EFFECTS OF GLYPHOSPHATE HERBICIDE ("ROUND UP") ON FINGERLINGS OF *Oreochromis niloticus* and *Clarias gariepinus.*

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ABSTRACT: Fingerlings of both *Clarias gariepinus and Oreochromis niloticus* fish species exposed to various concentrations of glyphosphate herbicide in the laboratory were found to have both suffered toxic effects. *Oreochromis niloticus* fingerlings were found to be more susceptible to death from the herbicide. For both species immediate reaction to toxicity was hyperactivity and monitored frequencies of opercular plate and pectoral fin of both fish species showed ascending and descending phases. Behavioural patterns exhibited by both fish species could be classified into: (i) the pattern common to both fish species like restlessness, loss of balance, attempts at jumping out and haemorrhaged gills (ii) the pattern peculiar to either fish species; for example, *Clarias gariepinus* was more prone to swimming in erect posture with snout popping out of water but *Oreochromis niloticus* was more prone to swimming on either of its sides. Death occurred in all concentrations used on a daily basis. Furthermore, all concentrations killed off 50% of test fish population within 60 hours for *O. niloticus* but for *C. gariepinus* except for lowest concentration (20ppm) which achieved this feat in 73 hours all other concentrations killed off 50% of test fish population within 60 hours. For *C. gariepinus* the 96hrLC_{50}, threshold (safe level), lower limit and upper limit values were determined to be 20.89ppm, 13.18ppm, 17.26ppm and 25.28ppm respectively. Whereas, for *O. niloticus* 13.49ppm, 10.47ppm, 10.94ppm and 16.63ppm were respectively obtained. Observed water quality parameters showed similar trend with both fish species.

INTRODUCTION

There is a close relationship between the land and the water bodies. Activities on land most often affect the water through surface runoffs which carry heavy loads of both organic and inorganic matters into the water bodies. Large scale use of pesticides in crop agriculture has resulted in the presence of xenobiotic compounds in aquatic ecosystems (Gurure 1987; Avoaja and Oti 1997). Water pollution by pesticides is hazardous to all forms of aquatic fauna and flora and even to man (Aguigwo 2002). Glyphosphate herbicide, an organophosphate chemical is a non-selective, broad-spectrum, post-emergent herbicide with systemic activity in plants and used for control of grasses, broad-leaved weeds etc. Generally, all agricultural and urban pollutions find their way into aquatic ecosystems as allochthonous inputs; by four main pathways: direct human voluntary introductions, direct human involuntary introductions, direct natural introductions and indirect human introductions (Chude 2008). Their effects in aquatic environment include causing several physiological and biochemical defects in fishes and can be carcinogenic even to man (GESAMP 1991).

Kushaba-Rugaaju (1985) had reported Diazinon (an organophosphate) as being a mutagen that cause birth defect in chick embryo and parrot beak as well as also cause abnormal feathering and development of disproportionately small limbs. Thereafter Moore and Scott (1992) and Moore and Waring (1998) exposed male Parr to both Diazinon and Carbofuran (a carbamate) pesticides and reported the inhibition of the olfactory detection mechanism in the males for the female priming pheromones. This inhibition resulted in the subsequent reduction in the levels

of plasma sex hormone concentrations and expressible milt in these males. This ultimately disrupted the synchronization of spawning between the sexes. Much work has been done on the toxicity of these allochthonous inputs in water (Konar *et al.,* 1990; Ufodike and Omoregie 1991; Faleye and Olaniran 1995; Okpokwasili and Odokuma 1996a & b, 1997; Annune and Ajike 1999; Boudreau *et al*., 2003; Oti 2003, Oti *et al*.,2005). There is no information on studies relating to the effect of glyphosphate herbicide on biota especially fish. Thus, this research therefore aims at investigating the effect of glyphosphate herbicide on the fingerlings of *Clarias garienpinus* and *Oreochromis niloticus* and water quality since it is frequently channeled into natural aquatic ecosystems.

MATERIALS AND METHODS

Oreochromis niloticus fingerlings with mean weight of 3.8 ± 0.13g and length range of 4-6cm used in this experiment were caught from the canal linking the National Root Crop Research Institute (NRCRI) reservoir, to the University fishfarm of Michael Okpara University of Agriculture, both in Umudike, Abia State. Fingerlings of *Clarias gariepinus* of the same brood stock and age were bought from African Regional Aquaculture Center (ARAC), Port Harcourt, Rivers State. They were of mean weight 4.80g ± 0.06g and mean length of 2.35cm ± 0.20cm S.E. The fish were retained in holding tanks for two weeks acclimation during which period they were fed twice daily (morning and evening) at 4% body weight with "BIOFOOD" – a commercially marketed pellet diet containing approximately 52% protein. The holding tanks had net covers and were aerated using a super 555 Oxyguard aquarium air pump. Water in these holding tanks was changed every morning (at feeding time) throughout the period of acclimation to remove uneaten food and faecal droppings. Feeding was discontinued 24hours before commencement of the actual experiment. The flow-through system (Chude 2008) was modified after Gharton (1980) to suit prevailing laboratory conditions which it consisted of a tub-stand with provision to hold six tubs, each tub appropriately fitted with a net covered lid. This set up was repeated in triplicate; into each of the six tubs of a tub-stand was randomly introduced ten fish. Water was kept up at the 20-litre level by the aid of an outlet regulator at the center of each tub. This regulator was simply a tube with net covered mouth and whose height was at the 20-litre mark. It therefore let out water continuously but not fish thereby keeping the tub water volume constantly at the required 20-litre mark. The experiment was a continuous flow-through bioassay with toxicant auto-delivery system rigged on a wooden T-shaped cross.

The fish were not fed during the period of the experiment and the tubs too were not artificially aerated as doing so would have encouraged degradation of the pollutant. After the initial exploratory tests, stock solution (SS) was prepared (1000mg or ml per litre) as pollutant-water-dispersion (P-W-D) modified from the method described by Keke (1986) which itself was a modification of Anderson *et al.,* (1974). Thereafter the experimental solutions (glyphosphate herbicide) used or test media were obtained by sequential serial dilution. Thus, glyphosphate herbicide concentrations used were 20ppm, 40ppm, 60ppm, 80ppm and 100ppm. The sixth tub was devoid of toxicant and served as control.

Effect of glyphosphate herbicide on both test fish species was monitored through frequency or beat rate of both opercular plate and pectoral fin. Fish mortality was recorded for 24hours, 48hours, 72hours and 96hours. Observations for loss of equilibrium, vigorous movement of gulping of air and other behavioural indices, death inclusive, were noted for fish in each tub. Death was ascertained when fish did not react to gentle prodding with a glass rod. Time of death of fish was recorded, dead fish removed and its weight and length measurements taken. Mortality record for each test tub was painstakingly kept. The test fish were not fed because remnants of uneaten food would have contaminated the water thereby increasing mortality rates. The acute experiment lasted for four days (96hours). The physico-chemical properties of the experimental water: pH, temperature, dissolved oxygen, total alkalinity and free carbon

dioxide were monitored using the standard methods described by AOAC (1980) and APHA (1989).

The experiment was designed as a split plot experiment in a randomized complete block design in three replicates. All data collected were statistically analyzed using Statistical Analytical Systems (SAS) of 2004 series and Fisher's Least Significant Difference (F-LSD) was used to separate differences between treatment "means" at $p<0.05$.

RESULTS

Observed water quality parameters showed similar trend with both fish species. Values of water quality parameters (Table 1) showed temperature being fairly stable ($p>0.05$) in all toxicant concentrations used. While pH remained stable especially in the lower concentrations of (20 and 40)ppm it also showed slight acidity with higher concentrations of 60-100ppm. Free carbon dioxide and total alkalinity values increased with increase in toxicant concentrations as dissolved oxygen values decreased.

Table 1: Water quality parameters during exposure of *Oreochromis niloticus* and *Clarias gariepinus* fingerlings to glyphosphate herbicide.

Parameters	Fish sp.	Glyphosphate concentrations (ppm) 0	20	40	60	80	100
pH	O.n	6.90± 0.1	6.90± 0.1	6.92± 0.1	6.71±0.1	6.65±0.1	6.61±0.1
	C.g	6.90±0.1	6.90±0.1	6.92±0.1	6.70±0.1	6.63±0.1	6.59±0.1
Temp(^oC)	O.n	25.2±0.1	25.2±0.1	25.2±0.1	25.2±0.1	25.2±0.1	25.2±0.1
	C.g	25.2±0.1	25.2±0.2	25.2±0.2	25.2± 01	25.2±0.2	25.2±0.2
D.O.(mg/l)	O.n	6.62±0.2	6.50±0.2	6.40±0.2	6.41±0.2	6.32±0.2	6.20±0.2
	C.g	6.60±0.2	6.40±0.2	6.37±0.2	6.35±0.2	6.15±0.2	6.10±0.2
Free CO_2(mg/l)	O.n	0.74±0.1	1.40±0.1	1.50±0.1	1.50±0.1	1.65±0.1	1.80±0.1
	C.g	0.78±0.1	1.40±0.1	1.48±0.1	1.50±0.1	1.62±0.1	1.75±0.1
Total Alkalinity	O.n	32.0±0.2	34.0±0.2	38.0±0.2	42.0±0.2	48.0±0.2	52.0±0.2
(mg/l$CaCO_3$)	C.g	32.0±0.2	34.0±0.2	38.0±0.2	42.0±0.2	48.0±0.2	52.0±0.2

O.n = Oreochromis niloticus C.g = Clarias gariepinus. Values are mean of three determinations.

Tables 2 and 3 show the behavioral patterns exhibited by both fish species which scan be classified into two - (i) Those patterns that were common to both species - for example: restlessness, seeming loss of balance, attempts at jumping out, hyperactivity of the opercula plates and hemorrhaged gills. (ii) Those patterns that were peculiar/specific to either fish species. For example; *Clarias gariepinus* was more prone to swimming in erect posture with snout popping out of water but *Oreochromis niloticus* was more prone to swimming on either of its sides.

Table 2: Opercular ventilation rates of *O. niloticus and C. gariepinus* fingerlings when exposed to various concentrations of glyphosphate herbicide.

Conc(ppm)	Ohrs		24hrs		48hrs		72hrs		96hrs	
	O. n.	C. g.	O. n.	C. g.	O. n.	C. g.	O. n.	C. g.	O. n.	C. g.
0	96	92	94	90	92	90	90	88	85	86
20	98	96	96	94	92	92	90	90	88	88
40	108	102	110	98	98	96	96	94	94	92
60	112	106	116	102	110	100	100	98	98	96
80	122	110	124	108	114	104	108	102	104	100
100	128	114	128	114	116	110	112	108	108	106

O.n. = Oreochromis niloticus C.g. = Clarias gariepinus. Values are mean of three determinations.

Table 3: Pectoral fin beat frequency of *O. niloticus* and *C. gariepinus* fingerlings when exposed to various concentrations of glyphosphate herbicide.

Conc(ppm)	Ohrs		24hrs		48hrs		72hrs		96hrs	
	O.n.	C. g.	O. n.	C. g.	O. n.	C. g.	O. n.	C. g.	O. n.	C. g.
0	98	128	100	130	98	124	94	120	90	116
20	130	136	134	134	132	128	128	132	122	120
40	134	142	136	138	134	134	132	136	128	130
60	140	148	142	144	140	142	138	140	130	134
80	148	152	150	148	146	144	144	142	136	140
100	152	156	156	152	152	148	148	146	140	144

O.n. = Oreochromis niloticus *C.g. = Clarias gariepinus.* Values are mean of three determinations.

Table 4 depicts critical observation of the mortality record which shows that death of both species started occurring even with 20ppm concentration but higher number of deaths and in shorter time too was recorded by *Oreochromis niloticus.* Furthermore, while all concentrations used killed off 50% of test fish population within 60 hours with *O.niloticus* all but the lowest concentration of 20ppm did the same feat with *C. gariepinus*. The death outcome spectrum showed *Oreochromis niloticus* more prone to death than *Clarias gariepinus.* On the other hand, there were no mortalities in the control tubs.

Table 4: Shows comprehensive mean mortality record obtained from observations of different concentrations of glyphosphate on *O. niloticus and C. gariepinus.*

F/sp	T.no	F/tub	C/(ppm)	Log.c	Mortalities				C.m	Surv.	C%m	P.k	T
					d.1	d.2	d.3	d.4					
O.n.	1	10	0	0	0	0	0	0	0	10	0	0	0
,,	2	10	20	1.30	2	2	1	1	6	4	60	5.25	60
,,	3	10	40	1.60	2	3	1	2	8	2	80	5.80	45
,,	4	10	60	1.78	3	2	2	2	9	1	90	6.30	40
,,	5	10	80	1.90	2	3	2	2	9	1	90	6.30	38
,,	6	10	100	2.00	3	2	3	1	9	1	90	6.30	38
C.g.	1	10	0	0	0	0	0	0	0	10	0	0	0
,,	2	10	20	1.30	1	1	2	2	6	4	60	5.25	73
,,	3	10	40	1.60	1	2	3	1	7	3	70	5.53	58
,,	4	10	60	1.78	1	2	3	2	8	2	80	5.84	53
,,	5	10	80	1.90	2	2	3	1	8	2	80	6.30	51
,,	6	10	100	2.00	2	2	2	3	9	1	90	6.30	51

F/sp = Fish Species; T. no. = Tub no.; F/tub = Fish per tub; C(ppm) = Conc. (ppm);
Log.c = Log conc .; d1 = 0-24hrs; d2 = 24-48hrs; d3 = 48-72hrs; d4 = 72-96hrs;
Surv. = survival; C.m = Cummulative mortality; C%m = Cummulative % mortality
P.k = Probit kill %; T = Time (hours) for 50 % mortality.

Table 5 shows the toxicity result of *C. gariepinus* at the 96hrLC_{50}, threshold (safe level), lower limit and upper limit values to be 20.89ppm, 13.18ppm, 17.26ppm and 25.28ppm respectively whereas, for *O. niloticus* the same respective values were found to be 13.49ppm, 10.47ppm, 10.94ppm and 16.63ppm respectively.

Table 5: 96hrLC_{50}, Threshold (safe level) and Lower/Upper limit toxic values of glyphosphate herbicide on *O. niloticus and C. gariepinus* fingerlings.

Toxicant	96hrLC_{50} (ppm)		Threshold (ppm)		Lower limit (ppm)		Upper limit (ppm)	
	O.n	C.g	O.n	C.g	O.n	C.g	O.n	C.g
Glyphosphate	13.49	20.89	10.47	13.18	10.94	17.26	16.63	25.28

O.n. = Oreochromis niloticus. *C.g. = Clarias gariepinus.*

DISCUSSION

Abiotic and biotic indicators have been used in detecting anthropogenic changes in the ecological integrity of aquatic systems. Human-induced perturbations can affect the compositional, structural and functional characteristics of these systems. Water quality attributes are prime factors that influence fish survival, reproduction, growth performance, and overall biological production (King, 1998; king and Jonathan 2003). They affect aquatic biotic integrity by directly causing mortality and / or shifting the equilibrium among species due to subtle influences such as reduced reproductive rates or alternations in competitive ability. This inverse relationship is interesting and indicative of higher demand for oxygen prompted by condition of hyperactivity as observed and explained by Ofojekwu *et al.,* (2001) and Oti (2003).

Behavioral patterns exhibited by both fish species can be classified into two - (i) those patterns that were common to both species - for example: restlessness, seeming loss of balance, attempts at jumping out, hyperactivity of the opercula plates and hemorrhaged gills. (ii) those patterns that were peculiar/specific to either fish species - for example; *Clarias gariepinus* was more prone to swimming in erect posture with snout popping out of water but *Oreochromis niloticus* was more prone to swimming on either of its sides. Chude (2008) who investigated the effects of six toxicants on these two fish species also reported same observations in his compendium of abnormal behavioural patterns exhibited by these two fish species. Since these patterns were not exhibited by any of the fishes in the control tubs it is safe to infer that these abnormal behavioural patterns were in consequence of the effect of the toxicant. The toxicant must have destabilized the fishes normal run of physiological activities. With the corresponding control values as baseline opercular ventilation rate and pectoral fin beat frequency initially increased for the first 24hours and thereafter decreased tending towards zero. These increase and decrease have been duly reported by past workers: Omoregie and Ufodike (1991); Wade *et al.,* (2002); Ndu (2004); Chude (2008).

Critical observation of the mortality record shows that death of both species occurred even with 20ppm concentration but higher number of deaths and in shorter time too was recorded by *Oreochromis niloticus.* Furthermore, while all concentrations used killed off 50% of test fish population within 60 hours with *O. niloticus* all but the lowest concentration of 20ppm did the same feat with *C. gariepinus*. The death outcome spectrum showed *Oreochromis niloticus* more prone to death than *Clarias gariepinus* (Omoregie and Ufodike 1991; Aguigwo 2002).

CONCLUSION

The inference therefore is that *Oreochromis niloticus* was more susceptible to glyphosphate herbicide than *Clarias gariepinus.* This inference is further established by the determined values of $96hrLC_{50}$, threshold and lower/upper limits. Values obtained for *Oreochromis niloticus* were lower than corresponding values obtained for *Clarias gariepinus.* However, while Ufodike and Omoregie (1991) reported that *Oreochromis niloticus* exposed to sub-lethal concentrations of Gammalin 20 and Acelic 2.5EC resulted in retarded growth, Oti (2001) reported on *Chrysichthys nigrodigitatus* and found the $96hrLC_{50}$ to be $2.3Ug/L^{-2}$ with lower and upper limits at 2.10 and $4.44Ug/L^{-2}$ respectively. Also, De Silver and Ranasinghe (1989) investigated the toxicity of four agrochemicals on *Oreochromis niloticus* fry and reported that younger fry were more sensitive to toxicants and that toxicity changed with fish development.

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