

**Behavioral Response and Acute Toxicity of Fingerlings of African Cat Fish, *Clarias Gariepinus*
Exposed to Paraquat Dichloride**

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Abstract

This study evaluated the behavioural response and toxicity of paraquat dichloride to fingerlings of *Clarias gariepinus*. The fishes were acclimatized for 14 days and exposed to sublethal concentration of 0.00 ppm, 16.56 ppm, 22.08 ppm, 27.60 ppm, 33.12 ppm and 38.64 ppm. A 24 hours' renewal bioassay was adopted in this study. Results showed that the fishes exhibited change in swimming, opercular movement, body pigmentation, surfacing and air gulping. Mortality rate increased significantly at $p < 0.05$ as the concentration of the toxicant increased as well as the exposure period. LC_{50} values at 24, 48, 72 and 96 were 59.95, 47.59, 38.12 and 26.18 ppm, respectively. Based on the results, Paraquat dichloride users need to discard the remains of empty cans properly to avoid contamination. Also there is need to exercise caution when using paraquat dichloride based herbicides in agricultural fields close to surface water resources.

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Introduction

In the recent times environmental resources (soil, water and ambient air quality) are threatened due to pollution resulting from anthropogenic activities [1-6]. The potability and domestic utilization of water resources especially by humans have been widely studied [7-11]. Surface water exists as freshwater, brackish and marine water. They are basically classified based on their physicochemical characteristics especially salinity, conductivity, hardness etc. Each of these surface water resources have their unique features they possess. This surface water is the major recipients of wastes streams resulting from human activities. Studies have indicated that surface water such as Epe creek, receives different kinds of wastes resulting from anthropogenic activities which has led to changes in the physical, chemical and biological characteristics of the surface water of the creek [12, 13]. Sewage is also discharged into the aquatic ecosystem through direct means especially in several coastal communities in Bayelsa state [14, 15]. After rainfall, runoff carries several materials and deposits them in the aquatic ecosystem.

Instances of substances recalcitrant to degradation (such as heavy metals) has been reported in surface water system in the Niger Delta [2, 16]. Some of these toxic substances have the tendency to bioaccumulate in fishes [17-21]. Typically, fishes are among the common protein source, and harvesting from the wild is a source of livelihood to indigenous people in many coastal regions of Bayelsa state, Nigeria.

Pesticides are substances that are used for controlling pest in homes and agricultural field. In the recent times, pesticides use is on the increasing trend. As such, pesticides are among the important pollutants of the aquatic ecosystem. Despite their roles in controlling pests in the environment, many of them pose a great problem to the environment probably due to their toxicity to animals and human. Their toxicity level depends on their chemical compositions and as well as the immune system and other metabolic pathways of the target organisms.

Paraquat, a non-selective, quaternary nitrogen based herbicide is use in controlling weeds [22, 23]. Paraquat dichloride act as a defoliant in plant

tissues [22, 23]. Paraquat dichloride kills herbs through contact processes. Apart from the toxicity in plants, its effects in human have been reported in literature. For instance, Ariyu et al. [24] reported that paraquat dichloride enter the body through inhalation and ingestion processes, and damaged skin contact and its detrimental to humans because it has the capacity to cause multi-organ dysfunctioning and respiratory problems.

The remains of cans of herbicides if not properly discarded could end up in the water ecosystem especially in coastal region where most of their municipal waste are dumped in aquatic ecosystem/ and or close to surface water resources. In addition, after rainfall, runoff could wash herbicides used in agricultural field close to surface water resources were they could impact on the receiving water quality.

Fish are sensitive to aquatic pollutants, and as such they have been widely used to ascertain the effects of contaminants on aquatic ecosystem. Many herbicides have the tendency to cause dysfunctioning in the reproductive, food conversion efficiency, growth and mortality rates in fishes. Fishes have been widely used as bio-indicator in assessing pollution level in aquatic ecosystem. Hence this study focused on the behavioural response and acute toxicity of *Clarias gariepinus* fingerlings exposed to paraquat dichloride.

Materials and Methods

Source of Fish, Transportation and Acclimation

Two hundred fingerlings of *Clarias gariepinus* with mean length of 6cm and weight of 6.5g was obtained from the Niger Delta University earthen fish farm using drag net. The fish was allowed to adapt for 2 weeks in a circular concrete aquarium. A daily renewal bioassay was carried out were test water, and sub-lethal concentrations of paraquat dichloride. Fish was also fed with their normal fish diet. During the process <1% death were recorded.

Range Finding Test (Trial Test)

A static renewal bioassay technique was employed in this experiment. During the process, the test toxicant (paraquat dichloride) and test solution (water) were renewed daily. A range finding test (trial test) was carried out using the toxicant in the following concentrations (15ppm, 25ppm, 35ppm and 45ppm) to

determine a safe sub-lethal concentration for the main experimental run. Each of these concentrations contains 4 fishes each.

Main Experiment

A 0.00 mls (control) 0.6mls, 0.8mls, 1.0mls, 1.2 mls and 1.4mls of paraquat dichloride (276g/L) (equivalent to 276000 mg/L) was pipetted into rectangular aquarium containing 10 litres of water (equivalent to 10000 mg/L). This brings the concentration to 0.00ppm (control), 16.56ppm, 22.08ppm, 27.60ppm, 33.12ppm and 38.64ppm for *Clarias garipinus*. Triplicate experimental groups (containing 10 fishes each) were carried out, and the test solution were renewed daily. The concentration of the toxicants was prepared based on the approach previously used by Inyang *et al.* [25 – 27]:

Mortality Determination

Mortality was established when they failed to respond to repeated prodding [28]. Mortality rate of the fish samples were calculated as:

$$\text{Mortality rate} = \frac{\text{Number of dead fish}}{\text{Total number of fish exposed to the paraquat dichloride}} \times 100$$

Behavioral Response

The behavioral changes were determined by physical observation of the fishes at different concentration of the paraquat dichloride. The behavioural response of the fish was assessed based on the features presented by Inyang *et al.* [27], Oyoroko and Ogamba [29], Nwani *et al.* [30, 31], Ladipo *et al.* [32], Ayoola [33].

Water Quality Analysis

The in-situ water quality parameters analyzed include pH, temperature, conductivity and salinity. All the in-situ characteristics were carried out following manufacturers guide. The pH, conductivity and salinity were determined using in-situ portable pH meter. The calibrated pH conductivity and salinity meter electrode was then dipped in water samples, and the readings were taken when the values that displayed in the meters were stable. While the temperature was measured using thermometer.

Statistical Analysis

The statistical analysis was carried out using Statistical package for social sciences version 20. The data were expressed as mean ± standard error, and one-way analysis of variance was used to show significant variations at p=0.05. Waller Duncan statistics was used to ascertain the source of variations at p=0.05. The percentage mortality was transformed to probit using Finney’s table [34]. Microsoft excel was used to carry out regression analysis of probit values against logarithm of the concentration. from there the LC₅₀ was obtained [22, 35].

Results and Discussion

The water quality characteristics of the aquarium for the various concentrations are presented in Table 1. The pH, temperature, conductivity and salinity of the water ranged from 6.3 – 6.8, 25 - 27 °C, 63.2 – 131.5 μS/cm and 0.02 – 0.04 ‰, respectively across the various concentrations. The water quality results including salinity and conductivity showed that the water is a freshwater and within the range that support fish life as variously reported in surface water in

Table 1. Water quality characteristics of the aquarium

Parameters	Concentration, ppm for <i>Clarias gariepinus</i>					
	0.00	16.56	22.08	27.6	33.12	38.64
pH	6.4	6.8	6.7	6.6	6.5	6.4
Temperature, °C	25.0	25.0	25.0	26.0	26.0	27.0
Conductivity, μS/cm	63.2	86.5	94.9	92.4	109.6	131.5
Salinity, ‰	0.02	0.03	0.03	0.04	0.03	0.00

Bayelsa state [7-14]. The pH and temperature is within the concentration to support fish life. Slight variation exists among the different concentrations but this could be due to the effect of increased concentration of the toxicant and/ or unstable nature of these parameters.

The Behavioral response of *Clarias gariepinus* exposed to varying concentration of paraquat dichloride. The fishes showed moderate swimming and opercular movement at lower concentration which decreased as the concentration increased. Body pigmentation, Intermittent swarming and Jerky movement were not observed in the control, but their rate increased as the concentration of the toxicant increased. The rate of surfacing and air gulping increased as the concentration of the toxicant increased. Behavioural response and mortality are some of the parameters used to examine the early toxicity of a toxicant to fishes. Several authors have reported the role of fish in monitoring pollution in aquatic ecosystem [36-39]. As such when the fishes are stressed they responds through their behaviours [29]. The behavioral changes observed in this study has some similarity with previous reports. Akinsorotan *et al.* [23] reported behavioral response such as restlessness, erratic swimming, loss of equilibrium, discolouration, and sudden fish death among *Oreochromis niloticus* fingerlings exposed to paraquat. Ladipo *et al.* [32] reported that herbicides containing paraquat causes abnormal behaviour in fish: erratic swimming, sudden quick movements and

restlessness. The authors further reported that high concentration of the toxicant caused general weakness and made the fish to settle at the bottom of the aquarium. In addition, the findings of this study also align with non-herbicide toxicants such as detergent as reported by Oyoroko and Ogamba [29], Ogundiran *et al.* [36], Chandanshive [37, 38], Ndome *et al.* [39]. The behavioural characteristics observed in this study suggests toxicological stress on the test organisms.

The percentage mortality of varying concentration of paraquat dichloride exposed to the fingerlings of *Clarias gariepinus* is presented in Table 2. The mortality rate at 0.00ppm, 16.56 ppm, 22.08 ppm, 27.60 ppm, 33.12ppm and 38.64 ppm was 0.00 %, 23.33%, 43.33%, 50.00%, 56.67% and 76.67%, respectively at 96 hours; 0.00 %, 20.00%, 26.67%, 33.33%, 36.67% and 46.67%, respectively at 72 hours; 0.00 %, 13.33%, 20.00%, 23.33%, 26.67% and 36.67%, respectively at 48 hours; and 0.00 %, 10.00%, 13.33%, 16.67%, 20.00% and 26.67%, respectively at 24 hours. Statistically, there was significant variation ($p < 0.05$) in mortality rate. As the concentration of the paraquat dichloride increased mortality rate of the fishes increased. In addition, the mortality rate also increased as the exposure period increased apart from the control. This trend has been reported by Ladipo *et al.* [32], Oyoroko and Ogamba [28], Seiyaboh and Izah [40], Aghoghovwia and Izah [22, 35]. In fingerlings of *Heterobranchus bidorsalis* exposed to paraquat

Table 2. Mortality rate of varying concentration of paraquat dichloride exposed to the fingerlings of *Clarias gariepinus*

Concentration , ppm	24, hours	48, hours	72, hours	96, hours
0.00	0.00±0.00a	0.00±0.00a	0.00±0.00a	00.00±0.00a
16.56	10.00±0.00ab	13.33±3.33ab	20.00±0.00b	23.33±3.33b
22.08	13.33±3.33b	20.00±5.77b	26.67±3.33bc	43.33±3.33cd
27.60	16.67±3.33bc	23.33±5.77bc	33.33±5.77c	50.00±5.77d
33.12	20.00±5.77bc	26.67±3.33bc	36.67±3.33cd	56.67±3.33e
38.64	26.67±3.33c	36.67±3.33c	46.67±3.33d	76.67±3.33

Data is expressed as mean ± standard error. Different letters indicate significant difference at $p < 0.05$ according to Waller Duncan statistics

dichloride Aghoghovwia and Izah [22] reported percentage mortality of 0.00 %, 23.33%, 43.33%, 66.67%, 80.00% and 93.33% at 0.00ppm, 15.00ppm, 18.00ppm, 20.00ppm, 22.00ppm, and 24.00ppm respectively.

Figure 1 presents LC₅₀ values of fingerlings of *Clarias gariepinus* exposed to paraquat dichloride. The LC₅₀ values at 24, 48, 72 and 96 were 59.95, 47.59, 38.12 and 26.18ppm, respectively. In this study there was no mortality observed at 0.00ppm of the toxicant (control) from 0.00 hours to 96 hours. The LC₅₀ values

observed in this study is higher than the value of 1.75mg/l at 96 hours of juvenile *Clarias gariepinus* exposed to paraquat [32]. Again the findings of this study is comparable to value of 30.2, 22.72, 20.23 and 18.44 ppm at 24, 48, 72 and 96 hours, respectively in fingerlings of *Heterobranchus bidosalis* exposed to paraquat reported by Aghoghovwia and Izah [22]. Akinsorotan *et al.* [23] reported that at 96 hours, LC₅₀ value of 40.768 mg/l. The variation could be due to the fishes species, age, length and weight, and biochemical makeup of the fishes as well as the chemical

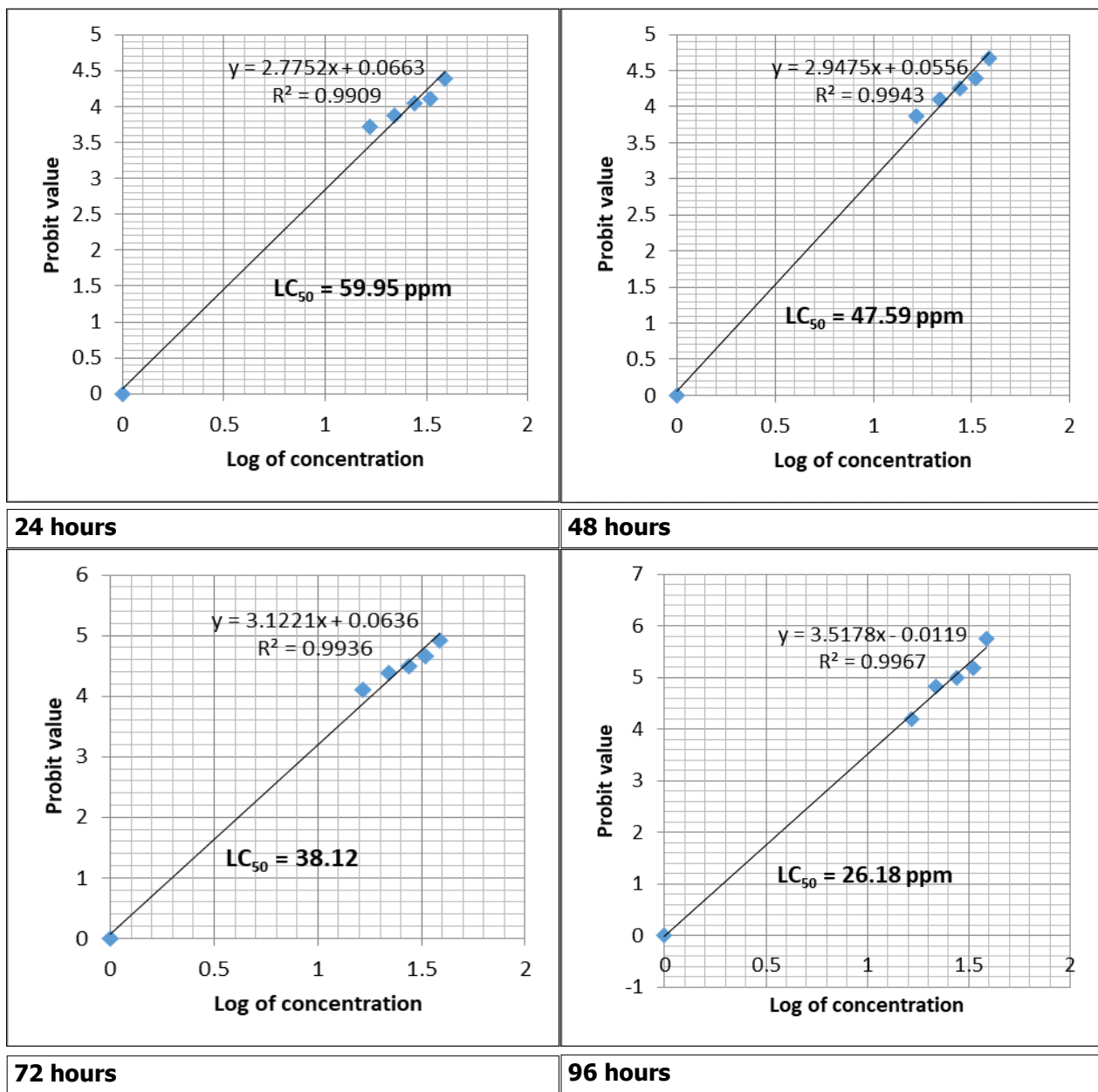


Figure1. LC₅₀ value of the paraquat dichloride exposed to fingerlings of *Clarias gariepinus* for different time intervals

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properties of the herbicide (toxicant) [22, 23, 35]. The decline in the LC₅₀ as the exposure period increased suggests the effect of stress [22, 23, 35]. Furthermore, paraquat containing herbicides could cause alteration in cellular and biochemical activities in the fish due to the effect of stress [41].

Conclusion

This study demonstrated that herbicides such as paraquat dichloride are toxic to fingerlings of *Clarias gariepinus*. The study also showed that the toxicity depends on fish species. The results showed that the mortality rate increased as exposure period and concentration increased. The LC₅₀ values showed that paraquat dichloride is lethal to fingerlings of *Clarias gariepinus*. Based on the results, Paraquat dichloride users need to properly discard the remains of empty cans to avoid contamination. Also there is need to excise caution when using paraquat dichloride based herbicides close to aquatic ecosystem.

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