29cm x 28cm) rectangular glass tanks of 60 litres capacity each filled with 20 litres aerated unchlorinated well water at twelve fish/tank for the experiment. The dose response of mortality was analyzed by probit analysis (Finney, 1971) based on a computer programme by Ge Le PaHoure, Imperial College, London and adopted by Otitoju (2001), This was used to derive the LC_{50}.

LC_{50} = Median lethal concentration that causes 50% mortality of exposed animals.

Results

The physico-chemical characteristics of the water

Tables 1 showed the results of the water parameters after Dizensate herbicide esposure of C.gariepinus fingerlings and adult. The pH, temperature and dissolved oxygen were determined at different time interval. The results obtained before the test were found to be close to the water quality parameters of the control experiment. The pH value obtained shows that Dizensate herbicide has slight effect on the pH of water. The pH reduced slightly from 7.2±0.2 in control to 6.4±0.1 in test treatment of concentration 24.0mg/l after the whole experiment as shown in tables 1. Temperature varies between 25±0.1to 28±0.2 in the 9.6mg/l concentration. The DO_2 decrease slight from 5.9±0.1 in control experiment to 4.4±0.1 in test treatment of concentration of 24.0mg/l

TABLE 1: Summary of Water Quality Parameters of glyphosate herbicide on fingerlings of C. gariepinus {Definitive test} (Mean ± S.D)

TIME	PARAMETERS	0 mg/l	9.6mg/l	14.4mg/l	19.2mg/l	21.6mg/l	24.0mg/l
1hr	Temp (0^oC)	25±0.1	25±0.1	25±0.1	25±0.1	26±0.2	26±0.1
	Ph	7.0±0.2	7.0±0.2	7.0±0.2	7.0±0.2	7.2±0.2	7.2±0.2
	DO_2 (mg/l)	5.9±0.1	5.9±0.1	5.9±0.1	5.9±0.2	5.8±0.1	5.9±0.1
2hr	Temp (0^oC)	25±0.2	25±0.2	26±0.1	25±0.1	26±0.1	26±0.1
	Ph	7.0±0.2	7.0±0.1	7.0±0.2	7.0±0.2	7.2±0.1	7.2±0.1
	DO_2 (mg/l)	5.9±0.1	5.9±0.1	5.9±0.1	5.8±0.1	5.9±0.1	5.9±0.1
3hr	Temp (0^oC)	25±0.1	26±0.1	25±0.1	26±0.1	27±0.1	26±0.2
	Ph	7.2±0.2	7.1±0.1	7.1±0.1	7.1±0.1	7.2±0.2	7.2±0.2
	DO_2 (mg/l)	5.9±0.1	5.9±0.1	5.9±0.2	5.8±0.2	5.9±0.1	5.9±0.1
4hr	Temp (0^oC)	26±0.2	27±0.2	27±0.1	27±0.1	27±0.1	27±0.1
	Ph	7.2±0.1	7.2±0.2	7.2±0.2	7.2±0.2	6.9±0.1	6.9±0.1
	DO_2 (mg/l)	5.9±0.1	5.9±0.1	5.9±0.1	5.8±0.1	5.7±0.1	5.8±0.1
8hrs	Temp (0^oC)	25±0.1	27±0.2	27±0.2	27±0.2	27±0.1	27±0.1
	Ph	7.2±0.2	7.2±0.1	7.2±0.1	7.2±0.1	6.8±0.1	6.9±0.1
	DO_2 (mg/l)	7.9±0.1	5.9±0.1	5.9±0.2	5.8±0.2	5.7±0.1	5.7±0.1
12hrs	Temp (0^oC)	26±0.2	26±0.1	26±0.2	26±0.2	27±0.1	27±0.2
	Ph	7.0±0.2	7.2±0.1	7.2±0.1	7.2±0.1	6.8±0.2	6.8±0.2
	DO_2 (mg/l)	7.9±0.1	5.9±0.1	5.9±0.2	5.8±0.2	5.7±0.1	5.7±0.1
16hrs	Temp (0^oC)	25±0.1	25±0.2	25±0.2	25±0.2	28±0.1	28±0.1
	Ph	7.0±0.2	7.2±0.2	7.2±0.1	7.2±0.1	6.7±0.2	6.7±0.2
	DO_2 (mg/l)	7.9±0.1	5.9±0.1	5.9±0.1	5.7±0.1	5.7±0.1	5.6±0.1
20hrs	Temp (0^oC)	25±0.1	25±0.2	25±0.2	25±0.2	28±0.2	28±0.2
	Ph	7.0±0.1	7.2±0.1	7.2±0.1	7.2±0.1	6.8±0.1	6.8±0.1
	DO_2 (mg/l)	7.9±0.1	5.9±0.1	5.8±0.2	5.5±0.2	5.6±0.1	5.6±0.1
24hrs	Temp (0^oC)	27±0.0	27±0.1	27±0.1	27±0.1	28±0.2	27±0.1
	Ph	7.2±0.0	7.2±0.1	7.2±0.1	7.2±0.1	6.8±0.1	6.8±0.1
	DO_2 (mg/l)	7.9±0.1	5.5±0.1	5.9±0.1	5.5±0.1	5.3±0.1	5.3±0.1
48hrs	Temp (0^oC)	27±0.1	27±0.2	27±0.2	27±0.2	29±0.2	28±0.2
	Ph	7.2±0.3	6.8±0.1	6.8±0.1	6.8±0.1	6.5±0.2	6.5±0.2
	DO_2 (mg/l)	6.9±0.1	5.5±0.1	5.9±0.1	5.5±0.1	5.2±0.1	5.2±0.1
72hrs	Temp (0^oC)	27±0.1	27±0.1	27±0.1	27±0.1	27±0.2	27±0.2
	Ph	7.2±0.1	7.2±0.2	7.2±0.2	7.2±0.2	6.6±0.1	6.6±0.1
	DO_2 (mg/l)	6.5±0.1	5.5±0.1	5.9±0.1	5.3±0.1	4.9±0.1	4.9±0.2
96hrs	Temp (0^oC)	27±0.2	28±0.2	27±0.1	27±0.1	27±0.2	27±0.2
	Ph	7.2±0.1	7.2±0.2	7.2±0.2	7.2±0.2	6.4±0.2	6.4±0.1
	DO_2 (mg/l)	6.4±0.1	5.4±0.1	5.7±0.1	5.3±0.1	4.4±0.1	4.4±0.1

Source: Field Survey, 2017

Acute Toxicity

The results of the acute toxicity test are presented in Tables 2&3. The LC_{50} value based on probit analysis was found to be 18.07 mg/L for 96 hrs of exposure to the glyphosate herbicide (Fig.1). The results obtained showed that there was no mortality (Table 2) of fish in the control experiment throughout the 96 hrs. There was 17% mortality of the fish exposed to 14.4 mg/L while at 24 mg/L, 92% mortality was observed. During this study the behaviour of the control fish was normal, while the fish introduced into the different concentrates of the herbicides showed different abnormal behaviour. Abnormal behaviour such as erratic swimming, sudden quick movements and restlessness were observed in fish exposed to the chemical. At high concentration of 24mg/L, the fish became very weak and settled at the bottom. Normal colour and behavioural response was observed in the control experiment.

Table 2: Lethal concentrations (96h- LC_{50}) values of glyphosate to which *C. gariepinus* fingerlings were treated after several hours' exposure time.

TIME (hours)	Log C Value	LC_{50}
24	1.387	24.38mg/l
48	1.366	23.23mg/l
72	1.318	20.80mg/l
96	1.257	18.07mg/l

KEY:
LC_{50}= Lethal Concentrations
Log C Value = Logarithm of the concentrations

Table 3: Estimation of the Log C and the LC_{50} value of glyphosate to which *C. gariepinus* fingerlings were treated after 96 hours exposure time.

Conc. (mg/l)	Log $_{10}$ Conc.	Total No	No.Dead	% Mortality	Corrected % mortality	Probit
T0 = 0	-	12	0	0		-
T1 = 9.6	0.982	12	0	0		-
T2 = 14.4	1.158	12	2	17		4.05
T3 = 19.2	1.283	12	7	58		5.20
T4 = 21.6	1.335	12	9	75		5.67
T5 = 24.0	1.380	12	11	92		6.41

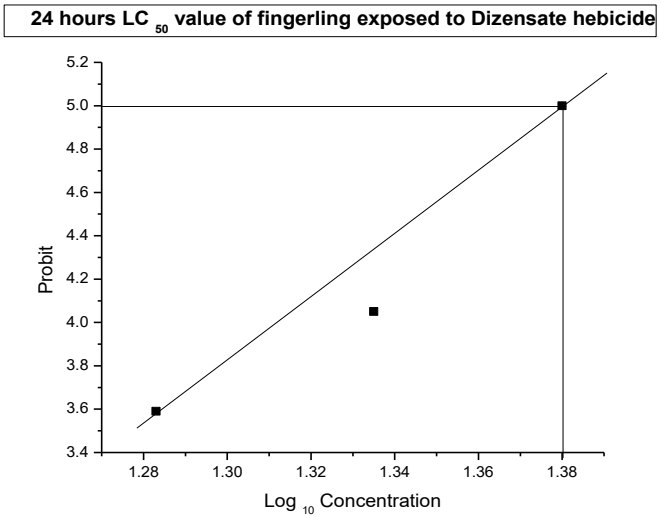

Fig. 1: LC_{50} for fingerlings of *C. gariepinus* at 24h exposure time to glyphosate

Mortality (%) of C. gariepinus fingerlings exposed to different concentrations of glyphosate herbicide (bioassay test) (Table 3) showed that the fish was sensitive to concentrations from 14.4 – 24.0 mg L^{-1}. The table indicated that within 96h, about 17% of the fish died in concentration of 14.4mg L^{-1}, while 58% died in concentration of 19.2mg L^{-1}, suggesting that the 96h LC_{50} of glyphosate herbicide might lie between 14.4 and 19.2mg L^{-1}. The concentration values were converted to Logit, while the mortality (%) was converted to Probit values according to methods of Hewlett & Plackett (1979), and the transformed values were used to determine the 96h LC_{50} graphically. Figure 1 presents the LC_{50} graph with the regression equation Y = 10.233x – 7.8583, where y = probit response and x = logit (log-dose). From the equation, the 96h LC_{50} was calculated as 18.07 mg L^{-1}.

Histopathological effect

LIVER: Transverse section through the liver showed no pathological lesion. Normal cellular pattern, normal central vein, billary epithelium, hepatic plate and hepatocytes. No lesion, necrosis, pigments, malignancy, inflammation or inclusion bodies were seen in the control (Fig.D1). However, patches of slight lesion, necrosis, malignancy, pigment and inflammation in the livers exposed to the glyphosate herbicide at 14.4mg/l and 19.2mg/l were observed (Fig D3 and D4). Vacuolation and hepatocyte enlargement of tissue was seen in concentration of 21.6mg/l of glyphosate herbicide treated fish. Shrinkage of cell and hyperplasia of cell was also observed. Complete degenerated tissue was observed in this highest concentration of 24.0mg/l within 96 hours (fig. D6)

GILL: There was normal cellular pattern in the control experiment. The gill arch, gill rakers, filament, sinus, and cartilaginous support were in order. Pseudo-brachial lamella, ceratobrachial bone of the arch, mucous epithelium lining on the membrane and branches of the afferent and efferent arterioles were all normal (Fig.A1). No lesion, necrosis, pigments, malignancy, inflammation or inclusion bodies were seen. However, as the concentration of the toxicant increase, Moderate and severe areas of lesion, necrosis, malignancy, pigment and inclusion bodies were observed. Fig. A2, A3 and A4 revealed degeneration of lamellar and hypertrophy of cellx. Complete degeneration was observed in concentration of 21.6mg/l of glyphosate

herbicide treated fish. Hypertrophy of gill arch and complete degeneration of filament was observed in highest concentration of 24mg/l of glyphosate herbicide treated fish within 96 hours period.

Discussion

The glyphosate herbicide exerted toxic effect on the fish in the present study and toxicity increased with increased concentration. The physico-chemical properties of glyphosate herbicide clearly indicated that it is a pollutant, as its presence in water changed the physical and chemical qualities of water to critical levels that could hardly support aquatic productivity. The maximum safe concentration (96h LC_{50}) range for fingerlings fish samples (18.23mg/l) compared favourable with those of Omitoyin et.al (2006) that reported the effect of gramoxone (paraquat) juvenile *Clarias gariepinus* with LC_{50} value of 18mg/l for 96h exposure. As expected, high concentration of glyphosate herbicide in the areas resulted in increased water temperature with corresponding reduction in dissolved oxygen concentrations.

Abnormal behaviours such as incessant jumping and gulping of air, restlessness, loss of equilibrium, increase opercular activities, surface to bottom movement, sudden quick movement and resting at the bottom observed in this study were similar to the observations of Ajani, *et. al.* (2002) and Fafioye (2001). The fish were stressed progressively with time before eventually dying. The stressful ailment of respiratory impairment due to the toxic effect of glyphosate herbicide on the gills was similar to the report of Omitoyin et al. (2006). The observed increasing state of inactivity with both increasing concentrations and exposure period agree with the report of Ayoola (2008). Water quality parameters had little variation, physicochemical parameter measured seemed to be within optimum range for fish culture as reported by Omitoyin et al. (2006) and Olaifa *et al.* (2003).

Accumulation of mucus in the gills of fish exposed to the different concentrations of Dizensate herbicide in this study might be responsible for the mortality recorded. This report was similar to the work of Muniyan and Veeraraghavan, 1990 who worked on the effect of insecticide ethofenprox on Nile Tilapia. Konar 1975 reported that the accumulation of mucus on the gills reduces respiratory activities in fish. Histopathology of the organs after 96 hours exposure revealed cell proliferation, lamellar fusion, lamellar, cell hyperplasia, and epithelial lifting. In the liver, there was vacuolation of hepatocytes and necrosis. The changes in these tissues occur predominantly in the 96 hours exposure. Respiratory stress, erratic swimming and instant death of fish were observed in exposed fish, which varied with the concentration of the toxicant.

Histopathological examination of the gill and liver of *C. gariepinus* fingerlings showed varied degrees of degenerative changes including vacuolation and necrosis which worsened with increasing concentration of the toxicant. The inability of the gill surface to actively carry out gaseous exchange might be responsible for the observed mortalities.

The mortality pattern recorded corroborates with that reported by Rand and Pectrocelli (1985) which stated that there should be less than 35% mortality in one of the concentrations and at least more than 65% mortality in the highest concentration.

Conclusion

The results of this study revealed that glyphosate herbicide is toxic to fish organs and causes histopathological changes in different vital organs such as Liver, a centre of deamination of toxic substances; gills, an interface for the exchange of gases; skin, an organ for protection and heart that is responsible for circulation of blood. They all had different levels of degeneration after exposure to the toxicant. This further establishes the

detrimental effects this toxicant has on the African catfish. Therefore, indiscriminate discharge into water bodies by farmers should be discouraged particularly in aquatic bodies.

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