

Acute Toxicity of Paraquat Dichloride on Fingerlings of *Oreochromis niloticus*

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Abstract: The toxicity effect of paraquat dichloride on fingerlings of *Oreochromis niloticus* was investigated. The fishes were allowed to acclimatize in the laboratory for 2 week. Range finding test was carried prior to final test. At the definitive test the fishes were exposed to lethal concentrations of 0.00mg/L, 27.60mg/L, 33.12mg/L, 38.64mg/L, 44.16mg/L and 49.64mg/L of paraquat dichloride for 96 hours following renewal bioassay. The toxicity bioassay showed that the 96 hours LC₅₀ was 40.768 mg/l. Behavioral responses which include restlessness, erratic swimming, loss of equilibrium, discoloration, and sudden fish death were observed in the exposed fish and these varied greatly with differences in concentrations of the toxicant. Mortality increases with increase in concentration. The differences observed in the mortalities of *Oreochromis niloticus* at varying concentrations were significant ($p < 0.05$), an indication that mortality could be a factor of concentration and time of exposure. The physico-chemical parameters of the water used for the experiment were within the tolerated limit. Based on the findings of this study there is the need to control the use of paraquat based herbicides close to aquatic ecosystems.

Keywords: Aquatic pollution, Fishes, Paraquat dichloride, Toxicity

1. INTRODUCTION

In recent times, population growth, industrialization, urbanization, extensive agricultural practices leading to deforestation and use of agrochemicals appears to be contributing to anthropogenic activities that could impacts on the environmental sustainability. Human activities due to deforestation have led to soil erosion in many regions of the world especially in developing nations. Most soil pollutant end up in nearby surface water including rivers, streams, creeks, creeklets, ponds, etc. Studies have suggested that water quality is rapidly declining due to human activities in the ecosystem especially in developing nations (Aghoghovwia *et al.*, 2018a-c, Izah *et al.*, 2016; Izah and Srivastav, 2015; Agedah *et al.*, 2015; Ben-Eledo *et al.*, 2017a,b; Seiyaboh and Izah, 2017a,b; Seiyaboh *et al.*, 2017; Ogamba *et al.*, 2015a-c).

Pesticides are used to control pests. Their roles depend on the target organisms. For instances, pesticides that are used in controlling weeds, ticks, insects and rodents are knows as herbicides, acaricides, insecticides and rodenticides respectively. In modern day agriculture the use of herbicides in agricultural fields, urban setting have increased (Aghoghovwia and Izah, 2018a,b). Most herbicides have the tendency to endanger non target organisms at certain concentration. This typically occurs when the herbicides end up in the aquatic ecosystem due to runoff after rainfall (soil erosion) (Inyang *et al.*, 2016a-f, 2017a-d, 2018; Ojesanmi *et al.*, 2017; Ogamba *et al.*, 2015d).

The increasing use of herbicides and pesticides in agriculture (including commercial and household production of vegetables) for the control of pest and herb causes chemical pollution of aquatic environment. The chemical pollution causes potential health hazards to live stock, especially to arthropods, fish, frogs, birds and even mammals. The toxicity level depends on route of exposure, concentrations, age of the organisms.

Paraquat is a common brand of non-selective herbicides used for the control of several broad leaved and grasses in plantations and other weeds in non-crop land/ urban and household settings worldwide (Aghoghovwia and Izah, 2018a). The authors further reported that paraquat dichloride acts as a defoliant for some plants. Basically paraquat is a quaternary nitrogen based herbicides that destroy plant tissues. Furthermore, paraquat acts on the tissue of several plant through contact processes (Banaee *et al.*, 2013; Aghoghovwia and Izah, 2018a).

The toxicity of paraquat based herbicides on human health is based on inhalation, ingestion and damaged skin integrity (Arivu *et al.*, 2016). The exposure due via damaged skin tissues could be associated to the corrosive nature (Aghoghovwia and Izah, 2018a). Toxicity of Paraquat through the use of leaking knapsack sprayer has been documented by Arivu *et al.* (2016). Despite the fact the manufacturers have considered it safe for use, Ariyu *et al.* (2016) have reported that its toxic to human health because of its tendency to cause multi-organ failure (viz: lungs, heart, kidneys, adrenal glands, central nervous system, liver, muscles and spleen) and induce respiratory disorder.

Toxic substances in aquatic environment can affect fish growth indirectly by reducing food availability, or directly by changing their metabolism processes (Nwaniet *et al.*, 2014). Generally most herbicides have the tendency to hamper reproduction, food conversion efficiency, growth and mortality among aquatic organisms. Fish are among the organisms used in ecotoxicological studies probably due to their sensitive to the aquatic pollutants. To this effect cat fish especially *Clarias gariepinus* (Ladipo, 2011), and *Heterobranchus bidorsalis* (Aghoghovwia and Izah, 2018a) are the most common group of fish use in toxicological studies in Nigeria. Tilapia, *Oreochromis niloticus* which belong to the family Cichlidae is a freshwater surface feeding omnivore fish. The fish has fast growth rate. *Oreochromis niloticus* is basically a freshwater fish some can adapt to brackish and/ or marine water. Hence, this study aimed at determining the toxicity of paraquat based herbicides on the fingerlings of *Oreochromis niloticus*.

2. MATERIALS AND METHODS

2.1. Source of the Experimental Fish and Acclimatization

The experimental fish, *Oreochromis niloticus* fingerlings were purchased at a fish farm in Ikole, Ekiti state and transported to laboratory with an open keg to the wet laboratory of Fisheries and Aquaculture Department, Federal University Oye-Ekiti, Ekiti State where the experiment was conducted. The average weight and length of the fish was 7.50g and 5.66cm respectively. The fingerlings were acclimatize in a 25litres rectangular plastic tank for 2weeks and feed on a pelleted feed. The water were changed daily during acclimatization and fed twice daily.

2.2. Experiment Design

A total of 216 *Oreochromis niloticus* fingerlings used were divided into six treatment (including 1 control and 5 different concentration of the toxicant) with three replicates. 12 fingerlings were stocked into each tank with replicate treatment. A range finding test (trial test) was carried out using the paraquat as a toxicant. Six concentrations of the paraquat were prepared from the original (276g/l).

2.3. Experimental Protocol

The range finding test previously described by Akinsorotan (2015) adopted in this study. The range finding test was necessary to determine the LC₅₀ value of the test chemical on the fish. This was carried out with the permission of the animal ethic committee of Federal University, Oye Ekiti, Ekiti State, Nigeria. Prior to the definitive test, the fish were starved for 24 hours. This was necessary to reduce water pollution as a result of decomposition of fecal droppings. It was also necessary to reduce external stress to the fish that could arise from feeding. The definitive concentration of 0.00mg/l, 1.5ml, 1.8ml, 2.1ml, 2.4ml, and 2.7ml were converted into 0.00mg/L, 27.60mg/L, 33.12mg/L, 38.64mg/L, 44.16mg/L and 49.64mg/L respectively and were measured with pipette from paraquat dichloride solution of 276g/l and were introduced into experimental tank of 15litres of water in each tank. The definite test was carried in triplicate. All procedures were in accordance with the ethical standard of the animal ethic committee of Federal University, Oye Ekiti, Ekiti State, Nigeria.

2.4. Behavioral Response

The behavioral and morphological response of the fish is observed after exposure of the fish to various concentrations of the toxicant. The control tank was monitored with the tanks of various concentrations of the toxicant, assessing any behavioral or morphological changes. The responses which are air gulping index, opercular ventilation count, tail fin movement, restlessness, erratic swimming, barbell deformation, loss of reflex were recorded from 24hrs-96hrs. The air gulping index, opercular ventilation count and tail fin movement rate was carried out for 96 hours which were counted using stop watch at 12, 24, 48, 72 and 96 hours per minutes (Okechukwu *et al.*, 2013). Two fish were used for the counting per tank and values were computed.

2.5. Mortality Rates

Mortality was also recorded from 24 - 96hrs. Mortality was confirmed when the fishes did not respond to repeated prodding (Oyoroko and Ogamba, 2017a). Mortality rate of the fish samples were calculated as:

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

2.6. Water Quality Analysis

The water quality parameters viz: temperature, dissolved oxygen, conductivity pH were determined using water analysis kit (Model SX751pH ORP/Conductivity/DO). Each of the parameters analyzed was calibrated with the corresponding fluids prior to analysis.

2.7. Statistical Analysis

Probit analysis (Finney, 1971) was used to determine LC₅₀. Data was subjected to one-way analysis of variance (ANOVA) using SPSS software version 16.0 to test for the significant differences between means. Graph analysis were plotted using Microsoft excel window 2010.

3. RESULTS AND DISCUSSION

Temperature and dissolved oxygen ranged from 22.53 – 24.13°C and 5.38 – 5.68mg/l respectively across all the exposure period. The pH was within the range recommended for water mean for aquaculture ranging from 6.53 – 7.10 for all the exposure period. pH typically has the tendency to cause an alteration biochemical activities of the aquarium water probably during oxidation and reduction processes. The conductivity range from 53.53 - 188.40 μS/cm for all exposure duration and concentration (Table 1). At exposure of the toxicant for 96hours, there was an increase in pH and conductivity, and decrease in dissolved oxygen. The fluctuations that occur between the various concentration and time could be due to the fact that these parameters are highly unstable. The water quality parameters under study are within the standard meant for aquaculture purposes. For instances, authors have reported pH of 6.5 – 9.0 to support fish life (Boyd, 1979; Okomoda and Ataguba, 2011; Oloruntuyi *et al.*, 1993). Bhatnagar and Devi (2013) also reported that catfishes and other air breathing fishes can tolerate low Dissolved oxygen concentration of 4 mg/l. Hence the dissolve oxygen content is within the values necessary for fish life. Basically in aquatic ecosystem temperature is very essential for the survival of fishes through metabolism. As such inability of fish to adapt to the environment could cause a change in their physiological response which could lead to mortality. The temperature is within the ambient temperature of the area and values of surface water resources in Nigeria (Agedah *et al.*, 2015; Ben-Eledo *et al.*, 2017a). Hence the fishes may not have died due to temperature.

Table1. Water quality parameters of paraquat dichloride herbicide on fingerlings of *Oreochromis niloticus*

Exposure period	Parameter	0.00, mg/l	2.76, mg/l	3.31, mg/l	3.86, mg/l	4.42, mg/l	4.97, mg/l
24hrs	Temperature, °C	24.13±0.11	24.03±0.29	24.07±0.06	23.93±0.06	23.97±0.06	23.76±0.1
	DO, mg/l	5.38±0.02	5.39±0.03	5.40±0.01	5.40±0.01	5.39±0.03	5.46±0.02
	pH	6.53±0.06	6.57±0.06	6.53±0.12	6.53±0.06	6.47±0.06	6.53±0.15
	Conductivity, μS/cm	53.53±2.89	75.32±1.14	139.60±16.39	132.50±4.30	139.83±11.85	137.43±19

48hrs	Temperature, °C	23.53±0.06	23.20±0.17	23.40±0.10	23.60±0.1	23.50±0.10	23.3±0.10
	DO, mg/l	5.52±0.02	5.55±0.01	5.54±0.01	5.50±0.03	5.35±0.30	5.55±0.01
	pH	6.70±0.10	6.73±0.06	6.87±0.06	6.77±0.06	6.87±0.06	6.87±0.06
	Conductivity, µS/cm	95.07±5.66	128.33±3.7	188.40±23.98	134.17±3.93	149.43±16.07	173.34 ±23.7
72hrs	Temperature, °C	22.87±0.06	22.77±0.23	22.70±0.10	22.80±0.44	22.77±0.29	22.67±0.1
	DO	5.59±0.06	5.59±0.00	5.60±0.06	5.58±0.02	5.62±0.00	5.61±0.01
	pH	7.10±0.10	7.07±0.06	7.00±0.06	7.10±0.06	7.03±0.01	6.93±0.0
	Conductivity, µS/cm	75.83±3.94	82.43±2.05	159.53±21.00	153.23±11.6	160.73±19.6	177.6±20.88
96hrs	Temperature, °C	22.73±0.32	22.60±0.10	22.57±0.07	22.47±0.16	22.53±0.06	22.56±0.11
	DO, mg/l	5.61±0.03	5.62±0.01	5.63±0.06	5.63±0.06	5.62±0.01	5.62±0.11
	pH	6.97±0.06	7.03±0.12	6.97±0.06	6.97±0.06	6.93±0.06	6.80±0.1
	Conductivity, µS/cm	117.67±4.4	102.80±32.5	141.53±17.55	89.50±11.26	96.03±24.3	112.70±19.0

Data were expressed as mean ± standard deviation

Oreochromis niloticus showed behavioral changes on exposure to paraquat herbicide (Table 2 – 4). Immediately the fish were introduced into the tank containing paraquat at concentrations 2.76 mg/l, 3.31mg/l, 3.86mg/l, 4.42mg/l and 4.97mg/l, they became restless and agitated. Fishes came to the surface of water much more frequently. They occasionally tried to jump out of the water. The fish showed abnormal swimming movements including loss of orientation, loss of buoyancy and spasms before death. Typically, acute toxicity of most fishes is characterized by excessive gulping of air and intermittent swarming, erratic swimming, restlessness, loss of movement, increased operculum movement, excessive secretion of mucus and body pigmentation, and jerky movement (Oyoroko and Ogamba, 2017b; Inyanget *et al.*, 2017a). Behavioural response could be affected by concentrations of toxicants, age, species and prevailing environmental condition (Omoniyi *et al.*, 2002).

Air gulping were observed and counted per minute. The result of air gulping of the exposed fish to the toxicant increases especially during 24 hours and 48hours when compared to the control (Table 2). At 0.00mg/l, 27.60mg/L, 33.12mg/L, 38.64mg/L, 44.16mg/L and 49.64mg/L concentrations, the air gulping index were 19.33±0.33, 59.33±0.58, 49.67±8.14, 48.00±5.57, 50.00±6.08 and 62.67 ±2.52 respectively for 24 hours treatment; 26.00±0.00, 83.67±7.57, 76.00±3.00, 67.52±2.08, 88.00±4.58 and 89.00±3.00 respectively for 48 hours treatment and 27.00±5.29, 82.67±0.00, 81.33±6.81, 80.00±6.08, 98.5±6.66 and 81.67±6.35 respectively for 96 hours treatment. The air gulping index showed a significant increase (p<0.05) between 24 hours and other exposure duration except for 2.76mg/l and 4.42 mg/l at 72 hours. The air gulping index showed respiratory distress probably due the effect of the toxicants.

Table2. Effect of acute concentration of paraquat with time on *Oreochromis niloticus* air gulping index movement

Conc(mg/l)	Exposure duration			
	24hrs	48hrs	72hrs	96hrs
0.00	19.33±0.33 ^a	26.00±0.00 ^b	25.00±1.00 ^b	27.00±5.29 ^b
2.76	59.33±0.58 ^a	83.67±7.57 ^b	61.67±5.51 ^a	82.67±0.00 ^b
3.31	49.67±8.14 ^a	76.00±13.00 ^b	79.35±2.08 ^b	81.33±6.81 ^b
3.86	48.00±5.57 ^a	67.52±2.08 ^d	58.33±2.08 ^b	80.00±6.08 ^c
4.42	50.00±6.08 ^a	88.00±4.58 ^b	51.67±5.50 ^a	98.5±6.66 ^b
4.97	62.67±2.52 ^a	89.00±3.00 ^b	84.33±7.23 ^b	81.67±6.35 ^b

Means with the same superscript across the row are not significantly different (p≥0.05). (Mean values ± SE)

The effects of acute concentrations of Paraquat with time on *Oreochromis niloticus* opercular ventilation count is presented in Table 3. At 0.00mg/l, 27.60mg/L, 33.12mg/L, 38.64mg/L, 44.16mg/L and 49.64mg/L concentration the opercular ventilation index were 29.00±0.00, 32.67±1.53, 34.67±4.51, 43.33±3.05, 47.67±3.21 and 52.00±2.00 respectively for 24 hours treatments; 32.33±2.09, 62.33±5.51, 75.00±6.93, 38.67±1.53, 56.67±2.08 and 55.33±5.69 respectively for 72 hours treatments; and 35.00±1.00, 42.33±3.05, 79.33±4.94, 56.67±2.08, 43.00±4.00 and

50.00±1.73 respectively for 96 hours treatments. Statistically, there was significant variation ($p < 0.05$) among the exposure period. Furthermore, the opercular ventilation count were lesser in the control group (0.00mg/l) compared to other concentration groups. The increase in opercular ventilation rate may be associated to the sudden response by the fish due to the shock which is an indication of hyperventilation according to Babatunde and Oladimeji (2014).

Table3. Effects of Acute Concentrations of Paraquat with Time on *Oreochromis niloticus* opercular ventilation count.

Conc(mg/l)	Exposure time			
	24hrs	48hrs	72hrs	96hrs
0	29.00±0.00 ^a	31.33±0.58 ^b	32.33±2.09 ^b	35.00±1.00 ^c
2.76	32.67±1.53 ^a	63.67±3.51 ^c	62.33±5.51 ^c	42.33±3.05 ^b
3.31	34.67±4.51 ^a	79.67±9.61 ^b	75.00±6.93 ^b	79.33±4.94 ^b
3.86	43.33±3.05 ^a	84.67±5.89 ^c	38.67±1.53 ^a	56.67±2.08 ^b
4.42	47.67±3.21 ^a	90.67±3.21 ^c	56.67±2.08 ^b	43.00±4.00 ^a
4.97	52.00±2.00 ^a	89.00±3.00 ^b	55.33±5.69 ^a	50.00±1.73 ^a

Means with the same superscript across the row are not significantly different ($p \geq 0.05$). (Mean values ± SE)

The effect of acute concentration of paraquat on *Oreochromis niloticus* on tail fin movement is presented in Table 4. At 0.00mg/l, 27.60mg/L, 33.12mg/L, 38.64mg/L, 44.16mg/L and 49.64mg/L concentration the tail fin movement were 30.00±6.08, 52.33±5.51, 65.00±5.29, 66.67±10.11, 66.00±1.00 and 64.00±3.61 respectively (24 hours), 47.00±1.00, 52.67±6.66, 97.67±1.53, 63.00±7.81, 67.00±1.00 and 65.67 ±2.08 respectively (48 hours), 22.0±1.00, 44.61±10.50, 85.33±5.51, 76.33±1.53, 68.00±1.73 and 74.00±2.00 respectively (96 hours). There was significant variation ($p < 0.05$) among the exposure period. Furthermore, the opercular ventilation count were lesser in the control group (0.00mg/l) compared to other concentration groups. The results also showed that the tail fin movement of the exposed fish to the toxicant at 24 hours and 72 hours were higher compare to the 96 hours for the control group. Adeogun (2012) opined that an increase in tail movement is due to quest to escape from the toxicant and in the processes causing restlessness. Typically the fluctuations in the tail fin movement, opercular ventilation and air gulping index could be due to survival/adaptation strategy by the fishes when they were exposure to the toxicants (Chindah *et al.*, 2004).

Table4. Effect of acute concentration of paraquat on *Oreochromis niloticus* on tail fin movement

Conc(mg/l)	EXPOSURE TIME			
	24hrs	48hrs	72hrs	96hrs
0	30.00±6.08 ^b	47.00±1.00 ^c	32.67±0.58 ^b	22.0±1.00 ^a
2.76	52.33±5.51 ^a	52.67±6.66 ^a	59.33±7.37 ^a	44.61±10.50 ^a
3.31	65.00±5.29 ^b	97.67±1.53 ^d	53.67±4.04 ^a	85.33±5.51 ^c
3.86	66.67±10.11 ^{ab}	63.00±7.81 ^a	70.00±0.00 ^{ab}	76.33±1.53 ^b
4.42	66.00±1.00 ^a	67.00±1.00 ^a	66.00±1.00 ^a	68.00±1.73 ^a
4.97	64.00±3.61 ^a	65.67 ±2.08 ^a	74.67±0.00 ^b	74.00±2.00 ^a

Means with the same superscript across the row are not significantly different ($p \geq 0.05$). (Mean values ± SE)

The mortality rates and probit value of *Oreochromis niloticus* fingerlings exposed to acute concentration of paraquat dichloride is presented in Table 5. Fish mortality was observed in all the tanks except in the control tanks. At 96 hours the mortality rate at 27.60mg/L, 33.12mg/L, 38.64mg/L, 44.16mg/L and 49.64mg/L of paraquat dichloride were 30.56%, 36.11%, 55.56%, 61.11% and 63.89 % respectively. The probit chart from where the LC₅₀ values was derived from substitution of the probit value of 50 in the equation in the chart and then taken the anti-logarithm value is presented in Figure 1. The result of the acute toxicity showed that paraquat was toxic to *Oreochromis niloticus* with LC₅₀ value of 40.7684 mg/l.

Table4.5. mortality rate and log of concentration in *Oreochromis niloticus* exposed to paraquat

Concentration of paraquat in (mg/l)	Log of concentration	Number of fish exposed	Number of mortality	%mortality	Probit value
0.00	0.000	36	0	0	0.00
2.76	0.441	36	11	30.56	4.00
3.31	0.510	36	13	36.11	4.64
3.86	0.587	36	20	55.56	5.15
4.42	0.645	36	22	61.11	5.28
4.97	0.696	36	23	63.89	5.36

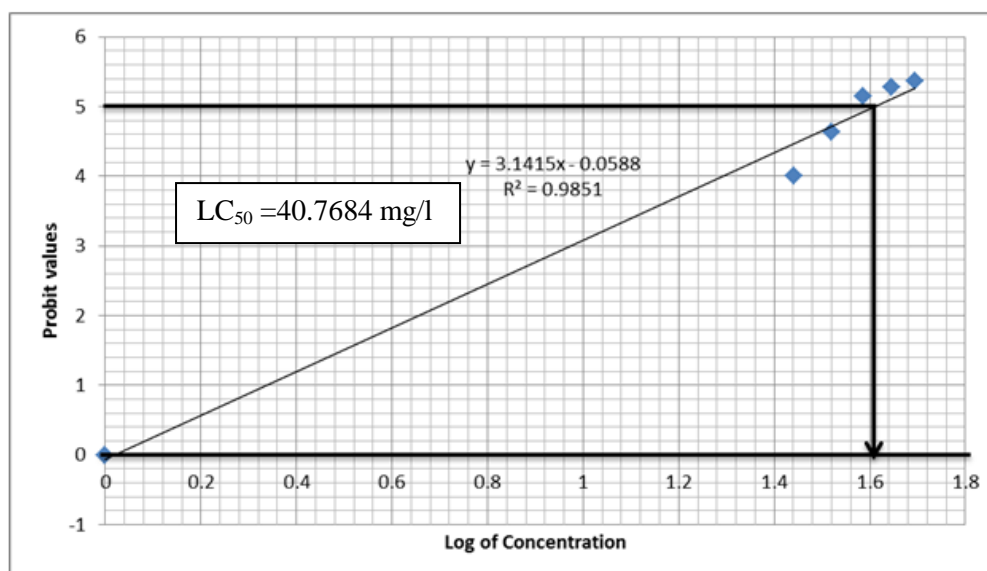


Figure 1. Probit value against log concentration of the toxicant

The LC_{50} values reported in this study is higher than the value of 30.28ppm, 22.72ppm, 20.23ppm and 18.44 ppm reported in 48 hours, 72 hours and 96 hours in fingerlings of *Heterobranchus bidorsalis* exposed to paraquat dichloride as reported by Aghoghovwia and Izah (2018a), 28.13, 27.61, 26.22 and 25.71 mg/l at for 24, 48, 72 and 96 hours respectively in fingerlings of *Labeo rohita* exposed to paraquat as reported by Arivu *et al.* (2016), 7.16 mg/l, 4.45 mg/l, 2.19 mg/l and 1.41 mg/l for 24 hours, 48 hours, 72 hours and 96 hours in adult *Trichogastertrichopterus* exposed to paraquat as reported by Banae *et al.* (2013), 1.75 mg/l after 96 hours of exposing juvenile *Clarias gariepinus* to Paraquat dichloride as reported by Ladipo (2011). Babatunde and Oladimeji (2014) reported LC_{50} value of 12.25mg/l in *Oreochromis niloticus* fingerlings exposed to paraquat. The variation could be due to size of the fish species (age), length and weight, and biochemical characteristics (Aghoghovwia and Izah, 2018a).

4. CONCLUSION

It was observed in this study that behavioural characteristics which include opercular ventilation count, tail fin movement rate and air gulping index were adversely affected by the toxicant used in this experiment. LC_{50} value of 40.7684mg/L was also found to be the acute toxicity of paraquat dichloride to *Oreochromis niloticus* fingerlings. This indicates high mortality threshold. It is therefore concluded that this toxicity, if not checked could lead to behavioural abnormalities as well as death in fish. Hence the usage must be monitored and controlled especially when in use close to any aquatic ecosystem.

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Citation: Ademola Michael Akinsorotan et al., " Acute Toxicity of Paraquat Dichloride on Fingerlings of *Oreochromis niloticus*", International Journal of Research Studies in Biosciences (IJRSB), vol. 7, no. 1, pp. 29-36, 2019. <http://dx.doi.org/10.20431/2349-0365.0701005>

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