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Toxicity of glyphosate based herbicides to fingerlings of *Heterobranchus bidorsalis*

Abstract

The use of herbicides to control weed has increased in many areas in the Niger Delta region of Nigeria. Sometimes the empty cans are carelessly discharged into the environment were some may end up in the aquatic ecosystem due to the runoff. Sometimes, the herbicides are applied in agricultural, urban or household settings close to the aquatic ecosystem. Fish rearing and cultivation both in homestead and catches from the wild are major sources of livelihood to several families in many coastal regions of the Niger Delta especially in Bayelsa state. This study investigated the toxicity of glyphosate (in the form of 480g/l isopropylamine salt) on *Heterobranchus bidorsalis* fingerlings. The fishes with mean length 6.00cm were allowed to acclimatize in the laboratory for seven days. Range finding test was carried out and from which seven concentrations of the toxicant were made viz: 0.00ppm, 12.00ppm, 15.00ppm, 21.00ppm, 24.00ppm and 27ppm. The experiment was carried out using renewal bioassay techniques for 96hours and LC 50 values were calculated following standard protocol. The percentage mortality rate was dependent on the dose. The LC50 values were 20.88ppm, 22.91ppm, 22.39ppm and 19.50ppm at 24hours, 48hours, 72hours and 96hours, respectively. Based on the findings of this study, there is a need to properly discard empty cans of herbicides and also caution should be exercised during its applications close to the aquatic ecosystem.

Keywords: aquatic ecosystem, glyphosate, fishes, mortality, toxicity

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Introduction

Environmental pollution is a major threat to its sustainability.[1] In the recent times, the use of agrochemicals in developing countries for the eradication of different pests/weeds has been on the increasing trend. For example, Stoyanova et al.,[2] Ojesanmi et al.,[3] and Aghoghovwia & Izah[4] reported that pesticides are among the most potentially harmful chemicals introduced into the environment through the activities of human. Some groups of pesticides are toxic to the non-targeted organisms. The toxicity depends on their chemical compositions. Beside their chemical compositions, agrochemicals can be grouped/classified based on target organisms. Classification based on targets organisms includes insecticides, rodenticides, herbicides and fumigants.[5–10]

Several brands of herbicides are available for the control of herbs/weeds in agricultural field. Some of these herbicides have negative ecological impacts on the organisms as well as humans that use the substances contaminated by such kind of chemicals unknowingly. Glyphosate [(N-phosphonomethyl-glycine) is a non-selective active and post-emergence herbicides that is used for the control of annual and perennial weeds in agricultural, urban and household settings.[11–14] Glyphosate is an organo phosphorus based herbicides that can be used for the control of broad-spectrum of plants. When glyphosate is applied on agricultural field, it is translocated from the leaves to other parts including tips of stems and roots, rhizomes and even tubers.[11] Subsequently, afterwards wither and dry up (which is characterized by chlorosis and necrosis). The drying up of the plant arising from applications of glyphosate is due to the decline in the activity of tryptophan, phenylalanine, and tyrosine, protein synthesis, indole acetic acid and chlorophyll production.[11] The ability of the herbicides to dry up the weed suggests that it has desiccation potentials against crop.

In the environment such as soil, glyphosate has the tendency to bind with the organic matters thereby making the toxicant unavailable in the soil for a very long period of time. Probably due to this effect, its activity on the soil is very low. Rzymski et al.,[11] also reported that glyphosate do not have chemical compounds that have highly reactive potentials, transports/ mobility and recalcitrant to biodegradation. In many part of the world it has been licensed as ideal herbicides for the control of weeds. Pieniążek et al.,[15] reported that glyphosate have been approved for use in more than 130 nations and commercially available as RoundupTM among others.

Glyphosate is basically soluble in water and can easily ionize as anions and can be adsorbed to sediments of pH > 3.5.[11] Like other pesticides, attentions have been draw to careless discharge of remains of empty cans used for storing pesticides. Most of these empty cans are discharged close to the aquatic ecosystem, or land and can also be transported to the aquatic ecosystem due to effects of runoff resulting from precipitation.[8–10,16–22] In some coastal regions of Niger Delta, some inhabitants mix glyphosate with other selective herbicides which they use for the control of some plant that thrives in swampy or moist areas. To this effect, the roles of glyphosate based herbicides have been widely studied using fishes as test organisms. In the Niger Delta region of Nigeria, fishes of the family Claridae especially *Heterobranchus bidorsalis* and *Clarias garepinius* have been widely used for ecotoxicological studies. The choice of these fish species has been attributed to their ability to thrive in harsh environmental conditions. Inyang and Ollor[13] reported that rhonasate 360sl containing glyphosate (isopropylamine salt) could cause an alteration in enzymes, urea and creatine of adult *Heterobranchus bidorsalis*. Furthermore, Inyang and Patani[14] also reported that glyphosate (isopropylamine salt) could cause an alteration in electrolytes and some haematological parameters of *Heterobranchus bidorsalis*. Inyang et al.,[19] also reported that rhonasate 360sl containing glyphosate could also cause

an alteration of organsomatic indices of *Heterobranchus bidorsalis.*

Acute toxicity of glyphosate based herbicides have been widely reported using different fish species as test organism including freshwater fish, *Cyprinus carpio*,[23] Arabian killifish, *Aphanius dispar*,[24] *Tilapia zillii*,[12] *Clarias garepinus*[25] but scientific information on *Heterobranchus bidorsalis* is scarce in literature. Hence, this study evaluated acute toxicity of 360g/l glyphosate (in the form of 480g/l isopropylamine salt) against fingerlings of *Heterobranchus bidorsalis.*

Materials and methods

Source of the fish and acclimation processes

The fingerlings of *Heterobranchus bidorsalis* with mean length 6.00cm were purchased from a private fish farm in Yenagoa metropolis, Bayelsa state. The fish samples were transported to the laboratory with 20 liter rubber cans in their natural water. In the laboratory, the fish were allowed to acclimatize for seven days in a rectangular aquarium. During the process, the fish were fed with their normal copen fish diet. During the acclimation period, the water was renewed daily.

Trial Test

During the trial test, a concentration of 10ppm, 35ppm, 50ppm and 75ppm of 360g/l glyphosate (in the form of 480g/l isopropylamine salt) was prepared in the aquarium. Thereafter, the bioassays were renewed every 24hours for 48hours. This was carried out to determine the sub-lethal concentration for the main experimental run.

Main experiment

Based on the trial test result, seven different concentrations of glyphosate (i.e. in the form of 480g/l isopropylamine salt) were made viz: 0.00ppm, 12.00ppm, 15.00ppm, 18.00ppm, 21.00ppm, 24.00ppm and 27ppm. These concentrations were made by using the formula previously described by Inyang et al.,[20–22] mls x stock solution (mg/L)=aquarium water (ml) x desired concentration (ppm ormg/l).

Then after, 12 individuals of fish were introduced into the aquarium containing the toxicant and the aquarium water was renewed at every 24hours. This was carried out in triplicates. Mortality was determined when the fish did not respond to repeated prodding.[26]

Mortality rate of the fish samples was calculated as:

$$\text{Mortality rate} = \frac{\text{Number of dead fish}}{\text{Total number of fish exposed to glyphosate}} \times 100\%$$

Some of the physiochemical properties of the water used for fish bioassay was analyzed following the scheme presented by APHA[27] and the results were in the range of Temperature 27°C, pH 6.07-6.45, conductivity 92.41– 139.08µ/cm, Turbidity 0.15–0.79 NTU and dissolved oxygen 5.21–7.32mg/L.

Statistical analysis

The percentage mortality was subjected to one way analysis of variance (ANOVA). Significant difference was established at P=0.05, and Waller Duncan statistics was used for multiple comparison among the means. The percentage mortality was transformed to probit using Finney's Table. Then regression analysis was carried out for probit values against logarithm of the concentration using Microsoft excel. From the regression analysis, the equation was derived based on the intercept (b) and x (value) obtained. The LC50 was then calculated by substituting the probit value of 50 in the equation Y= b+ ax in which variable x is known and a= unknown and b= intercept. The anti-logarithm value of "a" was taken as the LC50.

Results and discussion

The percentage mortality of *Heterobranchus bidorsalis* exposed to 360g/l glyphosate (in the form of 480g/l isopropylamine salt) is presented in Table 1. At 27ppm of the toxicants, 39.39ppm, 53.67 %, 73.33 % and 86.90 % of the fish died at 24hours, 48hours, 72hours and 96hours, respectively. Typically, as the concentration of the toxicant increased, the mortality increased significantly ($p<0.05$) for each of the period of bioassay (24hours, 48hours, 72hours and 96hours). The increased mortality of the fish as the concentration of the toxicant increased could be due to the effect of stressed and/ or alteration of the various organs/systems (viz: electrolytes, haematological, histopathology, enzymes and metabolites) of the fish. Previous studies have also indicated that toxicant could affect the various biochemical and physiological responses in fish.[28,29] The trend of this study is in consonance with the findings of Oyoroko & Ogamba[26] on the effects of detergents of some common Niger Delta fishes (*Heterobranchus bidorsalis, Clarias garipenus*), and work of Seiyaboh & Izah[29] on the effect of cassava mill effluents on mortality rate of *Heterobranchus bidorsalis*.

Table 1 Percentage mortality of *Heterobranchus bidosalis* exposed to 360g/l glyphosate (in the form of 480g/l isopropylamine salt)

Concentration, ppm	Hours				
	0	24	48	72	96
0.00	0.00±0.00	0.00±0.00^{a}	0.00±0.00^{a}	0.00±0.00^{a}	0.33±0.58^{a}
12.00	0.00±0.00	0.00±0.00^{a}	11.10±3.82^{ab}	15.53±3.87^{b}	19.78±6.34^{b}
15.00	0.00±0.00	8.90±3.81^{b}	13.33±6.65^{b}	15.53±3.87^{b}	26.67±6.65^{bc}
18.00	0.00±0.00	13.30±0.00^{b}	17.77±2.87^{bc}	24.47±3.87^{bc}	33.30±0.00^{c}
21.00	0.00±0.00	15.53±3.87^{b}	26.67±6.65^{cd}	33.33±6.65^{c}	48.92±3.82^{d}
24.00	0.00±0.00	24.44±7.70^{c}	35.83±10.63^{d}	53.33±6.65^{d}	62.23±3.87^{e}
27.00	0.00±0.00	39.93±6.60^{d}	53.67±6.16^{e}	73.33±6.65^{e}	86.90±6.30^{f}

Table 2 shows the LC50 values of *Heterobranchus bidorsalis* exposed to 360g/l glyphosate (in the form of 480g/l isopropylamine salt). The LC50 values were 25.88ppm, 22.91ppm, 22.39ppm and 19.50ppm at 24hours, 48hours, 72hours and 96hours of exposure, respectively. The LC50 values decreased as the exposure period increased. This suggests that as the exposure duration increased, the toxicant became more lethal to the experimental fish.

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Table 2 LC50 values of *Heterobranchus bidosalis* exposed to 360g/l glyphosate (in the form of 480g/l isopropylamine salt)

Hours	Equation for the regression analysis	LC 50
24	Y = 11.36396x-11.054	25.88
48	Y = 3.689982x-0.39639	22.91
72	Y = 4.531713x-1.1996	22.39
96	Y = 5.153527x-1.65075	19.50

The LC 50 values of this study were in consonance with the work of Gabriel & Erondu[25] that reported LC50 value of 19.58mg/l in fingerling of *Clarias gariepinus* with mean weight of 1.22g and length of 5.25cm exposed to Roundup (glyphosate) for 96hours. The results of this study were, however, not in conformance with previous works of Bawa et al.,[23] who reported LC50 value of 3.26ppm for freshwater fish, *Cyprinus carpio* with mean weight of 3.0g and length of 5.5cm exposed to glyphosate after calculating the 96hours. Messaad and Al Zailaie[24] reported LC 50 value of 115.25mg/l in Arabian killifish, *Aphanius dispar* with mean weight of 1.5 g and length of 4.5 cm exposed to glyphosate. Nwani et al.,[12] reported LC50 value of 477.79mg/l, 296.43mg/l, 253.21mg/l and 211.80mg/l at 24, 48, 72 and 96hours, respectively in *Tilapia zilli* with mean weight of 8.204g and length of 6.77cm exposed to glyphosate based herbicides (forceup). The variation could be due to the age and size of the fishes as well as the differences in their biochemical compositions.

Conclusion

This study investigated the toxicity of *Heterobranchus bidorsalis* exposed to 360g/l glyphosate (in the form of 480g/l isopropylamine salt). The findings showed that the toxicant effects on the fish increased (i.e. increased mortality) as exposure period increased. Hence, caution should be exercised during the use and discharge of empty cans of herbicides containing 360g/l glyphosate (in the form of 480g/l isopropylamine salt) close to the aquatic ecosystem especially in the coastal regions of the Niger Delta, Nigeria.

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None

Conflict of interest

Authors declare that there is no conflict of interest.

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