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Histo-morphological alteration of lethal and sub-lethal effect of glyphosate-based herbicide on catfish hybrid (*heteroclarias* sp.)

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Abstract

Herbicides are actively used in terrestrial and aquatic ecosystems to control unwanted weeds and their use has generated serious concerns and potential adverse effects on environment and human health. Toxicity of catfish hybrid (Heteroclarias sp.) juvenile exposed to glyphosate-based herbicide as it contributes to environmental degradation when reaches water bodies through direct applications, erosion, agricultural runoff and leaching, thereby endangers aquatic life such as fish was investigated. Ten pieces each of Heteroclarias sp juveniles with mean body weight of 8.45±2.82g and mean body length of 14.81±0.67cm were subjected to 0.00, 1.00, 1.25, 2.50, 3.75 and 5.00mg/l concentrations of glyphosate-based herbicide in a 50litres experimental water tank each for 96hours. The fish behavioural changes were noted and the lethal concentrations LC_{50} were calculated using probit analysis. Sub-lethal studies had 0.0, 1.0, 1.2, 1.5 and 2.0mg/L of the herbicide in a static renewal bioassay for 28 days. The body weight and length of Heteroclarias sp were measured and histological studies of gill, liver and muscle were processed. Fish in 1.0mg/L and 1.25mg/L concentrations showed normal swimming pattern, normal breathing and rapid response to external stimulus. While fish in the other concentrations (2.5mg/L, 3.75mg/L and 5.0mg/L) showed lateral swimming pattern, gasping breathing and sluggish responses. Highest mortality (70%) was observed in 5.0mg/L and 96h lethal concentration (LC_{50}) was 4.12mg/L. Mean body weight and length of the exposed fish were weekly insignificant ($P>0.05$). Fish in 2.0mg/L recorded 33.3% mortality, while 16.8% mortality was recorded in 1.2 and 1.5mg/L concentrations. Histology revealed degeneration and lifting of the epithelial cells, partial complete fusion and dilation of marginal channel of the secondary lamella, severe vasodilation with blood congestion and neutrophil infiltration of the Heteroclarias gills. Liver showed necrosis, vacuolization, lesion and damage of central vein, degeneration of hepatic connective tissue and presence of melanomacrophages. Meanwhile, muscle showed separation of muscle fibers, shortening of the muscle bundle, cellular infiltration and presence of rounded nuclei and severe derangement of myo-histology of the muscle. The herbicide is highly toxic to Heteroclarias sp juvenile when exposed to both lethal and sub-lethal concentrations.

Keywords: Glyphosate, *Heteroclarias* species, Herbicide, Histology, Hybrid, Lesion, Toxicity

Altération histo-morphologique de l'effet létal et sublétal de l'herbicide à base de glyphosate sur l'hybride de poisson-chat (heteroclarias sp.)

Résumé

Les herbicides sont activement utilisés dans les écosystèmes terrestres et aquatiques pour

contrôler les mauvaises herbes indésirables et leur utilisation a suscité de graves préoccupations et des effets néfastes potentiels sur l'environnement et la santé humaine. La toxicité des juvéniles hybrides de poisson-chat (Heteroclarias sp.) exposés à un herbicide à base de glyphosate, car il contribue à la dégradation de l'environnement lorsqu'il atteint les plans d'eau par des applications directes, l'érosion, le ruissellement agricole et le lessivage, met ainsi en danger la vie aquatique comme les poissons a été étudiée. Dix morceaux de juvéniles d'Heteroclariassp avec un poids corporel moyen de 8,45 ± 2,82 g et une longueur corporelle moyenne de 14,81 ± 0,67 cm ont été soumis à des concentrations de 0,00, 1,00, 1,25, 2,50, 3,75 et 5,00 mg/l d'herbicide à base de glyphosate dans un Réservoir d'eau expérimental de 50 litres chacun pendant 96 heures. Les changements de comportement des poissons ont été notés et les concentrations létales CL50 ont été calculées à l'aide d'une analyse probit. Les études sublétales avaient 0,0, 1,0, 1,2, 1,5 et 2,0 mg/L de l'herbicide dans un essai biologique de renouvellement statique pendant 28 jours. Le poids corporel et la longueur des Heteroclarias sp ont été mesurés et des études histologiques des branchies, du foie et des muscles ont été réalisées. Les poissons à des concentrations de 1,0 mg/L et 1,25 mg/L ont montré un schéma de nage normal, une respiration normale et une réponse rapide aux stimuli externes. Alors que les poissons aux autres concentrations (2,5 mg/L, 3,75 mg/L et 5,0 mg/L) ont montré une nage latérale, une respiration haletante et des réponses lentes. La mortalité la plus élevée (70 %) a été observée à 5,0 mg/L et la concentration létale à 96 h (LC50) était de 4,12 mg/L. Le poids corporel moyen et la longueur des poissons exposés étaient hebdomadairement insignifiants (P > 0,05). Les poissons à 2,0 mg/L ont enregistré une mortalité de 33,3 %, tandis qu'une mortalité de 16,8 % a été enregistrée à des concentrations de 1,2 et 1,5 mg/L. L'histologie a révélé une dégénérescence et un soulèvement des cellules épithéliales, une fusion complète partielle et une dilatation du canal marginal de la lamelle secondaire, une vasodilatation sévère avec congestion sanguine et infiltration de neutrophiles dans les branchies de l'hétéroclarie. Le foie présentait une nécrose, une vacuolisation, des lésions et des lésions de la veine centrale, une dégénérescence du tissu conjonctif hépatique et la présence de mélanomacrophages. Pendant ce temps, le muscle a montré une séparation des fibres musculaires, un raccourcissement du faisceau musculaire, une infiltration cellulaire et la présence de noyaux arrondis et un grave dérangement de la myo-histologie du muscle. L'herbicide est hautement toxique pour les juvéniles d'Heteroclarias sp lorsqu'il est exposé à des concentrations létales et sublétales.

Mots clés: Glyphosate, Espèce Heteroclarias, Herbicide, Histologie, Hybride, Lésion, Toxicité

Introduction

Fish is used as biomarker for checking or evaluating water quality in monitoring programmes. Catfish hybrid (*Hetero clarias* sp.) has great nutritional and commercial values, and mostly prefer by farmers. In areas where mixed farming is practiced or there is leaching or erosion of glyphosate-based herbicides in fish ponds, the effects of the use of this herbicide need to be studied. Herbicides are actively used in terrestrial and aquatic ecosystems to control unwanted weeds, and their use has generated serious concerns about the potential adverse effects on the environment and human health (Oleh *et al*., 2009). Herbicides may reach water bodies via agricultural runoff and leaching processes, as well as direct applications to control noxious aquatic weeds. Once in the aquatic ecosystems, herbicides may reduce environmental quality and influence essential ecosystem functioning by

reducing species diversity and community structures, modifying food chains, changing patterns of energy flow and nutrient cycling including stability and resilience of ecosystems (Perez, 2007). Alterations of the chemical composition of natural aquatic environments can affect all fauna present, especially fish (Oruc e*t al.,* 2004). Glyphosate based herbicide is a non-selective and used as post emergence herbicides (Dill *et al*., 2010). This herbicide is extensively used worldwide and known to be strongly adsorbed to soil, where it is subjected to microbial degradation. This is one of glyphosate's advantageous herbicidal properties, limiting agricultural input to surface waters in ideal conditions (Tsai, 2019). However, pulses of contamination can be expected when rainfall occurs directly after application and in flood events which also increases river sediment load (Byer *et al.,* 2008). Urban runoff and wastewater treatment effluent also account for considerable glyphosate input into rivers (Struger *et al*., 2008). Despite its widespread use, concentrations of glyphosate, or its associated formulation components, are not routinely monitored in surface waters. However, glyphosate concentrations have been regularly reported to occur up to 10 - 15 µg/L in rivers (Glusczak *et al*., 2009; Kelly *et al.,* 2010; Kreutz *et al.,* 2011). Fishes are widely used to evaluate the health of aquatic ecosystems and physiological changes serve as biomarkers of environmental pollution (Ndome *et al*., 2013). Hybridization is the products of progeny of parents from different lines, strains and species. *Heteroclaris* sp. is one of the genetic improvements in aquaculture industry which has been recognized as tool of stock improvement and management purposes. *Heteroclarias* sp is an hybrid from *Clarias gariepinus* ♀ x *Heterobranchus* spp ♂. *Heteroclarias* spp is in high demand by most farmers due to their hardness and fast growth (Solomon and Ezigbo, 2010) which has the best growth in both monoculture and polycultured systems when compared with the parent *Clarias* sp. and *Heterobranchus* sp. (Solomon and Faruwa, 2011). Rampant use of glyphosate-based herbicide for agricultural applications and non-agricultural operations such as home-based, industrial and municipal weed control has generated environmental degradation and health risk for living organisms. The International Agency for Research on Cancer (IARC) has reclassified glyphosate as Group 2A (probably carcinogenic to human) in 2015. Due to this, it is necessary to study the effect of this herbicide on *Heteroclarias* sp., since the hybrid is most preferred cultured fish by fish farmer. Research shows high concentration of glyphosate (5.2mg/l) in runoff waters when 8.6kg/ha of glyphosate was applied on farmland (Relyea, 2003). Detectable concentrations of glyphosate were reported in stream after application and longer half-lives were also reported in hard water after post application (Perez *et al*., 2007). Also, glyphosate has been grouped among carcinogenic agent when consumed or exposed to human being or other living organisms such as fish. However, this research was carried out to determine the toxicity of glyphosate-based herbicide on behavioural response, length-weight relationship and histopathological examination of organs of catfish hybrid *Heteroclarias* spp. since its most prefer fish species by fish farmer.

Materials and methods

The study was conducted at the Department of Zoology and Environmental Biology Laboratory, Lagos State University (LASU), Lagos, Nigeria, for four (4) months. The experiment was set up in twelve (12) glass tanks with dimension 70 cm x 40 cm x 40 cm cubic and capacity of 150litres of water. Force-up® (is a

glyphosate-based herbicides which contain 360g of glyphasate in form of 480gl^{-1} of isopropylamine) was purchased from Jubaili Agrotec, Ibadan, Nigeria for the experiment.

Experimental fish

200 samples of *Heteroclarias* sp juvenile with mean body weight of 8.45±2.82g and mean body length of 14.81±0.67cm were obtained from LASU fish farm. The fish samples were acclimatized to experimental conditions for fourteen (14) days prior to range finding test of the herbicides. They were fed with 2mm pellet of 45% protein of commercial feed once daily as suggested by Ndimele *et al*. (2015).

Acclimatization of the experimental fish

The experimental fish was held for 14days in the glass tank with capacity of 150 litres containing non-chlorinated water. The fish species were not fed within 24 hours after they were obtained from the fish farm. However, the fish were subsequently fed with 2mm pellet of 45% protein of commercial feed. During acclimatization, the water was changed daily in order to remove fecal materials and unconsumed feeds.

Range finding test and acute toxicity test

A preliminary range finding test was conducted with five (5) pieces of *Heteroclarias* sp in 5 glass tanks containing 50liters of water with different concentrations of Glyphosate herbicide (from 7.0mg/l down to 1.0mg/l) for few hours until lowest mortality was observed as opined by Micah *et al*. (2017). Ten samples of *Heteroclarias* sp were introduced into each glass tank containing 0.00, 1.00, 1.25, 2.50, 3.75, and 5.00mg/l of glyphosate herbicide in 50liters of experimental water for 96hours under laboratory conditions. The fish behavioural changes and mortality were reported after 24 hours, 48hours, 72hours and 96hours for each concentration (Makinde *et al*., 2015).

Chronic toxicity test

The following concentrations viz: 0.25, 0.30, 0.35, and 0.50 of the LC_{50} at 96hours were used in four weeks sub-lethal exposure. The corresponding concentrations were 1.0, 1.2, 1.5 and 2.0mg/l in 50litres of water respectively. Six experimental fish were introduced into each of the glass tank respectively. A renewable bioassay within 24 hours for four weeks was done. This setup was in duplicate.

Fish mortality

Fish mortality was recorded, and the percentage mortality was calculated.

$$\text{Mortality of Fish Species \%} = \frac{\text{Number of dead fish}}{\text{Total number of fish in the tank}} \times \frac{100}{1}$$

Morphometric measurement

The weight and standard length of the experimental fish were carried out weekly for each concentrations throughout the study period.

Histological study

Experimental fish was randomly dissected from each concentration (0.0mg/l, 1.0mg/l, 1.2mg/l, 1.5mg/l and 2.0mg/l) of the herbicide after four weeks of exposure. Liver, gill and muscle were collected from each fish species. The organs (liver, gill and muscle) were immediately fixed in 10% formal saline for 10 minutes and dehydrated in ascending grades of ethanol (40%, 50%, 60%, 70%, 80%, 90% and absolute), while embedded in soft paraffin block. Microtone equipped with a very sharp stainless-steel blade was used to cut the organs into sections of 2-5µm thickness and mounted on well labeled glass slide (Saravpreet *et al*., 2018). The slides were stained with haematoxylin and counter stained with aqueous eosin. Stained section was mounted on microscope (CETI microscope with imaging software version 12) for viewing and observation, thereafter photomicrograph. The photomicrograph

organs were read and recorded accordingly.

Statistical analysis

Data obtained were subjected to statistical analyses using SPSS (IBM Corp., 2011). Mean body length and body weight of *Heteroclarias* sp were compared using Analysis of Variance (ANOVA). Mean body length and body weight were presented as Mean ±Standard deviation. Post hoc test was done using the Student-Newman-Keuls (SNK). LC_{50} and LC_{90} values were also calculated using the Probit Analysis of the SPSS software version 20.0. Probability value (p-value) less than 0.05 was statistically significant.

Results

Acute toxicity test (LC_{50})

Behavioural changes of *Heteroclarias* exposed to glyphosate at different concentrations and durations are shown on Table 1 – 4. After 24hours, 48hours, 72hours and 96hours of exposure to 5.00mg/l, 3.75mg/l and 2.50mg/l of the herbicide, the fish responded sluggishly to external stimulus, gasping breathing and lateral swimming pattern. However, the fish responses to 1.00mg/l and 1.25mg/l of the herbicide were rapid to external stimulus, normal breathing and swimming pattern. Mortality was first recorded in the *Heteroclarias* sp exposed to 5.00 mg/l of glyphosate after 48 hours of exposure (Table 5). At 72 hours and 96 hours of exposure, mortality was recorded in 2.50 mg/l, 3.75 mg/l and 5.00 mg/l concentrations. The number of mortality observed increases with increase in the concentration of the herbicide exposure. On the other hand, no mortality was recorded in the *Heteroclarias* sp exposed to 1.0 mg/l and 1.25 mg/l of the herbicide throughout the 96 hours of exposure. Using the Probit analysis, LC_{50} of the herbicide on *Heteroclarias* sp was 5.55 mg/l at 48 hours, 4.55 mg/l at 72 hours and 4.12 mg/l at 96 hours. Also, the LC_{90} values were 6.42 mg/l, 6.36 mg/l and 5.93 mg/l at 48 hours, 72hours and 96 hours respectively.

Chronic toxicity test

No mortality was recorded in the control *Heteroclarias* sp group and 1.0 mg/l (24.3% of LC_{50} at 96 hours) after four weeks (Table 6). However, 16.8% mortality was recorded at 1.2 mg/l (29.1% of LC_{50} at 96 hours) and 1.5 mg/l (36.4% of LC_{50} at 96 hours) exposure. On the other hand, 33.3% mortality was recorded at 2.0 mg/l (48.5% of LC_{50} at 96 hours) of the herbicide exposure.

Mean body weight

The mean weekly body weight of *Heteroclarias* sp exposed to varying concentrations of glyphosate is shown in Figure 1. Weekly body weight of the control (0.0mg/l) and exposed *Heteroclarias* sp to the varying concentrations were observed to increase on weekly basis from the initial to week 4 of the exposure increasingly. Significantly lower ($p < 0.05$) mean initial and final body weight in *Heteroclarias* sp exposed to 1.0 mg/l of the glyphosate (Table 7). Mean initial and final body weight were not significantly different between the control and exposed concentrations. However, there was no significant difference ($p>0.05$) in the mean weight gain of the control *Heteroclarias* and those exposed to the varying concentrations of the glyphosate.

Mean body length

Mean weekly length of *Heteroclarias* sp exposed to varying concentrations of the herbicide for four (4) weeks experimental period, showed progressive increase in the mean length of all the experimental fish including the control as shown in figure 2. Mean initial and final length was significantly ($p<0.05$) lower at 1.0 mg/l concentration of the exposure (Table 8), but was not significantly ($p>0.05$) different among other experimental groups. On the other hand, 2.0 mg/l*concentration recorded* significantly ($p<0.05$) lowest, while mean

body length gain was higher but not significantly ($p>0.05$) different among the control and other exposure groups (Table 8).

Histological study

Plate 1, 2 and 3 showed photomicrograph of gills, liver and muscle of *Heteroclarias* sp exposed to different concentrations of glyphosate-based herbicide respectively.

Table 1: Behavioural changes of Heteroclarias sp after 24 hours exposure to glyphosate

Concentration (Mg/L)	Response to External Stimuli	Swimming Pattern	Breathing Mechanisms
1.00	All fish respond rapidly	Normal swimming pattern	Normal breathing
1.25	All fish respond rapidly	Normal swimming pattern	Normal breathing
2.50	All fish respond rapidly	Normal swimming pattern	Normal breathing
3.75	All fish respond rapidly	Normal swimming pattern	Normal breathing
5.00	Two fish respond sluggishly	lateral swimming pattern by two fish	Gasping breathing by two fish

Table 2: Behavioural changes of Heteroclarias sp after 48 hours exposure to glyphosate

Concentration (Mg/L)	Response to External Stimuli	Swimming Pattern	Breathing Mechanisms
1.00	All the fish respond rapidly	Normal swimming pattern	Normal breathing for all experimental fish
1.25	All the fish respond rapidly	Normal swimming pattern	Normal breathing for all experimental fish
2.50	One of the fish respond sluggishly	One of the fish had lateral swimming pattern while others had normal swimming pattern	Gasping breathing by one of the fish
3.75	Three of the fish showed sluggish movement	Three of the fish had lateral swimming pattern while others had normal swimming pattern	Gasping breathing by three of the fish
5.00	Four of the fish were relatively sluggish	Four of the fish had lateral swimming pattern while others had normal swimming pattern	Gasping breathing by four of the experimental fish

Table 3: Behavioural changes of Heteroclarias sp after 72 hours exposure to glyphosate

Concentration (Mg/L)	Response to External Stimuli	Swimming Pattern	Breathing Mechanisms
1.00	All the fish respond rapidly	Normal swimming pattern	Normal breathing for all the fish
1.25	All the fish respond rapidly	Normal swimming pattern	Normal breathing for all the fish
2.50	All the fish respond rapidly except one (1) with sluggish response	One of the fish had lateral swimming pattern while others had normal swimming pattern	Normal breathing except for one with Gasping breathing
3.75	All the fish respond rapidly	Normal swimming pattern	Normal breathing
5.00	Respond rapidly except one with relatively sluggish response	Normal swimming pattern except one with lateral swimming pattern	Normal breathing except one with gasping breathing

Table 4: Behavioural changes of Heteroclarias sp after 96 hours exposure to glyphosate

Concentration (Mg/L)	Response to External Stimuli	Swimming Pattern	Breathing Mechanisms
1.00	All the fish respond rapidly	Normal swimming pattern	Normal breathing for all the fish
1.25	All the fish respond rapidly	Normal swimming pattern	Normal breathing for all the fish
2.50	All the fish respond rapidly	Normal swimming pattern	Normal breathing
3.75	All the fish respond rapidly except one with sluggish response	Normal swimming pattern except one with lateral swimming pattern	Normal breathing except one with gasping breathing
5.00	All the fish respond rapidly	Normal swimming pattern	Normal breathing

Table 5: Acute toxicity test (LC_{50}) of the glyphosate herbicide on Heteroclarias sp

	Hours			
Concentration (mg/l)	24	48	72	96
1.00	0	0	0	0
1.25	0	0	0	0
2.50	0	0	1	2
3.75	0	0	3	4
5.00	0	2	6	7
LC_{50}	NA	5.55	4.55	4.12
LC_{90}	NA	6.42	6.36	5.93

Keys; NA - Not available

Table 6: Weekly mortality rate of Heteroclarias sp exposed to glyphosate

	Weeks				
Concentration (mg/l)	1	2	3	4	Total mortality (%)
0.0	0	0	0	0	0 (0.0)
1.0	0	0	0	0	0 (0.0)
1.2	1	0	0	0	1 (16.8)
1.5	0	0	0	1	1 (16.8)
2.0	1	0	0	1	2 (33.3)

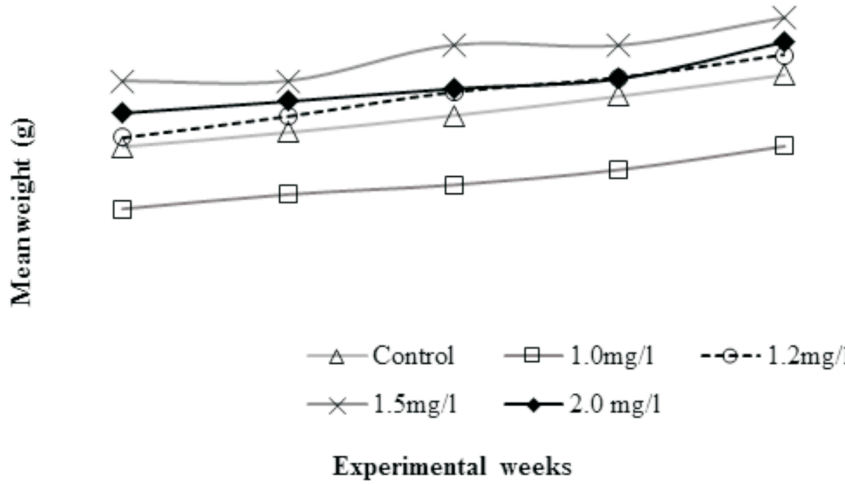


Figure 1: ***Weekly Mean Bodyweight of Heteroclarias sp exposed to glyphosate herbicide***

Table 7: ***Body weight (g) of Heteroclarias sp exposed to glyphosate-based herbicide***

	Weight		
	Initial	**Final**	**Gain**
Control	32.05±1.82[a]	39.75±1.78[a]	7.71±3.20[a]
1.0mg/l	25.38±2.10[b]	32.10±2.60[b]	6.73±4.06[a]
1.2mg/l	33.06±3.27[a]	41.88±4.47[a]	7.81±3.54[a]
1.5mg/l	39.13±3.26[a]	45.92±3.58[a]	5.79±5.09[a]
2.0 mg/l	35.65±4.02[a]	43.31±4.41[a]	7.42±2.37[a]

[abc]Mean (±Standard deviation) in the same column having similar superscripts are not significantly different at $p<0.05$

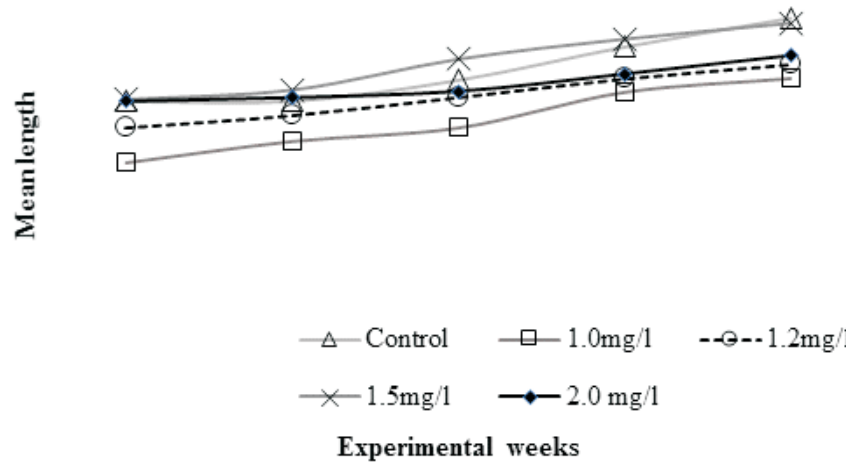
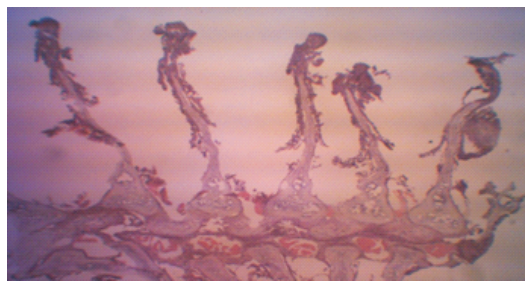


Figure 2: ***Weekly mean body length of Heteroclarias sp exposed to glyphosate herbicide***

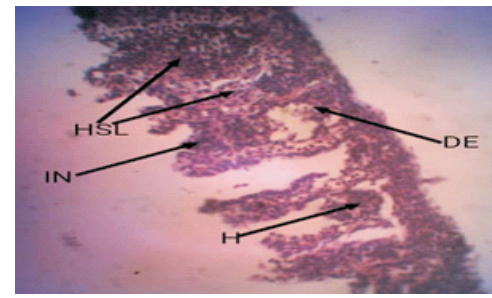
Table 8: ***Body length of Heteroclarias sp exposed to glyphosate herbicide***

	Length		
	Initial	**Final**	**Gain**
Control	15.63±0.56[a]	19.63±0.94[a]	4.00±1.25[a]
1.0 mg/l	12.67±0.19[b]	16.73±0.33[b]	4.07±0.43[a]
1.2 mg/l	14.37±0.75[a]	17.40±1.10[b]	2.98±0.83[b]
1.5 mg/l	15.72±1.00[a]	19.40±1.20[a]	3.54±1.48[a]
2.0 mg/l	15.64±0.87[a]	17.85±0.69[b]	1.63±0.42[b]

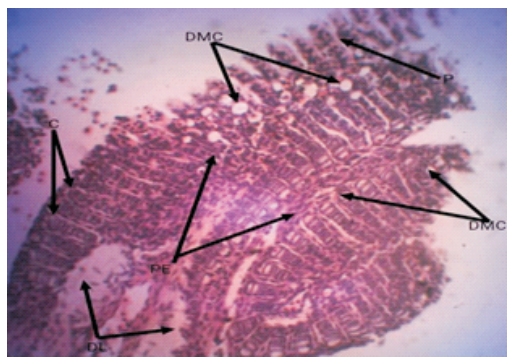
[abc]Mean (±Standard deviation) in the same column having similar superscripts are not significantly different at $p<0.05$



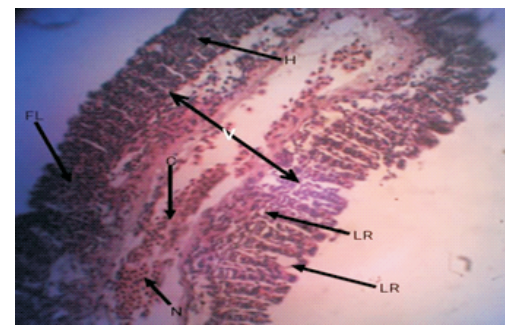
A: Photomicrograph (X400) of *Heteroclarias* gills exposed to 0.0mg/l glyphosate-based herbicide showed normal intact of the primary (PL) and secondary lamella (SL) freely separated without fusion. Central axis (CA), water channel (W) are visible without vasodilation. Epithelial cell (EC) are seen but detached in some place.



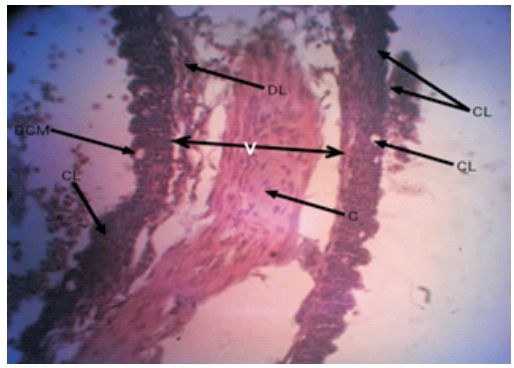
B: Photomicrograph (X400) of *Heteroclarias* gills exposed to 1.0mg/l glyphosate-based herbicide shows hyperplasia in secondary lamellae (HSL), hypertrophy (H). There is also partial fusion of secondary lamella (IN) with slight degeneration of epithelial cell (DE)



C: Photomicrograph of *Heteroclarias* gills exposed to 1.2mg/l glyphosate-based herbicide shows distortion of gill architecture characterized with numerous dilation of marginal channel of the secondary lamellae (DMC), complete (C) and partial (P) fusion of lamella (c). Hyperplasia of the epithelial cells (PE) is also seen. Degeneration and vacuolization of the lamellae (DL)

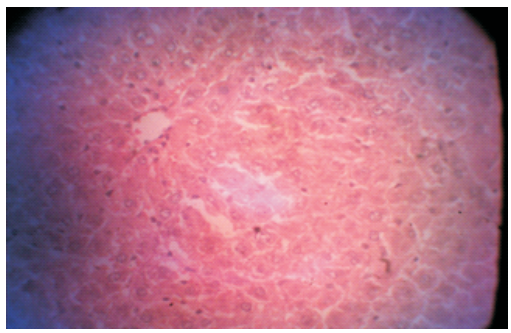


D: Photomicrograph (X400) of *Heteroclarias* gills exposed to 1.5mg/l glyphosate-based herbicide marked with severe **vasodilation (V) with blood congestion (C) and** neutrophils **infiltration (N),** lifting of the respiratory epithelium (LR), **cellular hypertrophy (H)** and several fusion of lamellae (FL)

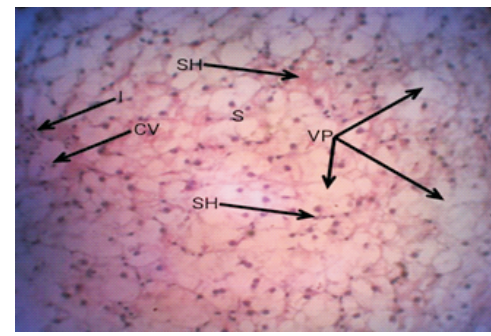


E: Photomicrograph (X400)of *Heteroclarias* gills exposed to 2.0mg/l glyphosate-based herbicide shows complete fusion of almost all lamellae (CL) as a result of cellular hypertrophy and hyperplasia, vasodilation (V), blood congestion (C), dilation of marginal channel of the secondary lamellae (DMC) and degeneration of primary lamellae (DL)

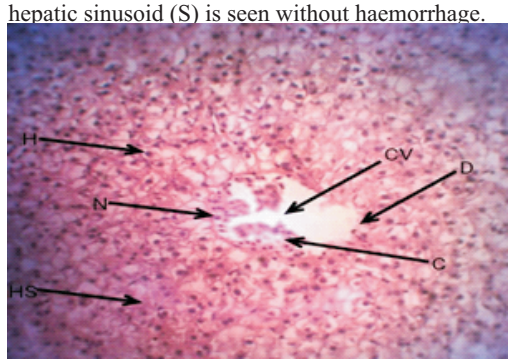
Plate 1(A – E): Photomicrograph of gills of *Heteroclarias* sp exposed to different concentrations of glyphosate-based herbicide



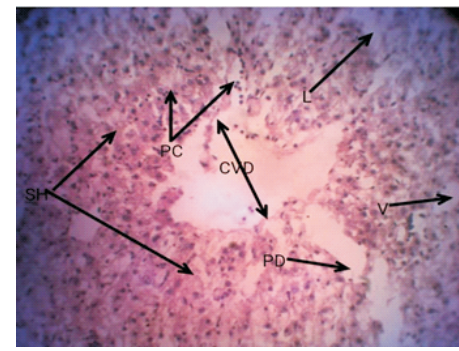
A: Photomicrograph (X400) of *Heteroclarias* liver exposed to 0.0mg/l glyphosate-based herbicide show a clear central vein (CV) with no congestion, hepatocytes are normal (H) arrange in a cord like manner toward the central vein (CV), normal hepatic sinusoid (S) is seen without haemorrhage.



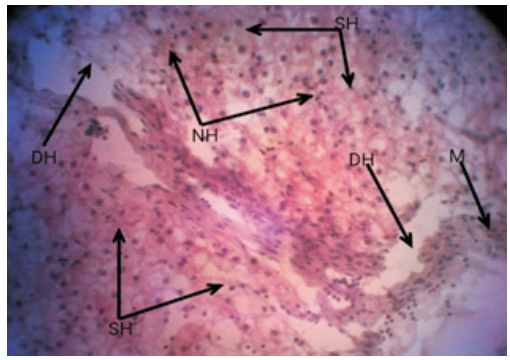
B: Photomicrograph (X400) of *Heteroclarias* liver exposed to 1.0mg/l glyphosate-based herbicide shows vacuolization of the hepatic parenchyma (VP), slight sinusoidal haemorrhage (SH), infiltration (I) around the central vein (CV) and pyknotic cell (PC)



C: Photomicrograph (X400) of*Heteroclarias*liver exposed to 1.2mg/l glyphosate-based herbicide shows derangement in hepatic histo-architecture with numerous sinusoidal haemorrhages (HS) central vein (CV) congestion (C) and infiltration with neutrophils (N). There is also slight degeneration (D) of hepatocyte (H).

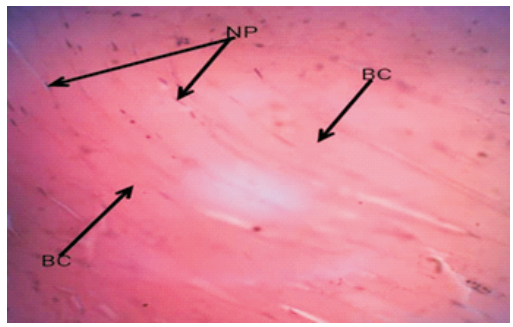


D: Photomicrograph (X400) of*Heteroclarias*liver exposed to 1.5mg/l glyphosate-based herbicide shows severe derangement characterized with parenchyma degeneration (PD), vacuolization (V) and lesion (L). There are presence of pyknotic cells (PC), damage central vein (CVD) and sinusoidal haemorrhage (SH)

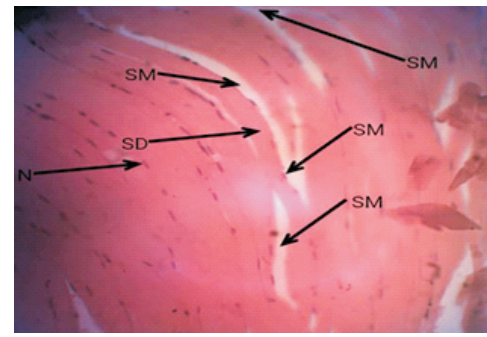


E: Photomicrograph (X400) of*Heteroclarias*liver exposed to 2.0mg/l glyphosate-based herbicide shows severe sinusoidal haemorrhage (SH), necrotic hepatocytes (NH), degeneration of hepatic connective tissue (DH) and present of melano-macrophages (M)

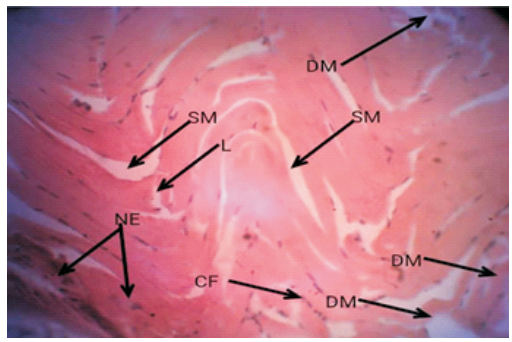
Plate 2(A – E): Photomicrograph of liver of *Heteroclarias* sp exposed to different concentrations of glyphosate-based herbicide



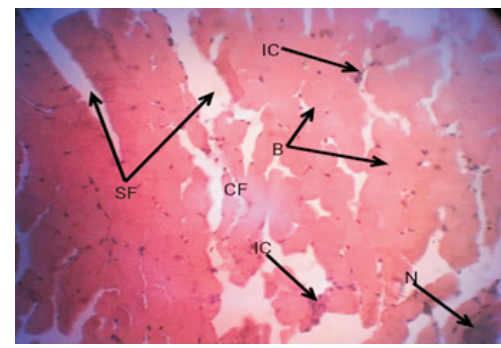
A: Photomicrograph (X400) of *Heteroclarias* muscle exposed to 0.0mg/l glyphosate-based herbicide shows muscle fibers that are cylindrical in shape and non-branching (BC), multiple elongated nuclei located peripherally (NP). Muscle bundles are closelypacked with no vacuolization and fragmentation.



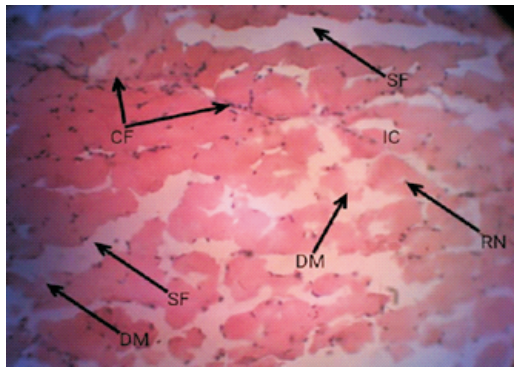
B: Photomicrograph (X400) of *Heteroclarias* muscle exposed to 1.0mg/l glyphosate-based herbicide shows slight shortening of muscle bundles (SD), splitting/separation of muscle fiber (SM), intact nuclei (N) which are located peripherally.



C: Photomicrograph (X400) of *Heteroclarias* muscle exposed to 1.2mg/l glyphosate-based herbicide showed mild degeneration of muscle fiber (DM), necrosis (NE), lesion (L), spitting of muscle fiber (SM) and cellular infiltration (CF).



D: Photomicrograph (X400) of *Heteroclarias* muscle exposed to 1.5mg/l glyphosate-based herbicide show sever derangement of myo-histology characterized with inflammatory cellular infiltration (IC), necrosis (N), muscle fiber are broken (B) and not
cylindrical in shape, fiber are fiber are Separated from each other (SF)



E: Photomicrograph (X400) of*Heteroclarias*muscle exposed to 2.0mg/l glyphosate-based herbicide showed sever distortion characterized by fragmentation and degeneration of muscle fiber (DM), increase cellular infiltration (CF), increase separation of fiber (SF) and presence of rounded nuclei and pyknotic (RN)

Plate 3(A – E): Photomicrograph of *Heteroclarias* sp muscle exposed to different concentrations of glyphosate-based herbicide

Discussion

Fish is used for ecosystem assessments, which behavioural changes can indicate the presence or absence of toxicant in the water body (Robinson, 2009). Behavioural changes of *Heteroclarias* sp juvenile is more pronounced with increasing concentrations of the herbicide which conforms to Ayanda *et al*. (2016) findings. These behavioural responses could be due to disruption of the nervous system of the fish which depend on the concentration of the toxicant (Fafioye *et al*., 2005). Also, behavioural changes may be due to change in biochemical body re-arrangement which includes hepatic functions of the fish liver (Ayoade *et al*., 2015). Mortality was observed with increase in concentration and duration of the exposure of *Heteroclarias* sp to the toxicant. The percentage mortality was in accordance with Ateeq *et al*. (2005) and Olurin *et al*. (2006) which opined that there should be less than 35% mortality in the lowest concentration, and at least, more than 65% mortality in the highest concentration for acute toxicity and chronic toxicity. Death of some of the fish could have occurred by direct or indirect poisoning which makes the experimental water not conducive for the fish (Ayande, 2016). The study showed 96h LC_{50} value of 4.12mg/l which is in contrast with Ayanda *et al*. (2016) who reported 96h LC_{50} of 0.530mg/L, Akinsorotan *et al.* (2013) with LC_{50} value of 43.65mg/L, Okowoda and Ataguda (2011) with LC_{50} of 17.5mg/L, Ayoola (2008) with LC_{50} of 1.05mg/L for *Clarias gariepinus*. However, the 96h LC_{50} value of this study is close to the LC_{50} value of Micah *et al*. (2015) which is 6.838mg/l, putting into consideration that toxicity of fish to toxicant depends on size, species and age (Konsar and Javed, 2012; Noga, 2012). The study indicated that *Heteroclarias* sp exposed to sub-lethal concentrations of glyphosate gained increase in body weight and length, which is not statistically significant. The mean weight gained ranges from 7.71± 3.20 (in control) to 7.42 ± 2.37 (in 2.0mg/l) and mean standard length of 4.00 ± 1.25 (in control) to 1.63 ±0.42 (in 2.0mg/l) concentration. This is in accordance with Owodeinde *et al*. (2012) that *Heteroclarias* exhibit superior growth, improved survival and general hardness than the true breed of either *Clarias gariepinus* or *Heterobranchus bidorsalis*. However, this is opposed to the report of Jimmy *et al*. (2014) which observed reduction in weight gain of fish exposed to chronic toxicity. The gill of *Heteroclarias* sp has large surface area which may be sensitive to the herbicide due to its vital role and direct exposure to the toxicant (Fergunson, 2006; Makinde *et al*., 2015). The damage of the gill secondary lamella

from partial to complete fusion as a result of cellular hypertrophy and hyperplasia may be due to irritation caused by the herbicide. However, numerous dilations of marginal channel of the secondary lamella observed with severe vasodilation with blood congestion and neutrophil infiltration might have caused increase of blood flow in the fish tissues which change the supply of the blood flow (Abdolahi *et al*., 2012). Alterations of the gill structures are adaptive and reduce the rate of absorption of toxic substances (Strzewska-Worotynska *et al*., 2017) which were also found altered in the parental (*Clarias gariepinus* and *Heterobranchus bidorsalis*) species of the *Heteroclarias* when exposed to toxicants (Fafioye *et al*., 2004; Ladipo *et al*., 2011; Olufayo and Alade, 2012; Makinde, 2015). Gills are metabolically active tissue for gaseous exchange, and its toxicants accumulation is significantly related to the level of the toxins that the fish inhabit (Manera *et al*., 2016). Epithelial hypertrophy increases water-blood distance which impairs oxygen uptake and increase the rate of the fish respiration by compensating for the low oxygen entrance (Flores-Lopes and Thomas, 2011; Agamy, 2013). Liver plays a role in breaking down of chemicals in the body system. Accumulation and detoxification of toxicant is a feature of liver, but when level of impediment in the experimental fish is reached, gradual degeneration of the hepatic connective tissue, derangement of the hepatocyte, vacuolization, lesion, damage of the central vein and presence of melanomacrophage was resulted. Decrease in the surface area of the liver cells could be a result of increase in degeneration of hepatic connective tissue which was in line with the findings of Ladipo *et al*. (2011) and Makinde *et al*. (2015). Ferguson (2006) reported osmoregulatory dysfunction from toxin-mediated diseases being responsible for necrosis of hepatocyte as observed in this study. The muscle of *Heteroclarias* sp is the most edible part of the fish that directly exposed to the herbicide as the gills. Separation and broken of the muscle fiber, slight shortening of the muscle bundles observed may be due to the displayed of lesions in muscle tissue which could have exposed the muscle to micro-organism invasion as reported by Saad *et al*. (2012). Increase cellular infiltration, presence of rounded nuclei and pyknotic, necrosis, elongation of muscle bundles, lesion and severe derangement of myo-histology recorded were structural changes in the muscle tissues as documented by Abbas and Ali (2007) and Patnaik *et al*. (2011). All these histo-architectural changes indicated that exposure of *Heteroclaria*s sp to lethal and sub-lethal concentrations of glyphosate-based herbicide were subjected to high environmental stress and toxicant. The alterations exhibit protective response which leads to reduction/degeneration or failure of the function which leads to death of the fish. Therefore, there should be a limit in the glyphosate-based herbicides exposure to aquatic ecosystem.

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