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SUBLETHAL EFFECT OF GLYPHOSATE [N-(PHOSPHONOMETHYL)GLYCINE] ON GROWTH PERFORMANCE AND BIOCHEMICAL ACTIVITIES IN SOME ORGANS OF *CLARIAS GARIEPINUS* (BURCHELL, 1822) FINGERLINGS

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ABSTRACT. The effect of the sublethal concentration of glyphosate [N-(phosphonomethyl)glycine] on growth and some biochemical indices in the organs of African catfish (*Clarias gariepinus*, Burchell 1822) fingerlings in a static bioassay setup was studied to provide information on the sublethal toxicity effect of glyphosate on *C. gariepinus* fingerlings. Two hundred and seventy (270) fingerlings of *C. gariepinus* (10.02 ± 0.20 g) randomly stocked at 30 fish per tank in triplicate were exposed to varying concentration (0, 2.75 and 5.00 ppm) of glyphosate for 70 days. The results of the study showed that the determined growth parameters decrease with increase glyphosate concentration (except specific growth rate). The highest mortality rate was recorded in the treatment with the highest phosphate concentration (5.50 ppm). The enzymatic analyses of the fish tissue revealed that Na^+/K^+-ATPase activity which ranged from 0.20 to 19.29 µM Pi min^{-1} mg^{-1} protein in all the fish tissues increase with increase glyphosate concentration in the fish muscle and liver, and decreases with increase in glyphosate concentration in the gills. However, the muscle and liver malate DH activities decreased with increase in glyphosate concentration while the lactate DH activity increases with increase intoxicant concentration in the muscle (with the highest treatment having a threefold increase). Generally, the enzymatic activities of fish tissues followed the order: Malate DH >Na+/K+-ATPase>Lactate DH. The study concluded that the glyphosate concentration negatively impacted the growth and survival of *C. gariepinus* and also had a pronounced effect on the enzymatic activities of the studied organs.

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Introduction

Water bodies around the world are increasingly getting polluted, more than ever in history, due to human activities, especially with the use of an organic substance which directly or indirectly enter into the aquatic ecosystem through human perturbations (Scott *et al.,* 2000; Pérez *et al.,* 2011; Ayanda, Egbamuwo, 2012). The use of pesticide especially herbicide for destroying, preventing, or mitigating insects, rodents, nematodes, fungi, or weeds (as applicable), is on the increase worldwide in modern agricultural practices (Kellogg *et al.,* 2000; Tomlin, 2006; Steinmaus, 2010; Okayi *et al.,* 2010; Pérez *et al.,* 2011; Benbrook, 2016). Due to the chemical structure, toxicity, leaching through root zones, mobility and persistency of organic pesticides, several authors have reported them to possess a threat to the underground, surface water and aquatic lives (Artiola *et al*., 2004; Brusseau *et al*., 2006; Brusseau, Tick, 2006; Yusuf *et al.,* 2017).

Ikpe (2012) reported that since fish and other aquatic organisms only flourish when the biological and chemical conditions of the water are stable, the health of these aquatic organisms and even human consumers are therefore at risk when the aquatic ecosystem is

polluted or contaminated. Several indicators have been used to study the effect of indiscriminate use, improper handling, accidental spillages or drifts of herbicide into natural waterways on aquatic organisms. Olaleye *et al.* (2005) reported the effect of glyphosate application to control *Eichhornia crassipes* on fish composition and abundance in Arabia Creek, Niger Delta, Nigeria while Ayanda, Egbamuwo (2012) reported the effect of glyphosate on histology of *Clarias gariepinus* liver and gill.

However, the effect of glyphosate on enzymes which are an organic catalyst that speeds up the rate of chemical reactions in a living cell (Michael, 2007), proteinous inactive and not used up during such biochemical reactions (Slenzka *et al*., 1994) have not been documented. Enzymes are known to play a very crucial role in the metabolic process in the fish body and are thus biochemical markers or indicators used to evaluate environmental contamination to avoid jeopardizing the health of aquatic lives (Barnhoorn, van Vuren, 2004). Moreover, the effect of toxicants on the enzymatic activity of fish is one of the vital biochemical parameters that are affected when under stress, and when an organ is diseased due to the effect of a toxicant, enzyme activity appears to be increased or it may be inhibited due to the active site(s) being denatured or distorted. Therefore, the increase or decrease in the levels of some enzymes which catalyze some steps in the metabolism of major macromolecules (such as carbohydrates and proteins) known to be present in most tissues, may be sufficient enough to provide information of diagnostic value (Adeyeni, 2010).

Some the enzymes that have been reported to have a close link between toxicants effects on organism include; glycolytic enzymes (Lactate Dehydrogenase, pyruvate kinase and phosphofructokinase); Transmembrane ATpases (sodium-potassium exchanger (Na^+/K^+ ATPases) and hydrogen-potassium ATPase – H^+/K^+ ATPase or gastric proton pump); Malate dehydrogenase (MDH) (Das *et al.,* 2004; Barnhoorn, van Vuren, 2004). These authors reported that increase or decrease in the level of these enzymes is enough biochemical indicators to qualify the damage done to an organism after exposure to a toxicant.

Glyphosate is widely used herbicides in Nigeria because of its efficacy for weed control (Miller *et al*., 2010). However, wholesale application of the herbicides for weed control in river catchment area ultimately result in the introduction of the herbicides and its residue into the aquatic ecosystem through erosion, spray drift and runoffs from the catchment areas and thus affect aquatic lives (Olaleye *et al*., 2005; Ayoola, 2009). Therefore, aquatic bioassays are necessary for water pollution control to determine whether a potential toxicant like glyphosate is harmful to aquatic lives, at varying concentrations (Olaifa *et al*., 2003). There is, therefore the need to evaluate the possible acute and sublethal toxic effect of glyphosate on aquatic life forms using fingerlings of *Clarias gariepinus,* which is the most widely cultured fish species in Nigeria due to its hardiness, economic values and ability to tolerate water of poor quality among others (Ayanda, Egbamuwo, 2012). The study aimed at determining the effect of sublethal concentration of glyphosate, popular herbicides on growth and oxidative enzymes of an important Nigeria food fish *Clarias gariepinus*.

Materials and methods

Range Finding and Acute Toxicity Test

The concentrations of the glyphosate used for the range finding test followed the methods described by Reish and Oshida (1986) and Obuotor (2004).

A stock solution of 4 gl^{-1} glyphosate was prepared by measuring 11.1 mL of Round-up® which was diluted to obtain 0.4, 0.04, 0.004, 0.0004 and 0.0 gl^{-1} glyphosate. 10 fingerlings of the test fish were randomly selected and introduced into each of the 10-litre tank containing 5 litres of dechlorinated tap water containing the test solution (toxicant). The containers labelled A to E contained the logarithmic concentration of 4, 0.4, 0.04, 0.004, and 0.0004 gl^{-1} glyphosate. Another tank labelled F which represented the control was without glyphosate addition. The exposure was carried out in triplicate. The fish were then observed for mortality after 1 hr, 3, 6, 12, 48, 72 and 96 hrs (Reich, Oshida, 1986). Toxicity range value was then estimated from the probit analysis and Spearman-Karber method of estimating mortality results (Carter *et al*., 2012). The result of the toxicity range obtained from the range-finding tests was used to prepare 8 graded concentration, 1.00, 4.00, 8.00, 12.00, 16.00, 20.00, 24.00 and 400.00 ppm of commercial glyphosate formulation (Roundup®) (containing isopropyl ammonium salt of glyphosate at 480 gl^{-1} as the active ingredient (equivalent to 360 g glyphosate per litre. The various concentrations were used for 96 hours acute toxicity bioassay in a static exposure system Obuotor, 2004). Ten (10) *Clarias gariepinus* fingerlings (10.02 ± 0.2 g) randomly selected were introduced carefully into each of the exposure tanks containing the test concentrations in triplicates. Mortality in each of the exposure chamber was monitored and recorded after 1 hour, 3, 6, 12, 24, 48, 72 and 96 hrs. Dead fish were promptly removed from the exposure chamber and the test was terminated after the 96 hrs period of exposure. The 96 hrs LC_{50} values were then calculated from the data collected using Trimmed Spearman-Karber Analysis (U.S.EPA, 1997).

Chronic toxicity testing

A total of two hundred and seventy (270) 6-weeks old African catfish (*Clarias gariepinus*) fingerlings (10.02 ± 0.2 g) obtained from Prime Aquaculture Ltd., Ikorodu, Lagos and transported to the Fish Culture Laboratory, Department of Zoology, Obafemi Awolowo University, Ile-Ife were acclimatized for two weeks in an aerated 150 L glass tanks. The fingerlings stocked in 30 fish per tank were fed with 2 mm Coppen's® feed containing (45% crude protein) at 4%

their body weight in two instalments. After acclimatization, the fish fingerlings randomly selected were carefully introduced into three exposure sets (*i.e.* control, 2.75 ppm (25% of the 96 hrs LC_{50} *i.e.* Lethal Dose 50%) and 5.50 ppm (50% of the 96 hrs LC_{50})) in triplicate at 30 fish per tank for 70 days. During these periods, the experimental test fingerlings were fed with Coppen's ® feed at the rate of 4% per body weight. The test solution in each tank was renewed every 72 hours with freshly prepared solutions. The water quality measured *in situ* daily were pH, temperature and Dissolved oxygen using a portable pH meter (Model with resolution 0.01 pH and accuracy of ± 0.05 pH), mercury in glass thermometer and Milkawauke D.O. meter respectively.

Fish growth performance was monitored fortnightly by collectively weighing and measuring the fish from each tank using a top-loading meter balance model P1210 (in grams). From the weight data collected and the quantity of the feed consumed, the growth performance and the feed utilization data were generated as follows:

Daily Feed Intake (DFI)

$$DFI\ (g\ fish^{-1}) = \frac{TFI}{t}, \quad (1)$$

where TFI = total feed intake (g),
t = rearing period of the experiment (days).

Mean Weight Gain (MWG)

This was calculated according to the method of Pitcher, Hart (1982) as:

$$MWG = W_f - W_i, \quad (2)$$

where W_f = final mean weight (g),
W_i = initial mean weight (g).

Daily Weight Gain (DWG)

The DWG was estimated according to the method of Pitcher and Hart (1982) as:

$$DWG\ (g) = \frac{MWG}{t}, \quad (3)$$

where: MWG = mean weight gained,
t = rearing period (days).

Percentage Weight Gain (PWG)

The percentage of weight gained was obtained according to the formula:

$$WG\ (\%) = \frac{MWG}{W_i} \times 100, \quad (4)$$

where: MWG = mean weight gain,
W_i = mean Initial weight.

Specific Growth Rate (SGR)

This was estimated from the logarithmic differences between the final and initial mean weight of fish (Brown, Guy, 2007).

$$SGR = \frac{100\ (Log_e W_f - Log_e W_i}{T}, \quad (5)$$

where W_f = final mean weight,
W_i = initial mean weight (g).
T = rearing period (days)

Feed Conversion Ratio (FCR)

The FCR was estimated according to the methods of Bruel *et al*. (2000) as:

$$FCR = \frac{Total\ feed\ intake\ (g)}{Total\ weight\ gain\ (g)}, \quad (6)$$

Percentage Survival

Survival was monitored daily by observing the experimental tanks each morning and recording the number of survivors and removing the dead fish. At the end of the experimental period, survived fish in each tank were counted and survival percentage was estimated as:

$$\%\ Survival = \frac{N_f}{N_i} \times 100, \quad (7)$$

where N_f = number of fingerlings at the end of the experiment,
N_i = number of fingerlings at the beginning of the experiment.

Biochemical Assay

After the 70th day of the experiment, five (5) fish from each tank were sacrificed and excised for the collection of liver, gills and muscle tissue. The tissues were rinsed in ice-cold 0.25M sucrose solution. The tissues were then weighed on a weighing balance, homogenized in iced-cold 0.15 M tris buffer, (pH 7.4) using a motor-driven glass-telfon potter-Elvejhem (TRI-R STIR-R K43) homogenizer, at 1000 rev min^{-1} to give a 10% homogenate. This resulting suspension was used for the assay of adenosine triphosphatase (ATPase), Malate Dehydrogenase and lactate dehydrogenase.

Enzyme Assays Procedure

The following oxidative marker enzymes were assayed for in the gills, liver and muscle homogenates for both the control and the experimental fish.

Malate Dehydrogenase (MDH) Assay

MDH was assayed for as described by Worthington Biochemical Corporation (1993). The assay mixture which consists of 0.1 ML oxaloacetate, 2.6 ml 0.1 M^{-1} phosphate buffer and 0.2 Ml NADH was incubated at temperature 25 °C in a Pharmacie Biotech Novaspec II spectrophotometer (Model 80-2088-64, Cambridge, England) at 340 nm for 3–4 minutes to achieve temperature equilibration and establish the blank rate. 0.1 ml of the liver homogenate sample was then added and a decrease in absorbance was read for 4 minutes at 30 seconds intervals. The $D_{340\ nm\ min^{-1}}$ was obtained from the linear curve of absorbance against time. The MDH activity was then calculated from the formula:

$$\mu l\ mg^{-1} = \frac{D_{340\ nm\ min^{-1}}}{6.22 \times mg\ enzyme\ ml\ reaction\ micture^{-1}}, \quad (8)$$

where $D_{340\ nm\ min^{-1}}$ = the slope from the graph of the absorbance against time.

Enzyme activity was expressed as u mg^{-1} where one unit of enzyme oxidizes 1.0 Nmol of NADH in 1 minute at 25 °C and pH 7.4 under the specified conditions.

Sodium, Potassium ATPase Assay (Na^+/K^+-ATPase)

The inorganic phosphate (P_i) liberated from the hydrolysis of the substrate adenosine triphosphate (ATP) at 37 °C was used for the measurement of Na, K – ATPase activities. Frozen gill filaments, (200 mg) was homogenized for 90 seconds in 0.3 m sucrose buffer (pH 7.4) were homogenized and centrifuged at 1000 g (r.m.p) for 10 minutes to remove debris. ATPase activity was monitored immediately on the resulting supernatant. Final assay concentrations of chemicals used were in (Mol l^{-1}) tris-HCL (pH = 7.4) 135, NaCl 100, KCl 10, $MgCl_2$ 6, ATP 3, EDTA 0.1 and Quabian 3.

After pre-incubation of the medium for 5 minutes, reactions were started by adding the samples and ATP appropriately. The reaction was continued for 30 minutes with the incubated medium shaken on a shaker using 100 rpm model of shaker. The reaction was terminated by putting the samples in ice and a Lubrol-molybdate mixture (1:1 w w^{-1}). The added sample was then vortexed to form the soluble yellow complex. An inorganic aliquot of the incubated mixtures. All assays were carried out in triplicate and ran with enzyme and reaction blanks. ATPase activity was expressed as Nmol Pi mg^{-1} protein hr^{-1} (Canli, Stagg, 1996; Obuotor, 2004).

Lactate Dehydrogenase (LDH) Assay

The assay for LDH was done on the gills, muscle and liver samples using the Randox Diagnostic Kit (Reitman, Frankel, 1957). The assay mixture contains either 0.04 ml gills, muscle or liver homogenate and 1.0 ml Randox buffer mixed at a temperature between 25–30 °C. The initial absorbance of the mixture was read at 340 nm after 30 seconds using a Pharmacia Biotech Novaspec II spectrophotometer, (Model 80-2088-64, Cambridge, England). The absorbance was then read every 30 seconds for 4 minutes. A regression line was obtained from the graph of absorbance against time while the slope was estimated. The LDH activity was calculated from the formula:

$$UI^{-1} = 4127 \times D_{340\,nm\,min^{-1}}, \qquad (9)$$

where $D_{340\,nm\,min^{-1}}$ is the slope from the graph of absorbance against time. Enzyme activities were expressed as UL^{-1}, where UL^{-1} is equal to the amount of enzyme required to convert 1.0 µmol of suitable to the product in 1 minute between 25–30 °C.

Data Analysis

Data generated were statistically analysed as a completely randomized design using each cage as an experimental unit. One-way ANOVA was used to determine if there were significant differences ($P<0.05$) among the treatments, followed by the Duncan test to identify where the differences occurred. The analytical tools used are SPSS software version 20.0 for statistical analysis, and Microsoft Excel for graphical representation.

Ethical Statement

The protocol and procedures employed in this study for the sacrifice of animal used were ethically reviewed and approved. The procedures also complied with directive 2010/63/EU of the European Parliament and of the Council on the protection of animals.

Results

The temperature, pH and dissolved oxygen measured during the experimental period were relatively stable ranging from 24.5 to 26.9°C, 6.24 to 7.62, and 4.00 to 8.00 mg L^{-1} respectively.

During the 96-hour acute toxicity test, fish mortality was recorded in each tank (Table 1). Swimming behaviour was observed to drastically change on introducing the fish into tanks treated with glyphosate. A first noticeable change in swimming pattern was observed in tanks with the highest treatments (Treatments F, G, H and I) which began with rapid sporadic movement characterized by darting movements in the tanks and lack of cruising movement. Vertical swimming, loss of balance, flaring of the opercula and gasping was later observed in the treatments before fish deaths which further increased the physiological stress of the fish. Feed avoidance was also noticed among the various treatments with very high glyphosate levels.

Table 1. The acute mortality rate of the experimental fish

Concentrations of glyphosate (ppm)	Time of Exposure (h)										Mortality rate	
	0.25	0.5	1	3	6	12	24	48	72	96	n	%
0	0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	1	1	10
4	0	0	0	0	0	0	0	1	0	1	2	20
8	0	0	0	1	0	0	0	0	2	1	4	40
12	0	0	0	0	0	0	1	0	0	2	3	30
16	0	0	0	0	0	0	0	1	2	1	4	40
20	0	0	0	0	2	0	0	1	4	2	9	90
24	0	0	0	0	3	0	1	2	3	0	9	90
40	3	2	5	0	0	0	0	0	0	0	10	100

Fish in the control tanks and lowest treatment (Treatment A and B) maintained an initial short 'burst' movement and in a few minutes, began the regular cruising movement. As shown in Table 1, absolute (100%) fish mortality was first recorded in a tank with the highest concentration of glyphosate after one-hour exposure. Treatments F (20.00 ppm) and G (24.00 ppm) recorded 90% mortality rate on the 96 hr while Treatments C (8.00 ppm) and E (16.00 ppm) recorded 40% mortality rate after the 96 hours of exposure respectively (Table 1). Treatments A (1.00 ppm), B (4.00 ppm) and D (12.00 ppm) had a mortality of 10%, 20% and 30% rates respectively at the end of the 96 hours short term static bioassay (Table 1).

The daily feed intake (DFI), mean daily weight gain (MDWG), mean weight gain (MWG) and food conversion ratio (FCR) values of the fish in various tanks decreased with increase in the concentration of the glyphosate (Table 2). However, the specific growth

rate (SGR) was independent of glyphosate concentration. The lowest SGR value was recorded in the fish exposed to 2.75 ppm glyphosate concentration while the highest SGR value was recorded in the control treatment tank. The mean weight gain of the exposed fish showed insignificant difference with the control treatment (P>0.05).

During the long term toxicity test, a highest mean mortality rate of 50% was observed at the end of the experiment in treatment with the highest concentration of glyphosate (5.50 ppm) while the control and treatment with 2.75 ppm had a mortality rate of 30% (Table 3).

Enzymatic Activities

Enzymatic activities of ATPase determined in various fish tissues (muscle, gill and liver) exposed in all the treatments tanks ranged from 0.20 ± 0.04 to 19.29 ± 1.86 μM Pi min^{-1} mg^{-1} protein. In all the tissue assayed, fish muscles recorded the lowest Na^+/K^+-ATPase activity which ranged between 0.20 ± 0.04 (control) and 0.43 ± 0.02 μM Pi min^{-1} mg^{-1} protein (5.50 ppm) while highest Na^+/K^+-ATPase activity was recorded in the gill with a range of 9.29 ± 1.33 (5.50 ppm) to 19.29 ± 1.86 μM Pi min^{-1} mg^{-1} protein (control) (Fig. 1). Among the treatments, Na^+/K^+-ATPase activity in the fish muscles and livers was observed to increase with an increase in glyphosate concentration while a decrease in the Na^+/K^+-ATPase activity with increasing concentration was observed in the gills of the exposed fish (Fig. 1). However, while the Na^+/K^+-ATPase activity in the muscle and liver of the exposed fish across the treatments showed no significant differences (P>0.05), the Na^+/K^+-ATPase activity level recorded in the gills of the exposed fish across the treatments were highly significant (P>0.05).

Malate DH activity in the muscle, gill and liver of the fish assayed in all the treatment tanks ranged from 5.50 ± 0.69 to 578.45 ± 33.47 μM Malate min^{-1} mg^{-1} protein (Fig. 2). In fish muscles, Malate DH activity significantly decreases (P<0.05) with the increasing concentration of glyphosate.

Table 2. Mean (±SE) Growth Performance* of *C. gariepinus* Juveniles Exposed to Variable glyphosate concentrations of for 70 days. Among-treatment comparisons are marked with different superscripts, which indicate significant differences (ANOVA Duncan test P<0.05).

Growth performance indices	Concentration of WSF		
	0% (control)	2.75 ppm	5.00%
Initial mean weight (g)	26.50^a ±0.42	28.50^a ±0.22	27.50^a ±0.53
Final mean weight (g)	138.10^a ±4.28	136.70^a ±2.82	132.40^a ±2.36
Mean weight gain (g)	111.50^a ±1.25	108.20^a ±2.24	104.00^a ±3.49
Mean daily weight gain (g)	1.59^a ±0.30	1.55^a ±0.17	1.49^a ±0.15
Specific Growth Rate (%)	0.31^a ±0.16	0.29^a ±0.12	0.30^a ±0.15
Feed Conversion Ratio	0.86^a ±0.08	0.86^a ±0.37	0.63^b ±0.60
% Mortality	30	30	50

Values with different superscripts are significantly different at P<0.05

Table 3. The mortality rate of the experimental fish during definitive exposure

Concentrations of glyphosate (ppm)	Weeks of exposure										Mortality rate	
	1	2	3	4	5	6	7	8	9	10	n	%
0.00	0	0	0	0	0	0	1	0	0	1	2	20
0.00	0	0	0	0	0	0	0	0	0	1	1	10
0.00	0	0	0	0	0	0	0	0	0	0	0	0
2.75	0	0	0	0	0	0	0	0	0	0	0	0
2.75	0	0	0	0	0	0	0	2	0	0	2	20
2.75	0	0	0	0	0	0	0	1	0	0	1	10
5.50	0	0	0	0	0	0	0	3	1	0	4	40
5.50	0	0	0	0	0	0	0	3	1	0	4	40
5.50	0	0	0	0	0	0	0	0	6	1	7	70

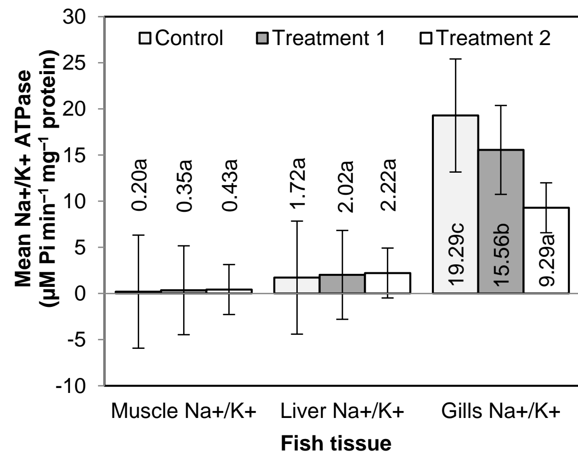

Figure 1. Comparative changes in the mean (±S.E.) Na^+/K^+ ATPase activity in the muscle, liver and gills of *C. gariepinus* following exposure to sublethal concentration of glyphosate. Treatment 1 – 2.75 ppm; Treatment 2 – 5.50 ppm.
*Bars of the same fish tissue with different superscripts were significantly different (P<0.05)

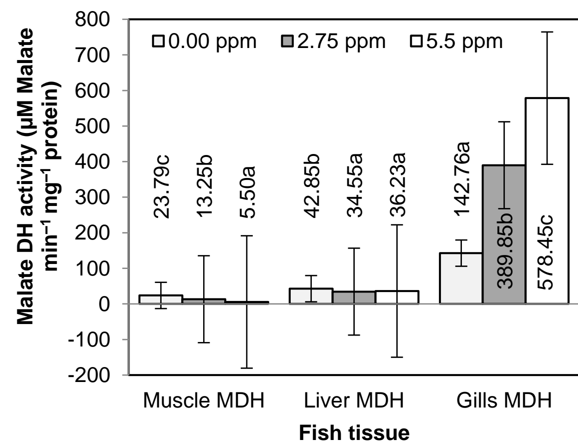

Figure 2. Changes in the mean (±S.E.) malate dehydrogenase activity in gills, muscle and liver of *C. gariepinus* fingerlings following exposure to sublethal concentration of glyphosate. Control – 0.00 ppm
*Bars of the same fish tissue with different superscripts were significantly different (P<0.05)

Highest mean Malate DH activity (23.79 ± 2.2 μM Malate min^{-1} mg^{-1} protein) was recorded in the control fish while the fish in 5.50 ppm treatment had lowest (5.50 ± 0.69 μM Malate min^{-1} mg^{-1} protein) Malate DH activity (Fig. 2). The Malate DH activity in the liver of

the fish from various treatment tanks ranged narrowly from 34.55 ± 2.11 μM Malate min^{-1} mg^{-1} protein (2.75 ppm glyphosate) to 42.85 ± 2.65 μM Malate min^{-1} mg^{-1} protein (control). Malate DH activity in the liver of the exposed fish which were not significantly different (P>0.05) from each other was however significant (p<0.05) compared to the activity level recorded in the control fish (Fig. 2). In the gill tissues, Malate DH activity was generally higher than in other tissues and significantly increases with increasing concentration of glyphosate with a range of 142.76 ± 10.14 to 578.45 ± 33.47 μM Malate min^{-1} mg^{-1} protein (Fig. 2). Fish in the control tanks recorded the lowest mean value of Malate DH activity in their gills with a mean value of 142.76 ± 10.14 μM Malate min^{-1} mg^{-1} protein while treatment 5.50 ppm glyphosate recorded the highest activities of the enzymes in the gills with a mean value of 578.45 ± 33.47 μM Malate min^{-1} mg^{-1} protein.

The lactate DH activity of the fish tissues is shown in Figure 3. The Lactate DH activity of all the fish tissues assayed revealed that the activity of this enzyme in the gills was glyphosate concentration-dependent. Among the various fish tissue assayed for lactate DH activity, the liver had the highest Lactate DH activity (1.91 μM Lactate min^{-1} mg^{-1} protein) while the least Lactate DH activity (0.26 μM Lactate min^{-1} mg^{-1} protein) was recorded in the muscle. In the muscle of the fish from various treatment tanks, Lactate DH activity of the fish exposed to Treatment 2.75 ppm glyphosate recorded the lowest (0.26 μM Lactate min^{-1} mg^{-1} protein) while Treatment 5.50 ppm glyphosate had the highest muscles' lactate DH activity of over a threefold increase (0.99 μM Lactate min^{-1} mg^{-1} protein).

However, the activities of gill lactate DH in the control fish was the lowest with a mean value of 0.34 μM Lactate min^{-1} mg^{-1} protein, while the highest gill lactate DH activity was recorded in Treatment 5.50 ppm glyphosate with mean value 0.78 μM Lactate min^{-1} mg^{-1} protein (Fig. 3).

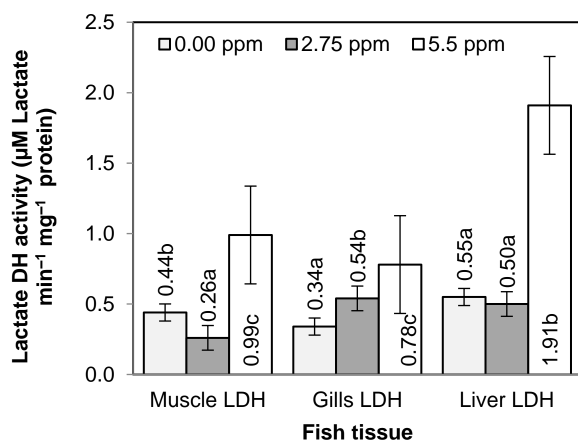

Figure 3. Changes in the mean (±S.E.) lactate dehydrogenase activity in gills, muscle and liver of *C. gariepinus* fingerlings following exposure to sublethal concentration of glyphosate. Control – 0.00 ppm
*Bars of the same fish tissue with different superscripts were significantly different (P<0.05)

The liver of the fish in treatment 2.75 ppm glyphosate had the lowest mean value of lactate DH activity (0.50 μM Lactate min^{-1} mg^{-1} protein) while the fish in the highest glyphosate treatment recorded a two-fold increase in the level of liver lactate DH activity with a mean value of 1.91 μM. Generally, significantly higher (P<0.05) lactate DH activity was recorded in all the tissues of the fish exposed to the highest concentration of glyphosate (Fig. 3).

Enzymatic activities of fish tissues followed the order viz malate DH > Na^+/K^+ ATPase > lactate DH.

Discussion

The overall mean water quality parameters determined in this study were within an acceptable range for aquaculture production (NSDWQ, 2007; Boyd, 1979).

The sporadic burst of movement, lacking cruising and balance, vertical swimming, flaring of opercula, gasping, and death of *C. gariepinus* fingerlings exposed during the acute toxicity test in this study is similar to the findings observed by (Annune *et al.*, 1994; Omitoyin *et al.*, 2006; Ayoola, 2008a) when fish are exposed to the higher concentration of glyphosate at 96 hr. Fish mortality was highest in treatments with glyphosate in the 96h acute toxicity test. This confirms the observation by Fryer and Makepeace (1997), that in all toxicants, a threshold is reached above which there is no drastic survival of the animal. Below the threshold, animal performance is not affected while above the tolerance zone is the zone of resistance. In the 96 h test, 100% of fish deaths were recorded in treatments ≥1.00 mg L^{-1}. This outcome was similar to reports by Okayi *et al.*, (2010) and Ayoola (2008a,b) in their study.

In sublethal toxicity test, fish mortality recorded the highest level with increasing glyphosate treatment. This is probably as a result of its toxicity to the gills and perhaps not necessarily decline in oxygen levels because the parameters had little variation and were evident that physicochemical properties of the water holding tanks were within the desirable range of fish culture (Boyd, 1979). Meleter *et al.* (1971) reported that herbicide affects the gas exchange of fish and other aquatic organisms.

Fish survival and growth performance indices which are useful tools to ascertain fish performance and condition(s) in a system were found to decrease with the increasing concentration of the glyphosate in this study. This corroborates the findings of Salbego *et al.* (2010) and Sweilum (2006) that herbicides concentration in the aquatic system cause physiological stress and have direct effects in decreasing the relative growth rate of fish. Several authors have reported that environmental stressors affect the biochemical and physiological capacities of fish to digest and transform the digested nutrients which in turn leads to a reduction in fish growth and feed efficiency (Adeyeni, 2010; Lazzari *et al.,* 2010; Fourie, 2006; Avoaj, Oti, 1997).

In this study, muscle Na^+/K^+-ATPase were lowest in the control compared to other treatments which indicate

that glyphosate affected the increase in production of Na^+/K^+-ATPase. The decrease in Na^+/K^+-ATPase synthesis in the fish muscles suggests normal cruising efficient swimming patterns which do not require excessive enzymatic energy production as well as maintaining adequate resting potential while the increase in the muscle's Na^+/K^+-ATPase in the glyphosate treatments was due to response to the chemical stress which was, as a result, increased swimming and activities. The increased movement has also been characterized as fish's response to chemical stressors (Ayoola, 2008a; Ogundiran *et al.,* 2009; Hadi, Alwan, 2012).

Gill Na^+/K^+-ATPase was generally high among the control groups without glyphosate. The gills which are active respiratory organs in fish are required to be active to sustain respiration hence the high levels of Na^+/K^+-ATPase activities. This agrees with the work of Obuotor (2004). The decrease of Na^+/K^+-ATPase activity with increasing concentration of glyphosate in the fish gills recorded in this study might be a resultant effect of Na^+/K^+- pump disruptions, which allows unusual or inconsistent movement of Na^+/K^+ into the cell along the concentration gradient. Nowak (1992) also reported that a decrease in Na^+/K^+-ATPase of fish exposed to a toxic substance may be metabolic or ionic regulation. Another reason for the reduction of Na^+/K^+-ATPase activity in the gills of the exposed fish might be due to damages of the cells which allow the leakages of ATP into the bloodstream and pathological changes in tissue (Olurin *et al*., 2006).

Chemical toxicants affect normal organ functioning either by decreasing or increasing its cellular activities which is a dose-dependent response to the toxicant (Sullivan, Somero, 1983). Liver functions in the storage of glucose in the form of glycogen through a process of glycogenesis and a centre for detoxification (Barnhoorn, van Vuren, 2004). Liver Na^+/K^+ ATPase activity showed a corresponding increase with an increase in glyphosate. The Na^+/K^+ ATPase enzymatic activities in the liver were increased by the presence of glyphosate probably in response to stress created by the herbicides. The liver is largely responsible for the detoxification of toxins from the body and usually the organ that is first damaged due to toxic exposures. Chemical toxicants significantly affect the physiology of the liver by some histological alterations as recorded in earlier studies (Ayoola, 2008a,b; Ogundiran *et al*., 2009)

Malate dehydrogenase plays an important role in the pathway of the tricarboxylic acid cycle which is also known as the Kreb's Cycle which is critical to cellular respiration in cells (Minárik *et al*., 2002). Malate DH which is a biomarker of cellular respiratory activities in animal tissues could be affected by pathological and toxic conditions which tend to decrease or upsetting cellular respiratory activities (Panepucci *et al.,* 2002). Muscles generally have higher cellular respiratory activities hence as seen in the fish tissues in this study compared to other tissues. Minárik *et al.* (2002) opined that cellular respiratory activities through the tricarboxylic acid cycle can be triggered by normal and abnormal environmental and physiological conditions such as stress. In this study, glyphosate exposure caused a decrease in muscle and liver tissues while gill tissues increased cellular respiratory activities with exposure to glyphosate.

Lactate dehydrogenase is another very important enzyme that plays a significant role in the interconversion of pyruvate, the final product of the glycolytic pathway to lactate in no or short supply of oxygen. At high concentrations, the enzyme exhibits feedback inhibition and the rate of conversion of pyruvate to lactate is decreased. Increased lactate dehydrogenase synthesis observed in all tissues of fish exposed to glyphosate is an indication of hypoxia in cells due to reduction in feed conversion and cellular respiration efficiency.

Conclusions

The study concluded that the increase in glyphosate concentration negatively impacted feed nutrient utilization by the fish which consequently affected their survival and growth, and also had a pronounced effect on the enzymatic activities of the studied organs.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Author contributions

AOA – conducted the research and investigation process, collected and analyzed the data;
HAA – formulated the research goals and aim, assisted in experimental procedures and interpretation of data, and draft the manuscript;
VFO – supervised the research work and reviewed the manuscript for final submission;
GEE – assisted in experimental procedures and interpretation of data.

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