Biotechnol Res 2016; Vol 2(4):161-165
eISSN 2395-6763

ORIGINAL RESEARCH

Effect of glyphosate on some enzymes and electrolytes in *Heterobranchus bidosalis* (a common African catfish)

Iniobong Reuben INYANG[1], Nancy C. OKON[1], Sylvester Chibueze IZAH[1]*
[1]Environmental Toxicology Research Unit, Department of Biological Sciences, Niger Delta University, Wilberforce Island Bayelsa State
*Corresponding Author email: chivestizah@gmail.com

• *Received: 29 July 2016 • Revised: 30 August 2016 • Accepted: 10 September 2016 • Published: 16 September 2016* •

ABSTRACT
Pesticide constitute one of the most important pollutants in the aquatic environment pesticide can find their way into food chain, hence the need to study the effect of glyphosate on *Heterobranchus bidorsalis* enzymes and electrolytes. Adult *Heterobranchus bidorsalis* (mean length 15.07±0.28cm, mean weight, 18.05±0.4g) were acclimatized individually in a rectangular aquaria for seven days and then exposed to varying concentrations of the toxicant in a static bioassay for 30 days. Alkaline aminotranferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were determined in the plasma and kidney while electrolyte (Ca^{+}, K^{+}, Na^{+}) were determined in the liver. All the enzymes tested were statistically significant ($p<0.05$). Liver K+ values increased as the concentration of the toxicant increases (in a dose dependent pattern). While $Ca^{2+}$and Na^{+} values were not statistically significant ($P>0.05$), rather a slight fluctuation in values within the experimental group were observed. It is concluded that glyphosate could be toxic at high concentration. Enzymes tested are more useful biomarker of sublethal effect of glyphosate than the electrolytes. Further studies are required to evaluate the toxicity of glyphosate in *Heterobranchus bidorsalis* fingerlings, juveniles and recovery response in adults.

KEY WORDS: *Toxicity, Pesticides, Electrolytes, Enzymes, Heterobranchus bidorsalis*

Introduction

Pesticides have contributed a lot to pest management and agricultural enhancement. Activities pests have the tendency to severely compromise the health and wellbeing of individual animals and by extension, man (Ojezele and Abatan, 2009). In course of controlling pest, pesticides often affect non-target organisms' e.g. fish and other aquatic organisms. Unfortunately, pesticides are designed to alter specific body processes and they are not target specific (Daren and Cynthia, 2007; Lang, 1993).

Typically pesticides are substances used with the intention to control, prevent, and mitigate pest (Seiyaboh et al., 2013; Lawson et al., 2011; Akan et al., 2013; Ogamba et al., 2015). Pesticides applied in the field or from careless discarding of pesticide containers. These pesticides find their way into the aquatic ecosystem via runoff after precipitation. Sometimes they are carried by wind as aerosols. Like other pollutants, pesticides could alter water quality parameters with regard to heavy metals and general physicochemistry. However, heavy contamination of water by pesticide often result in Oxygen depletion, poisoning and mass mortality of fishes has been reported (Inyang et al., 2014, Atamnalp et al., 2001).

Glyphosate (N-(Phophomethyl) glycine (IUPAC) is an acid that belongs to chemical group of phosphonoglycine or more generic: Organophosphate herbicide. Glysophate is a post

emergent, systemic and non-selective (a broad spectrum) herbicide used in both agricultural and non-agricultural areas. It is commonly used in Nigeria and other West African countries for the control of unwanted weeds including perennials and wood plants. It is mainly absorbed into the plant via the leaves and then transported throughout the plant where it acts on plant enzyme system.

Glyphosate can contaminate surface water either directly as a result of aquatic weed control or indirectly when glyphosate bound to soil particles is washed into rivers or streams (WHO, 2005), glyphosate are commercially formulated products containing surfactants which are toxic to fish and to some aquatic invertebrates (Servizi et al., 1997). Studies have shown that the acute toxicity of glyphosate varies according to species and age of fish and under different environmental conditions, such as pH and temperature (Cox, 1995). Additionally, sub lethal effect in fish unveiled changes in some enzyme activity in the serum, liver, kidneys and morphological changes in gills, liver and kidneys (Neskovicet et al., 1996).

Various chemicals entering the aquatic ecosystem via human activities, either accidentally or by design causes adverse effect on the aquatic biota including deleterious changes which disrupts the metabolic activity at biochemical levels (Das and Mukerjee, 2000). The physiological processes in fish have been monitored by determining changes in the activities of enzymes in plasma/serum and functional organs. The biochemical changes in plasma/serum is often regarded as the most important indices of the status of the internal environment of the fish (Inyang et al. 2014). Some enzyme analysis are used as a biomarker in assessing the toxicity of xenobiotics in Pisces. Biomarker may be any measurable biochemical, cellular, physiological or behavioural change in an organism or population that indicates exposure to chemical pollutant (Depledge, 1994). By focusing on the intermediate, sublethal effects of a pollutant, revealed the environmental threat before obvious toxic effects such as death of organisms (Aly and El-Gendy, 2015).

This present study is aimed at looking at the plasma and kidney as well as electrolytes in the liver as a biomarker in these vital organs in *Heterobranchus bidorsalis*.

Materials and methods

Source of fish used in the experiment

Fish samples for this study were obtained from a private fish farm at biotechnology research center, Odi, Bayelsa state. They were transported to the wet laboratory of the Department Of Biological Sciences, Niger Delta University, where the assays were conducted. Thirty adult Heterobranchus bidorsalis (mean weight 98.05±0.4gSD mean length 15.07±0.23cmSD) were acclimatized individually in a rectangular aquaria for eight days (9.00-11.00h) with 35% crude protein diet at 1% biomass.

General bioassay technique

Sublethal concentrations of glyphosate for the assay (0.16, 0.32. 0.46mg/L were determined based on the range finding test (Inyang et al., 2010). These were prepared by transferring 0.01, 0.02, 0.03mls with borehole water in the test aquaria. 30L of the diluent water was used as control. For replications of each treatment level (concentration) and control were set up by introducing fishes individually into each aquarium. The exposure period lasted for 30 days during which the exposure media was renewed daily. The physio-chemical characterization of the water used for fish bioassay was carried out using standard methods (APHA, 1998) and the following values were obtained: Temperature 26.00-26.05oC, pH 6.20-6.37, alkalinity, 12.33-15.30mg/l, conductivity 97-128µs/cm and turbidity 0.42-0.58NTU.

Biochemical and electrolyte profile following exposure to glyphosate.

After the 30 days exposure period blood sample for enzyme analysis were collected from each fish (behind the anal fin) with 23G size needle and syringe. Fish were not fed prior to blood collection, sample were preserved in EDTA. Fish were dissected for the collection of kidney and liver. 0.5g of each organ was macerated (grounded) with pestle and mortar, physiological saline and deionized water was used for preservation and stabilization. Samples were centrifuged at the rate of 300rpm for 10 minutes. The supernatants were then removed and store in plain bottles at -20oC for analysis. The activities of aspartate amino transferase (AST), and alanine amino transferase (ALT) were assayed using the colorimetric method of Reitman and Frankel (1957) while alkaline phosphatase (ALP) was assayed via previously

described method by King and Armstrong (1934). All the electrolyte were assayed via Logawary et al. (2006) and APHA (1998) methods.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) where differences exist, Duncan multiple range test (DMRT) were used to test for pairwise significant differences ($P<0.05$) between treatments (Wahua, 1999).

Results and Discussion

Enzyme analysis is widely used for rapid detection and prediction of early warning of chemical toxicity in organisms. Enzyme analysis of organs such as kidney, liver and plasma in fish can provide important information about any change that occurs in an organism. Enzyme activities affect various chemical and biological reactions in the body of fish. A shift in the activities of enzyme from the control is also used as a relevant stress indicator.

Table 1 and 2 presents the level of enzymes in the blood and kidney of Heterobranchus bidorsalis exposed to glyphosate for 30m days respectively. Plasma ALT values decreases as the concentration of the toxicant increased, while the kidney ALT values increased slightly (abeit not significant) as the concentration of the glyphosate increases. Plasma ALT values decreases as the concentration of the toxicant (glyphosate) increased. ALP values also decreases within the experimental group in a dose dependent pattern. Kidney AST values were significant ($p<0.05$) in a dose dependent pattern.

Alanine amino transferase (ALT) and aspartate amino transferase (AST) are liver specific enzymes and they are more sensitive measure of hepatoxicity and histopathologic changes and can be assessed within a short time. Any increase or decrease in the values of ALT and AST indicate tissue damage in liver and kidney (Inyang, 2008). Alterations in alkaline phosphatase (ALP) activities in tissue, organs and plasma have been reported in fish exposed to toxicants of varying concentrations (Inyang, 2008, Inyang *et al*., 2010). Alterations of these enzymes (ALT, AST and ALP) in fish as a result of toxicant or contaminant effect in various organs in fish have been reported (Inyang, 2008, Sunmonu and Oloyede, 2012, Aly and EL-Gendy 2015). Alterations in ALT and AST in the kidney may be due to the nephrotoxic effect of the toxicant on the function of the kidney which results in the liberation of the intracellular enzymes. A clear reduction in values of ALP is an indication of inhibition of this enzyme in the kidney cells. Additionally, this may indicate inactive transamination and onidative deamination in the kidney and liver cells. Elevated values of AST in the kidney has been reported by Ojezele and Abatan (2009) when they exposed bird to chloropyrifos. The authors concluded that the effect reflect damage of liver cells. According to Takaori (1993), elevation of AST in the plasma levels unveiled a clear acute hepatocellular damage, entrahepatic obstruction or both. The liver contains numerous enzymes, some of which are also present in serum in very small concentrations. These enzymes have no known functions in the serum/plasma other than to provide information about the hepatic state and disorders. This disorders could be as a result of injury or liver damage. The injury could cause by reactive metabolites, resulting from xenobiotic metabolism in the liver.

Electrolytes are molecules found throughout the blood tissues and cells of fishes including *Heterobranchus bidorsalis.* These molecules which are either positive or negative ions conduct electric current and help to balance pH and acid base levels in the fish. Electrolytes also facilitate the passage of fluid between and within cells through a process known as osmosis and also play a part in regulating the functioning of neuro-muscular, endocrine and excretory system of fishes. Electrolyte concentration is indicative of the ability of the fish to osmoregulate. This ability is often compromise with stress (Ogamba et al., 2011). Since the electrolyte are responsible for proper functioning of all type of tissue, the presence of alkali metal series (Na^+, K^+, Ca^{2+}) are essential for the activation of any enzyme. Critical loss of body electrolyte reduces the osmotic concentration and leads to circulatory collapse.

Electrolytes in the liver of *Heterobranchus bidorsalis* exposed to glyphosate for 30m days is presented in Table 3. Values of calcium (Ca^{2+}) ions and Sodium (Na^+) were not statistically significant ($p>0.05$) compared to the control. Albeit values fluctuate within the experimental group (not in dose dependent pattern). Liver K^+ values increased as the concentration of the toxicant increases in a dose dependent

Table 1: ALT, AST, and ALP in plasma of *Heterobranchus bidorsalis* exposed to glyphosate for 30 days (mean ± SD).

Conc of Glyphosate (mg/l)	ALT (µ/l)	AST (µ/l)	ALP (µ/l)
0.00	110.00±0.59^{a}	443.00±0.93^{a}	443.00±0.91^{a}
0.16	91.00±0.16^{b}	161.00±1.02^{c}	395.00±1.21^{a}
0.32	32.00±0.03^{c}	231.00±0.65^{b}	385.00±1.56^{a}
0.48	43.00±0.21^{c}	243.00±2.10^{b}	281.00±0.86^{b}

Means within column with same superscript are not significantly different (p>0.05).

Table 2: ALT, AST, and ALP in Kidney of *Heterobranchus bidorsalis* exposed to glyphosate for 30 days (mean ± SD).

Conc of Glyphosate (mg/l)	Plasma/organ	ALT (µ/l)	AST (µ/l)	ALP (µ/l)
0.00	Kidney	6.50±0.03^{a}	80.00±0.31^{c}	928.50±2.86^{a}
0.16	Kidney	8.00±0.01^{a}	130.00±0.83^{b}	402.00±3.02^{c}
0.32	Kidney	7.50±0.00^{a}	239.00±2.39^{a}	742.00±2.35^{b}
0.48	Kidney	8.21±0.01^{a}	231.00±0.98^{a}	705.00±0.71^{b}

Means within column with same superscript are not significantly different (p>0.05).

Table 3: $Ca^{2+,}$ K^{+} and Na^{+} in the liver of *Heterobranchus bidorsalis* exposed to glyphosate for 30m days.

Conc of Glyphosate (mg/l)	Na^{+}(mmol/l)	Ca^{2+} (mmol/l)	K^{+} (mmol/l)
0.00	2.00±0.00^{a}	8.10±0.10^{bc}	413.50±3.20^{a}
0.16	1.40±0.01^{a}	12.20±0.03^{b}	409.50±2.32^{a}
0.32	1.80±0.02^{a}	15.00±0.02^{a}	417.00±6.20^{a}
0.48	1.35±0.01^{a}	16.00±0.02^{a}	418.35±2.60^{a}

Means within column with same superscript are not significantly different (p>0.05)

pattern. Fluctuation and stabilization of values of electrolytes was also reported by Inyang *et al*. (2013), when they exposed *Clarias gariepinus* to dichlorvos. This may be due to the toxic nature of the toxicant. According to Ogamba *et al*. (2011), stabilization and increase in K^{+} ions could be a stress induced response occasioned by the chronic exposure of fish to toxicants which may have activated certain physiological and metabolic mechanisms that could lead to a rapid uptake of the electrolyte from water, food material and a possible reduction of ion efflux.

Sodium and Potassium are essential for the activity of many enzymes and also in the transport of ATP which participates in several metabolic processes. Fluctuation of values recorded in this present study is a clear indication of distortion of metabolic functions of this vital organ (Inyang et al., 2013). Typically, electrolytes perform a vital role in gaseous exchange and inter-compartmental water balance, therefore elevated or low levels observed in this study may lead to hyper or hypo function of the organs or tissues.

Conclusion

This research work has unveiled the toxicity of glyphosate on some enzymes and electrolytes in *Heterobranchu bidorsalis.* These parameters could serves as useful biomarkers of sublethal effect of glyphosate on African catfish. Additionally, the use of glyphosate for annihilation of weeds close to aquatic environment should be done with caution.

Acknowledgement

This publication is based on undergraduate project work of the second author (Nancy C. Okon) supervised by the lead author (Dr Iniobong R. Inyang) at the Niger Delta University, Nigeria.

References

Akan, J.C., Mohammed, Z., Jafiya, L. and Ogugbuaja, V.O. (2013) Organochlorine Pesticide Residues in Fish Samples from Alau Dam, Borno State, North Eastern Nigeria. J. Environ. Anal Toxicol., 3: 171. doi:10.4172/2161-0525.1000171.

Aly, N. and El-Gendy, K. (2015). Impact of parathion exposure on some biochemical parameters in rabbit as a non targeted organism. Alexandna Journal of medicine, 15: 11-17.

APHA (American Public health Association) (1998). Standards methods for examination of water and waste water, APHA, Washington DC.

Atamnalp, M., Keles. M.S and Aras, H.I. (2001). The effects of cypermethrin (a sysnthetic pyrethroid) on some biochemical parameters (Ca, P, Na, Tp) of rainbow trout (*Oncorhynchus mykiss)* Canadian Journal of fish and Aquatic science 45:219-222.

Cox, C. (1995). Glyphosate Part 1: Toxicology. Journal of Pesticide Reform 15(3): 14-20

Daren, M.R. and Cynthia, K.A. (2007). Management of acute organophosphorus pesticide poisoning. Journal of Toxicology, 334:629-634

Das, B.K. and Mukharjee, S.C. (2000). Sublethal effects of organophophorus pesticides (quinalphose and Dimethroate) on selected blood parameters of Labeo rohita (Ham fingerlings) The Asian Fisheries Science Journal*,* 13(3):225–233.

Depledge, M.H. (1994). The rational basis for the use of biomarkers as ecotoxicological tools. In: Fossi MC, Leonzio C, Eds. Nondestructive Biomarkers in Vertebrates. Boca: FL Lewis, pp. 271-95.

Inyang I.R, Daka, E.R. and Ogamba, E.N. (2010). Effects of sublethal concentrations of diazinon on total protein and transaminase activities in *Clarias gariepinus.* Current Research Journal of Biological Science, 2(6):390-395.

Inyang, I.R. 2008. Haematological and biochemical responses of *Clarias gariepinus* to diazinon. Ph.D thesis, Rivers State University of Science and Technology. Port Harcourt, Nigeria

Inyang, I.R., Ekweozor, I.K.E. and Ollor, A. (2014). Physiological effects of diazinon on *Clarias gariepinus.* BEST Journal, 11(1):171-176.

Inyang, I.R., Ogamba, E.N. and Frank, V.E. (2013) Biochemical charges and electrolyte stabilization in *Clarias gariepinus* (Juveniles) induced by dichlorvos. International Journal of Biochemistry, 108: 244-248.

King, E. J. and Armstrong, A. R. (1934). Determination of serum and bile phosphatase activity, J. Can. Med. Ass. 31: 376–378.

Lang, L. (1993). Are pesticide a problem? Env. Health Perspectives, 101:578-583.

Lawson, L.O., Ndimele, P.E., Jimoh, A.A. and Whenu, O.O. (2011). Acute Toxicity of Lindane (Gamma Hexachloro-Cyclohexane) to African Catfish (*Clarias gariepinus,* Burchell, 1822). International Journal of Animal and Veterinary Advances, 3(2): 63-68.

Logawary S, Redha, G. and Subheshal S. and Longankumar, K. (2006). Alterations in the levels of ions in blood and liver of fresh water fish *Cyprinus carpio* exposed to dimethroate. J. Env. Mont. Assess., 131, 1-3

Neskovic, N.K., Poleksic, V., Elezovic, I., Karan, V. and Budimir, M. (1996). Biochemical and histopathological effects of glyphosphate on Carp. Cyprinus Capio L. Bull. Env. Cont. and Toxicol. 56.295-302.

Ogamba, E.N, Inyang I.R and Azuma, I.K. (2011). Effect of paraquat dichloride on some metabolic and enzyme parameters of *Clarias gariepinus.* Current Research Journal of Biological Sciences, 3(3):186-190.

Ogamba, E.N., Izah, S.C. and Nabebe, G. (2015). Effects of 2, 4-Dichlorophenoxyacetic acid in the electrolytes of blood, liver and muscles of *Clarias gariepinus.* Nigeria Journal of Agriculture Food and Environment, 11(4): 23- 27.

Ojezele, M.O. and Abatan, O.M. (2009). Toxicological effects of Chloropyrifos and methidathion in young chickens. African Journal of Biochemistry Research, 3: 48-51.

Reitman, S. and Frnakel, C. (1957). Colorimetric method for the determination of setrum glutamic oxaloacetic and glutamic pyruvate transaminase. Am. J. Clinical Pathologies, 28: 56-63.

Seiyaboh, E.I., Inyang, I.R., Gijo, A.H., Adobeni, G.D. (2013). Acute Toxicity of Paraquat Dichloride on Blood Plasma Indices of *Clarias gariepinus.* IOSR Journal of Environmental Science, Toxicology and Food Technology, 7(6): 15 – 17.

Servizi, J.A., Gardan, R.W and Martens, D.W. (1997). Acute toxicity of Garion and Roundup herbicides to salmon, Daphnia and trout. Bulletin of Environmental Contamination and Toxicology, 33: 355-361.

Sunmonu, T.O. and Oloyede, O.B. (2012). Monoclotophos-induced enzymatic charges as toxicity biomarkers in Winster Rat liver. Agriculture and biology. Journal of North America, 37: 302-305.

Takaori, H. (1993). "Thiophanate-methyl combined chronic toxicity/oncogenicity study in rats" Unpublished report No. RD-9327 from Nisso Institute for Life Sciences, Kanagawa, Japan. Submitted to WHO by Nippon Soda Co. Ltd, Tokyo, Japan, 1993.

Wahua, T.A.T. (1999). Applied Statisitics for scientific studies. Africa link books. Ibadan.

WHO (World Health Organization). (2005). Glyphosate, Environmental health criteria. 159. International programme on chemical safety (IPCS) WHO Geneva.