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Behavioural and Some Physiological Assessment of Glyphosate and Paraquat Toxicity to Juveniles of African Catfish, *Clarias gariepinus*

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AOI designed and executed the experimental work and wrote the article. OSJ supervised the work and helped in preparation of the manuscript. AJ was involved in designing of experimental work.

Key words
Glyphosate, Paraquat, OVC, TMR, AGI.

ABSTRACT

The impact of acute exposure of *Clarias gariepinus* juveniles to commonly used herbicides, glyphosate and paraquat was evaluated through changes in fish behaviour and mortality. Juveniles of the African catfish were exposed to varying acute concentrations of glyphosate and paraquat. The fishes responded, exhibiting different behavioural abnormalities like hyperactivity, abnormal swimming, restlessness, loss of equilibrum and haemorrhage. Observation of opercular ventilation count (OVC), tail fin movemant rate (TMR) and air gulping index (AGI) showed a marked difference between control and exposed fishes, indicating that the herbicides negatively impact on these parameters. These behavioural and morphological anomalies became more pronounced with increasing concentrations of the herbicides. Mortality was also observed to be concentration dependent. After 96 h of exposure, the 96hr LC_{50} for paraquat was found to be 0.07mg/L while that of glyphosate was found to be 0.530mg/L. The result revealed that glyphosate and paraquat have the ability to induce unusual behaviours in fish and can therefore serve as reliable indicators of toxicity in environmental impact assessment programmes.

INTRODUCTION

Freshwater contamination with a wide range of pollutants has become a matter of urgent concern over the last few decades (Al-Weher, 2008). There is increasing awareness of the potential hazards that exist due to the contamination of freshwater, especially toxic chemicals associated with mining, industrial and agricultural practices (Corbett, 1977; Du Preez *et al*., 2003). Run-off of herbicides from agricultural lands into natural water bodies have become a worldwide phenomenon. Due to the different pollutants entering the aquatic ecosystems, the organisms there are subjected to environmental stresses which may be deleterious to them, to a population or to a community and eventually causing an alteration in the structure of natural ecosystems (Imoobe and Adeyinka, 2010). The quality of fish food is inexorably linked to the health of fish which itself is dependent on the level of pollutants in the aquatic environment (Verma *et al*., 1981).

Glyphosate is a non-selective post-emergence herbicide that is commonly applied in agriculture and forestry for the control or destruction of herbaceous plants in fish-ponds, lakes, canals, slow running water, etc. This herbicide due to the changes of metabolic, oxidative and haematological parameters, may alter the ecological balance causing damage to non-target organisms (Neškovic *et al*., 1996). Paraquat (1,1-dimethyl-4,4-bipyridininum ion) is one of the most common contact and non-selective herbicide for exterminating vegetative pests, is used for controlling terrestrial weeds and aquatic plants in different countries and its presence is reported in many water sources of the world (Filizadeh, 2002; Ye *et al*., 2002; Gao *et al*., 2010; Ismail *et al*., 2011).

Indices for measuring stressed conditions in fish include physiological, morphological, behavioural, serum parameters, histopathology, genotoxicity, cytotoxicity among others. Behavioural responses are very important indicators in the natural and/or external environment of animals. It is first visible sign of stress noticed in an organism. It is a promising tool in ecotoxicology. Behavioural and morphological changes in fish have been used as a diagnostic endpoint for screening and differentiating chemicals according to their mode of action (Drummond *et al*., 1986).

When environmental contaminants like pesticides and other chemicals enter water bodies, they are able to cause depletion of the dissolved oxygen content present

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in it. Pesticides, in sublethal concentrations in the aquatic environment may probably be too low to cause rapid death directly but may affect the metabolism of the organisms, disrupt normal behaviour and reduce the fitness of natural population. The respiratory potential or oxygen consumption of an animal is the important physiological parameter to assess the toxic stress. As aquatic organisms have their outer bodies and important organs such as gills almost entirely exposed to water, the effect of toxicants on the respiration is more pronounced (Panigrahi *et al*., 2014).

C. gariepinus is an important fish for aquaculture in Nigeria because it meets up a partial solution for the increasing demand of protein. It has been artificially reproduced and cultured under various Nigerian aquaculture systems. Ayoola (2008) and Omitoyin *et al*. (2006) have both looked at aspects of glyphosate toxicity to *C. gariepinus* – histopathology and biochemical effects. Doherty *et al*. (2011) also studied histopathological changes in the liver and gills of *C. gariepinus* after exposure to paraquat. However, because these herbicides are widely used, it is imperative to look at other aspects of the unpleasant effects they are able to exert in fish. It is in this light that the present study was undertaken.

MATERIALS AND METHODS

Collection and maintenance of fish

Juveniles of *C. gariepinus* were purchased from a fish farm in Ota, Ogun State, Nigeria. The juveniles which averaged 7.35±2.33 cm standard length and body weight of 3.94±1.51g were used for the study. They were conveyed in a well aerated from the fish farm to the holding units in the laboratory. Ten fish each were held in 25L tanks and allowed to acclimatize for two weeks in dechlorinated water. During this period, the fishes were fed with pelleted diet containing 35% crude protein twice per day at 4% body weight. The fishes were thought to have adapted to laboratory conditions when less than 5% death was recorded during the period; feeding was discontinued 24 h before the start of the experiment.

Preparation of test solutions and exposure of fish

Both herbicides were purchased from a commercial outlet in Lagos, Nigeria. Acute renewal bioassay was conducted in the laboratory following OECD guidelines No 203 to determine the toxicity of glyphosate and paraquat to *C. gariepinus*. Five concentrations each, 0.36, 0.48, 0.60, 0.72 and 0.84mg/L for glyphosate; and 0.018, 0.037, 0.055, 0.110 and 0.221mg/L for paraquat and a control (0.00mg/L) were dispensed into 150L tanks containing dechlorinated water connected to three 25L tanks. Ten fishes were randomly distributed into each test tank and replicated 3 times. The physicochemical parameters of the diluting water (temperature, pH, dissolved oxygen, total hardness, total alkalinity and conductivity) during the acute test period were measured. The control solutions were made up of only dechlorinated tap water. The five concentrations of each herbicide were administered to the fish-holding units once and the response of the fishes was monitored for 96 h.

Behavioural studies

The fishes were exposed to the different concentrations of the toxicants, and observations were made on their behavioural and morphological responses at 12, 24, 48, 72, and 96 h (Drummond *et al*., 1986). The control fishes were simultaneously monitored along with the exposed fishes to establish a reference for any behavioural or morphological change. Responses different from the control and occurring in at least 10% of the fish in each test tank were recorded. The behavioural and morphological responses monitored included loss of equilibrium, startle responses, hyperactivity, abnormal swimming, haemorrhage and general restlessness. Sufficient time was allowed for observation so as to ensure accurate evaluation of each fish. Startle responses were monitored using the following stimuli of overhead moving visual stimulus, vibration stimulus and tactile stimulus. Air gulping index, opercular ventilation count, tail fin movement and mortality.

Air gulping index (AGI) was determined as the number of air gulping activity of fish per tank per minute, divided by the number of fish or surviving fish in the exposed groups. The opercular ventilation count (OVC) and tail fin movement rates (TMR) were determined using a stop watch for two minutes and the average recorded. Fishes were considered dead when the opercular movement ceased and there was no response to gentle probing. This was used as a measure of mortality. The LC_{50} was determined graphically from a table of probit values.

RESULTS

Effect of herbicides on fish behaviour

The response of the fish juveniles to different behavioural and morphological features after exposure to acute concentrations of glyphosate and paraquat are presented in Table I. The control fishes did not show any signs of abnormal behaviours. Acute concentrations of both herbicides were toxic to juveniles of *C. gariepinus*. The behavioural and morphological indexes of toxicity studied, (loss of equilibrium, startle responses, hyperactivity, abnormal swimming, haemorrhage and general

Table I.- Behavioural abnormalities of Juveniles of *Clarias gariepinus* after exposure to acute concentrations of glyphosate and paraquat.

Behavioural anomalies	Concentrations (mg/l)					
	0.00	**0.36**	**0.48**	**0.60**	**0.72**	**0.84**
Glyphosate						
Loss of equilibrium	-	+	+	+	++	++
Startle responses	-	+	+	+	++	+++
Hyperactivity	-	+	+	++	++	+++
Abnormal swimming	-	+	+	+	+++	+++
Haemorrhage	-	+	+	++	+++	+++
Restlessness	-	+	++	++	+++	+++
	0.00	**0.018**	**0.037**	**0.055**	**0.110**	**0.221**
Paraquat						
Loss of equilibrium	-	+	+	++	++	++
Startle responses	-	+	+	++	+++	+++
Hyperactivity	-	++	++	++	+++	+++
Abnormal swimming	-	+	++	++	+++	+++
Haemorrhage	-	++	+	++	+++	+++
Restlessness	-	+	++	++	+++	+++

–, None; +, Weak; ++, Moderate; +++, Strong.

restlessness) were all positive to varying degrees. The behavioural abnormalities of the juvenile fishes increased with increasing concentrations of both herbicides. The effects were more noticeable with paraquat.

The different concentrations of glyphosate and the durations of exposure also had significant effects on the rate of opercular ventilation of *C. gariepinus* (Table II). The highest OVC was observed in the first period of observation *i.e.* 12 h after exposure. The OVC reduced again by the 24th h, a period which represents the lowest OVC. The OVC increased again by the 48th h and peaked at the 96th h. Exposure of fish to acute concentrations of paraquat also has significant effects on the OVCs. In all the concentrations, the OVCs were lowest within the first 12 h and then increased with time, peaked at 72 h and then fell at 96 h (Table II).

TMRs were significantly affected by acute concentrations of glyphosate. Interplay between concentration and time revealed that the TMR was highest in the first 12 h of exposure to glyphosate. After this period, TMR reduced by the 24th h and increased again till the 96th h (Table III).

Comparing with the control, the TMRs in exposed fish were generally lower except during the first 12 h. Similarly, TMRs changed significantly with duration of exposure and with concentration in fish exposed to paraquat. Interaction between concentration and time showed that all the concentrations had the lowest TMR in the 24th h

Table II.- Effect of acute concentrations of glyphosate and paraquat opercular ventilation count of *C. gariepinus* for 12-96 h.

Conc (Mg/L)	Time (h) and OVC				
	12	**24**	**48**	**72**	**96**
Glyphosate					
0.00	48.00 ± 1.00^{Aa}	50.00 ± 1.00^{Aa}	49.00 ± 1.00^{Aa}	50.00 ± 0.58^{Aa}	50.00 ± 1.00^{Aa}
0.36	46.00 ± 1.15^{Aa}	52.00 ± 1.15^{Ab}	50.00 ± 1.06^{Ab}	54.00 ± 1.15^{Ab}	58.00 ± 0.58^{Ac}
0.48	47.00 ± 1.15^{Aa}	40.00 ± 0.58^{Bb}	48.00 ± 1.03^{Aac}	50.00 ± 1.15^{Bac}	51.00 ± 1.15^{Bac}
0.60	60.00 ± 0.58^{Ba}	35.00 ± 0.58^{Cb}	47.00 ± 1.00^{Ac}	48.00 ± 1.15^{Bc}	50.00 ± 0.58^{Bc}
0.72	61.33 ± 1.15^{Ba}	38.00 ± 0.58^{Cb}	44.00 ± 1.00^{Bc}	45.00 ± 0.58^{Cc}	50.00 ± 0.58^{Bd}
0.84	62.00 ± 1.15^{Ba}	38.00 ± 0.58^{Cb}	45.00 ± 1.02^{Bc}	47.00 ± 1.15^{Bc}	49.00 ± 1.15^{Bc}
Paraquat					
0.00	46.00 ± 1.00^{Aa}	49.00 ± 1.15^{Aa}	49.00 ± 1.00^{Aa}	50.00 ± 0.58^{Aa}	50.00 ± 1.00^{Aa}
0.012	12.00 ± 0.58^{Ba}	35.33 ± 1.15^{Bb}	69.00 ± 1.15^{Bc}	67.33 ± 0.58^{Bc}	60.00 ± 1.00^{Ac}
0.037	11.33 ± 0.58^{Ba}	24.00 ± 1.15^{Cb}	69.33 ± 1.15^{Bc}	70.00 ± 1.00^{Bc}	68.67 ± 1.15^{Bc}
0.055	10.00 ± 1.00^{Ba}	21.67 ± 0.58^{Cb}	49.67 ± 0.58^{Ac}	60.00 ± 1.00^{Cc}	50.00 ± 1.00^{Cc}
0.110	10.00 ± 1.00^{Ca}	11.00 ± 1.15^{Db}	75.33 ± 1.15^{Cc}	79.00 ± 1.15^{Dc}	70.00 ± 1.00^{Bc}
0.221	10.00 ± 1.00^{Ca}	10.00 ± 1.00^{Db}	50.00 ± 1.00^{Ac}	57.33 ± 0.58^{Ac}	49.67 ± 0.58^{Cc}

Means with the same capital letter superscript along same column and small letter superscript on the same row are not significantly different ($p\geq0.05$); (Mean values ± SE) n =3; OVC, opercular ventilation count.

Table III.- Effect of acute concentrations of glyphosate and paraquat on tail fin movement rate of *C. gariepinus* over a period of 12-96 h.

Conc (Mg/L)	Time (h) and TMR				
	12	24	48	72	96
Glyphosate					
0.00	102.00±1.00^{Aa}	100.00±0.58^{Aa}	103.00±1.00^{Aa}	102.00±1.00^{Aa}	103.00±0.58^{Aa}
0.36	130.00±0.58^{Ba}	66.00±1.15^{Bb}	75.00±1.15^{Bb}	77.00±1.15^{Bb}	92.00±0.58^{Bc}
0.48	98.00±0.58^{Ca}	56.00±1.15^{Cb}	78.00±1.15^{Bb}	79.00±0.58^{Bb}	90.00±0.58^{Bc}
0.60	128.00±1.15^{Ba}	71.33±1.15^{Db}	85.00±1.15^{Cb}	87.00±0.58^{Cb}	89.00±1.15^{Bb}
0.72	131.00±0.58^{Ba}	73.67±0.58^{Db}	86.00±1.15^{Cc}	81.00±1.15^{Dd}	88.00±0.58^{Bce}
0.84	127.00±1.15^{Ba}	82.00±1.15^{Eb}	88.00±0.58^{Cc}	90.00±0.58^{Cc}	92.00±1.15^{Bc}
Paraquat					
0.00	104.00±1.10^{Aa}	102.00±0.58^{Aa}	104.00±1.00^{Aa}	103.00±0.58^{Aa}	105.00±1.00^{Aa}
0.012	129.00±0.58^{Ba}	90.00±1.00^{Bb}	110.00±1.00^{BCc}	105.00±1.15^{Ad}	102.00±1.15^{BCc}
0.037	125.00±1.15^{Ca}	87.00±1.15^{Bb}	112.00±1.15^{Cc}	106.00±1.15^{Ad}	104.00±0.58^{Cd}
0.055	128.00±1.15^{Ba}	92.00±0.58^{Cb}	108.00±1.15^{Bc}	110.00±1.00^{Bc}	100.00±1.15^{Bd}
0.110	112.00±0.58^{Aa}	89.00±0.58^{Bb}	108.00±0.58^{Bc}	115.00±1.15^{Ca}	102.00±1.15^{BCd}
0.221	130.00±1.00^{Ba}	81.00±0.58^{Db}	107.00±1.15^{Bc}	118.00±0.58^{Dd}	102.00±0.58^{BCe}

Means with the same capital letter superscript along same column and small letter superscript on the same row are not significantly different (p≥0.05); (Mean values ± SE) n =3; TMR, tail fin movemant rate.

Table IV.- Effect of acute concentrations of glyphosate and paraquat on air gulping index of *C. gariepinus* over a period of 12-96 h.

Conc (Mg/L)	Time (h) and AGI				
	12	24	48	72	96
Glyphosate					
0.00	0.30±0.05^{Aa}	0.40±0.05^{Aa}	0.30±0.06^{Aa}	0.40±0.05^{Aa}	0.40±0.05^{Aa}
0.36	0.50±0.05^{Ba}	0.50±0.05^{Ba}	0.50±0.05^{Aa}	0.30±0.05^{Ab}	0.30±0.06^{Bb}
0.48	0.70±0.05^{Ba}	0.80±0.12^{Cb}	0.80±0.06^{Bb}	0.90±0.05^{Bc}	0.90±0.12^{Bc}
0.60	0.90±0.06^{Ca}	0.70±0.05^{Cb}	0.70±0.05^{Bb}	0.50±0.06^{Cc}	0.50±0.05^{Ac}
0.72	0.80±0.05^{Ca}	0.90±0.05^{Ca}	0.70±0.12^{Bb}	0.30±0.05^{Ac}	0.40±0.05^{Ac}
0.84	1.20±0.05^{Da}	1.20±0.06^{Da}	0.80±0.12^{Bb}	0.50±0.05^{Cc}	0.70±0.05^{Cb}
Paraquat					
0.00	0.40±0.05^{Aa}	0.30±0.05^{Aa}	0.40±0.00^{Aa}	0.40±0.05^{Aa}	0.30±0.00^{Aa}
0.012	0.70±0.05^{Ba}	0.80±0.05^{Ba}	0.80±0.05^{Ba}	0.70±0.12^{Ba}	0.40±0.06^{Bb}
0.037	0.80±0.05^{Ba}	0.70±0.05^{Ba}	0.50±0.06^{Ab}	0.30±0.06^{Ac}	0.20±0.05^{Ad}
0.055	1.00±0.05^{Ca}	1.10±0.12^{Ca}	0.40±0.06^{Ab}	0.50±0.05^{Cb}	0.40±0.06^{Bb}
0.110	1.20±0.05^{Da}	1.30±0.05^{Ca}	0.50±0.05^{Ab}	0.40±0.05^{Ab}	0.33±0.00^{Ac}
0.221	1.20±0.05^{Da}	0.70±0.06^{Bb}	0.20±0.05^{Bc}	0.70±0.05^{Bb}	0.33±0.00^{Ac}

Means with the same capital letter superscript along same column and small letter superscript on the same row are not significantly different (p≥0.05); (Mean values ± SE) n =3; AGI, air gulping index.

of exposure. Furthermore, the first 12 h showed the highest TMR, thereafter, it dropped significantly in the 24th h and then increased from the 48th h (Table III).

The interaction between duration and concentration

effects of this toxicant is presented in Table IV. Acute concentrations of glyphosate showed significant effects on the AGI of *C. gariepinus*. The control fish showed lower AGI when compared with the exposed fish. The highest concentration also showed the highest AGI between the 12th and 48th. This difference is statistically significant. There was no definite pattern in the 72nd and 96th h. The air gulping index in exposed fish was highest within the 12th h and 24th h of exposure. Similar pattern of effects as observed in the fishes exposed to glyphosate, was noticed in the AGI of fish exposed to acute concentrations of paraquat. The interaction between the effects of paraquat per duration and concentration is as presented in Table IV. Generally, the control fish showed lower air gulping index as compared with the exposed fish. Fish also showed the highest AGI within the first two periods of observation *i.e.*, 12th and 24th h after exposure.

96-h LC_{50} values

Table V show the effect of acute concentrations of both herbicide on the mortality and probit values of juveniles of *C. gariepinus*. Mortality and probit values increased with increasing concentrations of both herbicide with the highest mortality recorded in the highest concentrations. The 96 h LC_{50} values for both herbicides were calculated based on these values and were found to be 0.530 mg/L for glyphosate and 0.07 mg/L for paraquat.

Table V.- Mortality and probit values of *Clarias gariepinus* exposed to acute concentrations of glyphosate and paraquat for 96 h.

Conc. (mg/L)	Log $_{10}$ conc.	Total No. of fish exposed	No. of dead fish	% mortality	Probit value
Glyphosate					
0.00	0	30	0	0	0
0.36	-0.444	30	5	16.67	4.05
0.48	-0.319	30	10	33.33	4.56
0.60	-0.222	30	20	66.67	5.44
0.72	-0.143	30	24	80	5.84
0.84	-0.076	30	26	86.67	6.13
Paraquat					
0.00	0	30	0	0	0
0.018	-1.744	30	2	6.67	3.12
0.037	-1.432	30	5	16.67	3.87
0.055	-1.260	30	10	33.33	5.44
0.110	-0.959	30	24	80	5.84
0.221	-0.656	30	27	90	6.28

DISCUSSION

Behavioural abnormalities were induced in fishes as a result of exposure to acute concentrations of glyphosate and paraquat. These behavioural abnormalities could be due to the disruption of nervous system activity depending on the impact of the toxicants on fish (Fafioye *et al*., 2005) or may be due to biochemical body derangement which may include compromising hepatic functions (Fadina *et al*., 1991). These behaviours point to an environmental stressor (the toxicant), hence fish provides important indices for ecosystem assessment (Robinson, 2009). In addition, these abnormal behaviours maybe caused by the neurotoxic effects and also by the irritation to perceptive system of the body due to accumulation of acetylcholine at synaptic junctions through the inactivation of acetylcholinesterase and stimulation of peripheral nervous system leading to higher metabolic rate (Rao *et al*., 2005). Result in this study agrees with the reports of Ayoola (2008) and Ojikutu *et al*. (2013).

The results of the study show that both herbicides have significant effects on the OVC of fish, TMR and AGI. The stressful ailment of respiratory impairment due to the toxic effect of glyphosate herbicide on the gills has been reported by Omitoyin *et al*. (2006). That the highest opercular ventilation rate (OVR) was observed within the first 12 h of exposure might be suggestive of a physiological response to oxygen stress. There were rapid opercular movements because of improper ventilation or impairment in the mechanism of exchange of oxygen from the environment. Opercular movement reduced by the 24th h which probably means that the fish are gradually coping to the new environment, trying to overcome the initial shock/harsh condition. A similar explanation may probably suffice for the increase in tail movement rate by the 12th h and reduction by the 24th h. Ogueji *et al*. (2013) reported that surviving fish was maximally intoxicated at this period due to maximum bioconcentration and bioaccumulation. Also, the marked increase in opercular ventilation and tail fin beats per minute may be that the exposed fish needed more oxygen for the increased metabolic rate especially within 12 h of exposure. This behaviour suggests respiratory impairment, due to the hypoxic environment of the toxicant and the effect on the gill and the body physiological processes. Lloyd (1992) reported that an increase in oxygen consumption may be associated with additional energy requirements for detoxification or it may be caused by the extra activity necessary for an avoidance reaction to the toxicant and also, an attempt to escape from the toxicant environment.

In fishes exposed to paraquat, OVCs were very low in the first two periods of observation *i.e.* at the 12th and

24th h (Table II). Such decreases probably help in reducing absorption of pesticide through gills (Venkata and Nagaraju, 2013). Opercular ventilation was not observed in the two highest concentrations (0.11 and 0.221mg/L) in the first 12 h of exposure due to the colour of paraquat which renders water to be very turbid at these two concentrations. Observation from the base of the holding tanks revealed cessation of opercular movement during these periods; thereafter, OVC rose significantly probably as the effect of the toxicant became limited with time and the turbidity of water reduced as a result of exposure to atmospheric oxygen. Furthermore, it could be associated with the inhibitory action of the toxicant on respiration as well as malfunctioning of some vital organs which may reduce the available energy for respiration. The fish could have increased ventilation rates in an attempt to make up for the loss in oxygen content in the gill. High opercular ventilation has been reported as an index of stress when fish come in contact with unfavourable environmental conditions (Sprague, 1973).

OVR increased from 12 and 24 h then further increased from 48th to 72nd h and then a sharp drop as death approached at about the 96th h. This indicates hyperventilation towards the 96th h and a decrease as death approached (Babatunde and Oladimeji, 2014). It is likely that the high OVR noticed between the 48th and 72nd h is due to thickened mucus layer as a result of continuous mucus production, which subsequently hinders gas exchange, which might be compensated for by elevating OVC. Mucus production has been reported as a response to many toxicants for providing a barrier that prevents toxin interaction with epithelial cells (Mallatt, 1985; Mcdonald and Wood, 1993).

Tail fin movement changed significantly with duration of exposure only (Table III). The first 12 h showed the highest TMR. After this, it dropped significantly in the 24th h and thereafter increased till the 96th h. The reason for this may be that the fishes are responding to the new toxic environment by lowering their physical activities and so as time extends, and they adapted to the new environment, they reverted to normal activity.

AGI is lower in the control than in exposed fishes. This is an indication that the fish requires increase supply of oxygen and had to swim to the surface to gulp air. This activity was observed to be at its highest within 12 to 24 h after exposure (Table II). This period coincides with a period of reduction in opercular ventilation, stressed cellular respiration and hence the need for alternative oxygen source. The AGI significantly reduced after 48 h, which may suggest physical fatigue due to swimming and other cumulative physiological effects of the toxicants. This scenario was also reported by Ogueji *et al*. (2013). The AGI in fish exposed to paraquat also increased significantly but the peak of this activity was within the first 24 h of exposure (Table IV). This period coincides with a period of reduction in opercular ventilation in which case the fishes were avoiding contact with the herbicide. This observed respiratory distress may have been due to either the decreasing dissolved oxygen contents of diluted water, or the decreasing ability of the exposed fish to respiration or both (Ojutiku *et al*., 2013).

Mortality was observed to be concentration and time dependent in this study (Table V). According to Fryer (1977), a threshold is attained above which there is no survival of animals. Below this threshold, animal is in a tolerance zone. The mortality pattern is between 17% and 87% for glyphosate, and 7% and 90% for paraquat, both pattern being similar to the report by Rand and Pectrocelli (1985) that there should be less than 35% mortality in the lowest concentration and at least more than 65% mortality in the highest concentration. These ranges of percentages were similarly observed by Ateeq *et al*. (2005) and Olurin *et al*. (2006). As the physico-chemical parameters of water used were found to be within range of *C. gariepinus* culture in this study, death could therefore have occurred either by direct poisoning or indirectly by making the medium unconducive for the fishes or even by both. Warren (1997) had earlier reported that the introduction of a toxicant into an aquatic system might decrease the dissolved oxygen concentration, which will impair respiration, leading to asphyxiation and ultimately death.

The present study shows that the 96 h LC_{50} value of glyphosate was 0.530mg/L. This is in contrast to previous studies by Akinsorotan *et al*. (2013) who reported LC_{50} value of 43.65mg/L, Okomoda and Ataguba (2011) reported LC_{50} value of 17.5mg/L and Ayoola (2008) reported a 96 h LC_{50} value of 1.05mg/L of glyphosate to *C. gariepinus* fingerlings. The 96 h LC_{50} value of paraquat as observed in this study was 0.07mg/L, which is also at variance with previous studies. Doherty *et al*. (2011) reported a value of 1.75mg/L while Omitoyin *et al*. (2006) reported a value of 18mg/L of paraquat to *C. gariepinus* fingerlings. The LC_{50} values depend on fish species and the test conditions as well as herbicide formulations (WHO, 1994). The variation may also be due to age of the experimental fish. Neibor and Richardson (1980) reported that the level of toxicity of any pesticide depends on its bioaccumulation, the different chemistries of the compound forming the pesticide and the reactions of the organisms receiving the toxicant.

CONCLUSIONS

Glyphosate and paraquat can negatively impact fish morphology and behaviour, reducing their aesthetic

value and vigour. The different concentrations of both herbicides have negative effect on air gulping index, tail fin movement rate and opercular ventilation rate. The abnormal behaviour may ultimately lead to death. It is imperative to be cautious about the quantity of these toxicants that get into our aquatic environments.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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