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Ecotoxicological Impact of Sub-lethal Concentrations of Glyphosate–based Herbicide on Juvenile *Clarias gariepinus*

C. O. Ujowundu[1*], D. M. Koloseke[1], N. O. Uba[1], J. J. Achilike[1], H. D. Ogbuagu[2] and E. A. Ubuoh[3]

[1]Environmental Biochemistry and Toxicology Unit, Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
[2]Department of Environmental Technology, Federal University of Technology, Owerri, Nigeria.
[3]Department of Environmental Management and Toxicology, Michael Okpara University of Agriculture, Umudike, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Authors COU, DMK, NOU and JJA designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors COU, HDO and EAU performed the statistical analysis and managed the analyses of the study. Authors DMK and NOU managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The presence of pesticides in both aquatic and terrestrial ecosystems has become an important issue globally. This study determined the possibility of bioaccumulation of glyphosate, the active compound of Roundup pesticides in muscle tissues of juvenile Catfish (*Clarias gariepinus)*, and the effects of exposure on some oxidative stress parameters. Forty fishes were grouped and exposed to graded (sub-lethal) concentration of Roundup pesticides for two weeks. Pesticides were extracted from the muscle tissues by cold extraction and detection and determination were performed using Gas chromatography- Electron captured detector (GC-ECD). Oxidative stress

**Corresponding author: E-mail: ujowundu@yahoo.com;*

parameters such as catalase, superoxide dismutase, reduced glutathione, glutathione peroxidase and malondialdehyde were analyzed. Fish tissue glyphosate concentration increased significantly ($p<0.05$) as dosage increased and residues of organochlorine pesticides such as p-p^{1}DDT, p-p^{1}DDE, HCB, α-HCH, γ-HCH, t-nonachlor and γ-chlordane were detected at concentration far below the provisional tolerable daily intake (PTDI) value. The biochemical studies showed significant increase ($p<0.05$) in activity of oxidative stress enzymes and lipid peroxidation product of fish groups exposed to Roundup pesticides in a dose dependent pattern. This study reveals that the use of Roundup pesticides in ways that could expose aquatic environment to its residues could result to adverse biochemical changes. Therefore, the use of this pesticide should be properly regulated and monitored to limit chronic exposure of fish consumers.

Keywords: Glyphosate; organochloride; pesticides; oxidative stress; xenobiotics; Roundup.

1. INTRODUCTION

The intense utilization of pesticides and herbicides to increase agricultural productivity has contributed to water pollution. One commonly used herbicide in Nigeria is Roundup®, a glyphosate-based herbicide, containing polyethoxyleneamine or polyethoxylated tallow amine (POEA) as surfactant [1]. Glyphosate (N-phosphonomethyl glycine) is a broad-spectrum herbicide, intensively applied on numerous fields, urban and industrial areas to control unwanted plants, including the macrophytes in aquatic systems [2,3]. Aside commercial agriculture, herbicides are used in small gardens as well as for de-weeding railway lines, urban pavements and roadsides. Glyphosate inhibits the activity of 5- enolpyruvylshikimate-3-phosphate synthase (EPSPS) that catalyzes the synthesis of aromatic amino acids in plants [4,5]. Glyphosate is a common terrestrial and aquatic pollutant considered persistent and mobile in soil and water. The presence of pesticides in aquatic environment leads to bioaccumulation in fish tissues due to the lipophilicity of the pesticide, the fat content of tissues, feeding habit, habitat, exposure, biotransformation capacity of the organism [6].

Toxicological studies have shown that chronic use of Roundup® is potentially harmful to fish living in shallow water [7] and non-target organisms [8,9]. Agricultural, industrial and domestic activities are the major sources of freshwater ecosystems pollution [10,11,12]. Ten percent of globally accessible runoff is used, generating a stream of wastewater, which flows or seeps into groundwater, rivers, lakes, or the oceans [10]. However, the agricultural sector's annual application of over 140 billion kilograms of fertilizers and large amounts of pesticides creates massive sources of diffuse pollution of freshwater systems. Pesticides interfere with organisms metabolic function [13,14], and may be responsible for a number of developmental anomalies in a wide range of species, from invertebrates to higher mammals [15]. Pesticides and its metabolites can cause oxidative stress [16] by induction of reactive oxygen species (ROS) production [17] via diverse mechanisms [18,19]. Most commercial pesticide formulated with surfactants or adjuvants are considered more toxic by oral route exposure to animals than the active compound (glyphosate) because of these additions state earlier [20,21,22]. Therefore to ascertain the toxicity of this herbicide on living organisms it is important to assess their whole formulations as mixtures because, the commercial formulation is implicated in cytotoxicity, oxidative effects, and apoptosis [23]. Some animals presented anorexia, lethargy, hypersalivation, vomiting, and diarrhoea on exposure to commercial glyphosate herbicides [24,25].

Fish is important in human diets, animal and poultry rations. Fish diet is palatable and easily digestible, rich in protein, essential oil, vitamins, calcium, phosphorous and iodine. Fish is often considered as a pollution marker for the natural aquatic environment [26,27,28], because it can metabolize, concentrate and bioaccumulate water pollutants. This study determined the possibility of bioaccumulation of glyphosate (an important constituent of Roundup herbicide) in fish muscles and the oxidative changes on fish exposed to varying concentration of Roundup, a glyphosate-based herbicide.

2. MATERIALS AND METHODS

2.1 Experimental Animals and Pesticide

Roundup®, a glyphosate-based herbicide was purchased from Abia State Ministry of Agriculture, Umuahia, Abia State Nigeria, and were preserved in an ambient temperature prior

to use. Forty juvenile Cat fish (*Clarias gariepinus*) weighing 135±20 g were purchased from the Department of Fishery and Aquaculture, Federal University of Technology, Owerri, Imo State of Nigeria. The fish and their sexes were identified by Mr. C. Ezeafulukwe of Fishery and Aquaculture Department. The male *Clarias gariepinus* have a distinct sexual papilla located above the anus. All animals received professional humane care in compliance with the guidelines of Ethical Animal Handling [29], and approved by the ethics committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.

2.2 Treatment

The animals were divided into four groups of ten fishes each, and were placed into different aquariums labelled A (Control) B, C and D. The aquariums with fishes were kept at the Department of Fishery and Aquaculture, for proper caring by the Hatchery personnel. They were allowed to acclimate for one week under close observation and were maintained on standard fish feed. After acclimatization, each aquarium was treated as follows: Group A fishes were put into 100 litres of water only (Control), Group B was dosed, 2.78 ml Roundup per 100 L of water (10 mg glyphosate/100L), Group C received 16.68 ml Roundup per 100 L of water (60 mg glyphosate/100L), and Group D received 83.4 ml Roundup per 100 L of water (300 mg glyphosate/100L) at alternate days for two weeks. They were fed accordingly and the water changed at alternate days. At the third week no death was recorded and the aquariums and fishes were transported to the Laboratory of Department of Biochemistry. Blood samples were collected from the fishes after immersion in ice cold water. The blood samples were processed and stored. Afterwards, the fishes were sacrificed and muscles and gills were collected for chemical and biochemical analyses.

2.3 Preparation of Samples and Gas Chromatography (GC) Analysis

2.3.1 Pesticides extraction and preparation of sample for GC analysis

Pesticides were extracted from fish muscle tissues by Sohxlet extraction method as described by AOAC [30]. Ten grams of the muscle sample was homogenized and mixed with 60g of anhydrous sodium sulphate in agate mortar to absorb moisture. The homogenate was placed into a 500 ml beaker and the extraction was carried out with 300 ml of n-hexane for 24 hours. The crude extract obtained was filtered, concentrated to 1 ml with a vacuum evaporator at 40℃. The extract (1 ml) was dissolved in 50 ml chloroform, transferred to a 100 ml volumetric flask and diluted to the mark. Most of the chloroform was evaporated at room temperature and 1 ml of the reagent (20 vol % benzene and 55 vol% methanol) was added. The mixture was sealed and heated in a water bath at 40℃ for 30 min. After heating, the organic sample was extracted with hexane and water, so that the final mixture of the reagent, hexane and water were in a ratio of 1: 1: 1. The mixture was shaken vigorously by hand for 2 min and the stable emulsion formed was broken by centrifugation. A portion of the hexane phase was transferred to a small test tube for injection into the gas chromatograph.

2.4 Identification and Quantification of Pesticides Residue with Gas Chromatograph

Buck 530 Gas Chromatograph fitted with Electron captured detector (ECD) was used for the identification and quantification of pesticides residue as described by Harris and Daniel, [31]. Residues were quantified by comparing peak heights with the corresponding peak heights of standard (Accu standard for pesticides residue, USA). One microlitre of the sample was injected into the injection port of the GC. The sample was subjected to gas chromatography analysis on a HP 88 capillary column with dimensions (100m x 0.25 um film thickness). The inlet and detection temperatures were set at 250℃ and 280℃. The equipment was run on split injection, with 20: 1 split ratio and utilized nitrogen as the carrier gas. The hydrogen and compressed air pressures were 22 psi and 35 psi respectively. The oven temperature was initially run in 180℃. The first ramping was at 2℃/min for 10mins and second ramping was at 20℃/min for 5 min and was maintained for another 2 min. The integrator chart speed was at 2 cm/min. The gas chromatography analysis was done in duplicate.

2.5 Biochemical Studies

The gills were cut into smaller pieces and homogenized in a phosphate buffered saline (PBS) to give a 10% (w/v) homogenate. The crude homogenates were centrifuged at 12,000 rpm for 15 minutes. The supernatant obtained was labelled gill sample and was used for the

determination of some oxidative stress parameters. The activity of superoxide dismutase (SOD) of gill sample was assayed according to the method of Xin et al. [32]. Briefly, using a clean test tube, a stock solution was made with 0.1 ml gill homogenate and 0.9 ml distilled H_2O. Afterwards, 0.1 ml of the stock solution was mixed with 0.9 ml carbonate buffer, and 75 µl xanthine oxidase. Absorbance of reaction was taken at 500 nm for 3 min at 20 seconds intervals. Rate of absorbance change indicated activity of SOD. The activity of catalase (CAT) was assayed according to the method of Aebi [33]. Briefly, 0.5 ml gill sample, 2.5 ml phosphate buffer and 2.0 ml H_2O_2 were mixed in a test tube and labelled stock. Then, 1.0 ml of the stock and 2 ml dichromate acetic acid reagent were added into a test tube and mixed appropriately. Four absorbances of the mixture at 240 nm were taken at a minute interval. The concentration of glutathione was determined according to the method of King and Wootton [34]. Briefly, 0.1 ml of gill sample and 0.1 ml distilled H_2O were delivered into test tubes labelled test and blank respectively. Afterwards, 0.9 ml distilled H_2O and 0.02 ml 20% sodium sulphite were delivered to both tubes. The setup was appropriately mixed and allowed to stand at 25℃ for 2 min. Afterwards, 0.02 ml of lithium sulphate and 0.02 ml of 20% Na_2CO_3 were added to all test tubes and mixed. Also, 0.2 ml phosphor-18-tungstic acid was added, mixed and allowed to stand for another 4 min for maximum colour development. Finally, 2.5 ml of 2% sodium sulphite was added and absorbance taken at 680 nm within 10 min.

The activity of glutathione peroxidase (GPx) was assayed by the method of Paglia and Valentine [35]. Briefly, 0.1 ml gill sample was delivered into a test tube containing 3 ml phosphate buffer, 0.55 ml guaiacol and 0.03 ml H_2O_2. The setup was appropriately mixed and absorbance of the mixture taken at 436 nm for 2 min at 30 seconds intervals. Malondialdehyde (MDA) concentration was determined by the method of Wallin et al. [36]. Briefly, 0.1 ml gill sample and 0.45 ml normal saline were added into test tubes labelled sample and blank, respectively. The setup was appropriately mixed before adding 0.5 ml, 25% trichloroacetic acid (TCA) and 0.5 ml of 17% thiobarbituric acid (TBA) in 0.3% NaOH. To the Blank tubes 0.1 ml distilled H_2O and same quantity of TCA, TBA and normal saline were added. The mixture was incubated at 95^0C for 40 min, cooled and 0.1 ml 20% sodium dodecyl sulphate was added and absorbance read spectrophotometrically at 532 and 600 nm against blank.

2.6 Statistical Analysis

The results obtained from this study were analyzed using one way analysis of variance (ANOVA) and expressed as mean ± standard deviation in bar charts. Values were considered statistically significant at $p \leq 0.05$.

3. RESULTS

3.1 Pesticide Accumulation

The result (Fig. 1) of glyphosate concentration in *C. gariepinus* shows a significant ($p < 0.05$) dose dependent increase from group B to group D. Group treated with 83.4 ml Roundup per 100 L (group D) showed the highest concentration of glyphosate while group treated with 2.78 ml Roundup per 100 L (group B) had the lowest, whereas no glyphosate was detected in control.

Fig. 2 presents a significant increase of HCB in groups B and C when compared to control and group D groups. For α-HCH, it shows a non-significant increase of α-HCH in groups B and D but group B when compared to control. The concentration of γ-HCH was significantly higher in all treated groups when compared to the control with the highest concentration presented by group B.

Fig. 3 presents a significant decrease of γ-chlordane in group B and C when compared to control and group D. Also, significant increase of t-nonachlor was observed in group B and C when compared to control and group D. The concentration of p-p^1-DDT (Fig. 4) increased significantly in group D when compared to other groups. No difference in p-p^1-DDT concentration was observed amongst control, group B and C. Furthermore, a significant increase in concentration of p-p^1-DDE (Fig. 4) was observed in group B and C when compared to control and group D. However, organochlorine pesticides such as p-p^1-DDD and p^1-p^1-DDD were measured but not detected.

3.2 Oxidative Parameters

Fig. 5(A1) presents the SOD activity of fish gill exposed to Roundup herbicide. The activity of SOD increased significantly ($p<0.05$) in group B, C, and D compared to group A (control). The control has the lowest activity while group D has the highest activity. Fig. 5(A2) presents catalase activity of gill exposed to Roundup herbicide.

Comparing group D and group C, catalase activity of gill increased significantly ($p<0.05$) in group B, C and D compared to group A (control). The group exposed to 83.4 ml Roundup/100 L had the highest activity. Fig. 6(B1) represents glutathione concentration in fish gill exposed to Roundup pesticide. It shows significant decrease in GSH of Roundup herbicide exposed groups in dose dependent pattern when compared to control group.

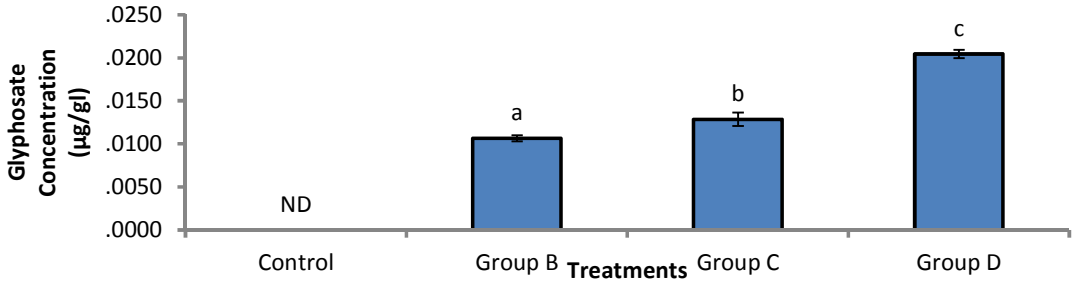

Fig. 1. Glyphosate concentration of muscle tissue of *C. gariepinus* exposed to graded doses of Roundup herbicide

Bars represent the mean ± standard deviation of duplicate determination. Bars with different alphabets indicate significant difference ($p< 0.05$). ND= Not detected

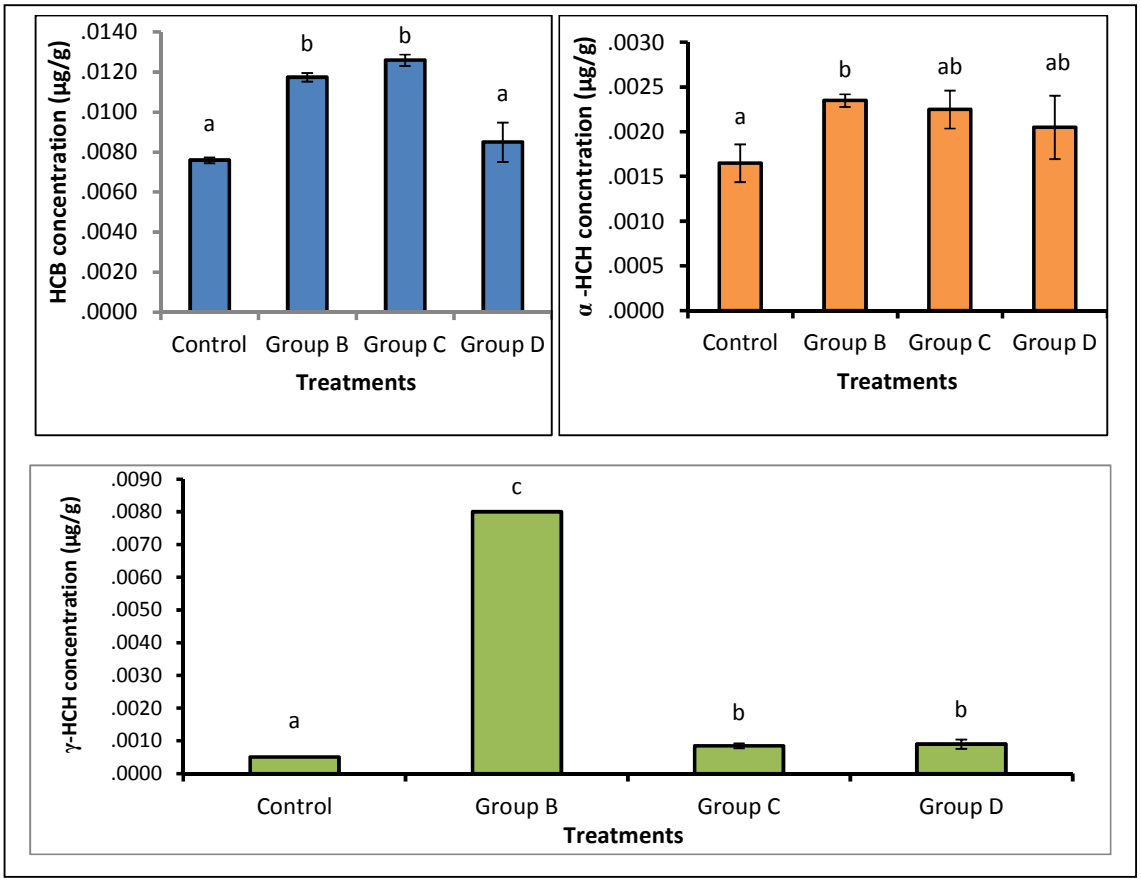

Fig. 2. Concentration of HCB, α-HCH and γ-HCH of muscle tissue of *C. gariepinus* exposed to graded doses of Roundup herbicide

Bars represent the mean ± standard deviation of duplicate determination.
Bars with different alphabets indicate significant difference ($p< 0.05$)

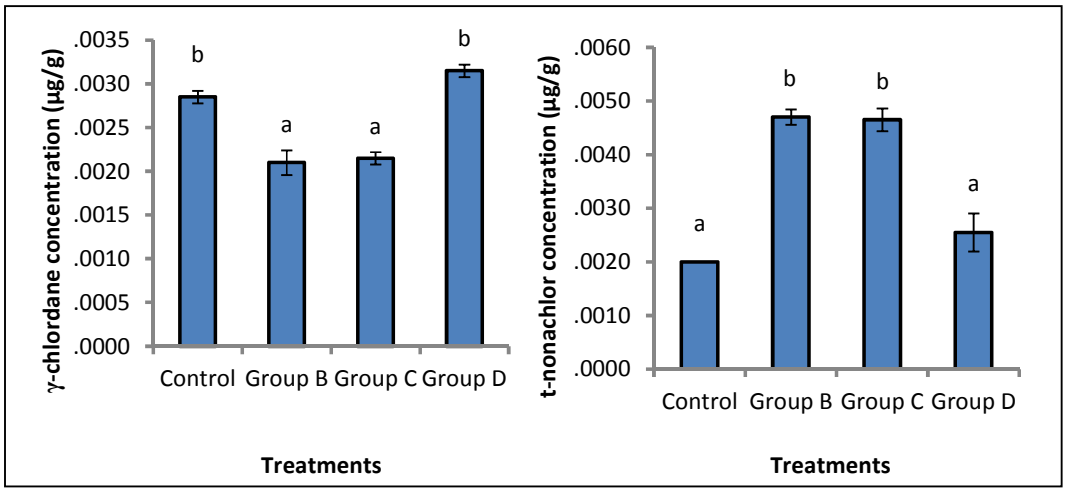

Fig. 3. Concentration of γ-chlordane and t-nonachlor of muscle tissue of *C. gariepinus* exposed to graded doses of Roundup herbicide

Bars represent the mean ± standard deviation of duplicate determination.
Bars with different alphabets indicate significant difference (p< 0.05)

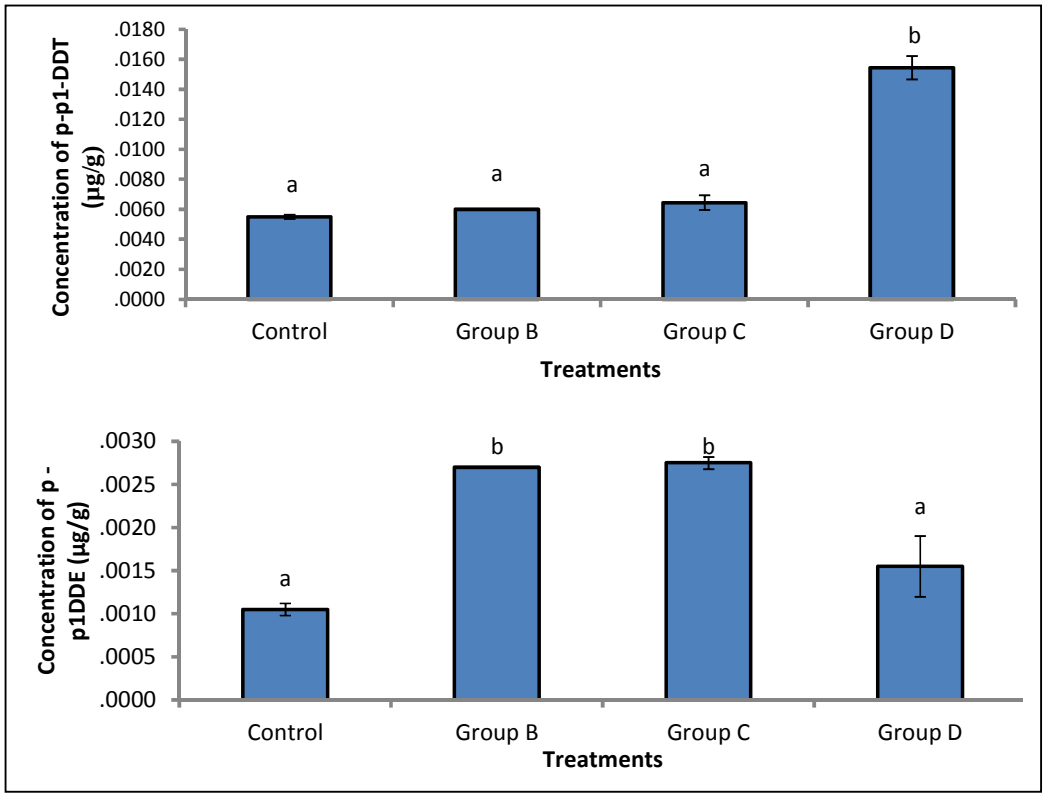

Fig. 4. Concentration of p-p^1-DDT and p-p^1-DDE of muscle tissue of *C. gariepinus* exposed to graded doses of Roundup herbicide

Bars represent the mean ± standard deviation of duplicate determination.
Bars with different alphabets indicate significant difference (p< 0.05)

Fig. 6(B2) shows glutathione peroxidase (GPx) activity (U/g gill) in gills of cat fish in group C and D increased significantly when compared to group A and group B. However, groups A and

group B showed no significant difference. Fig. 7 shows that the concentration of malondialdehyde was highest in group D and lowest in group A. The concentration of MDA increased significantly ($p<0.05$) as the concentration of Roundup herbicide increased in B, C and D.

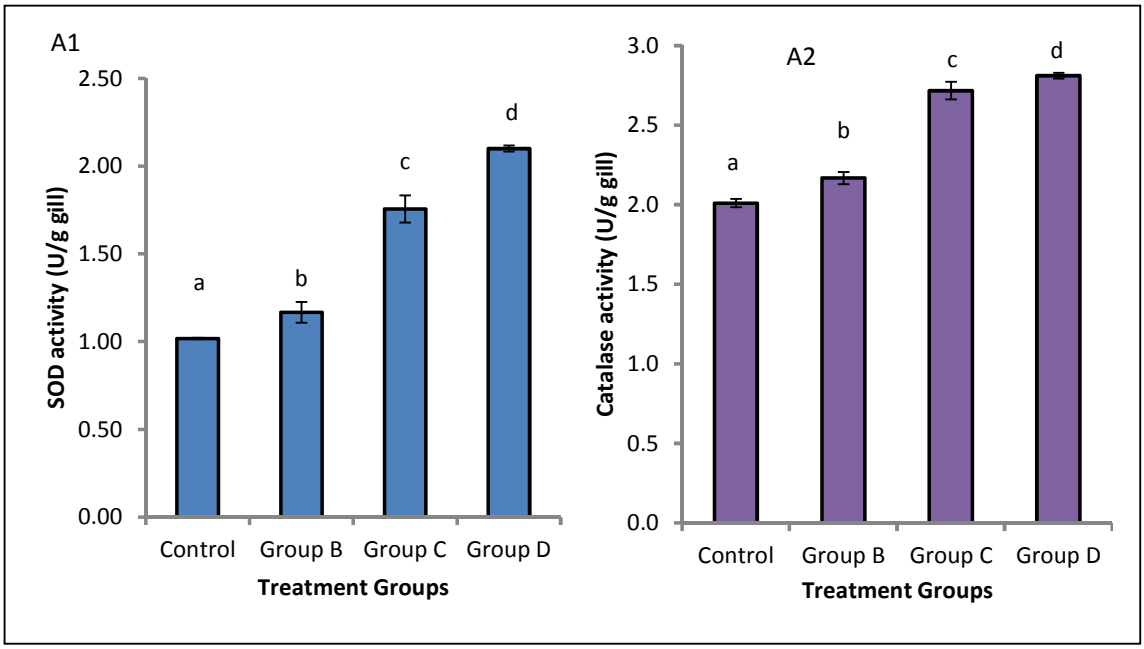

Fig. 5. SOD and CAT activities of gills of Cat fish exposed to graded doses of Roundup herbicide

Bars represent mean±standard deviation of triplicate determinations.
Bars with different alphabets are significantly different ($p<0.05$)

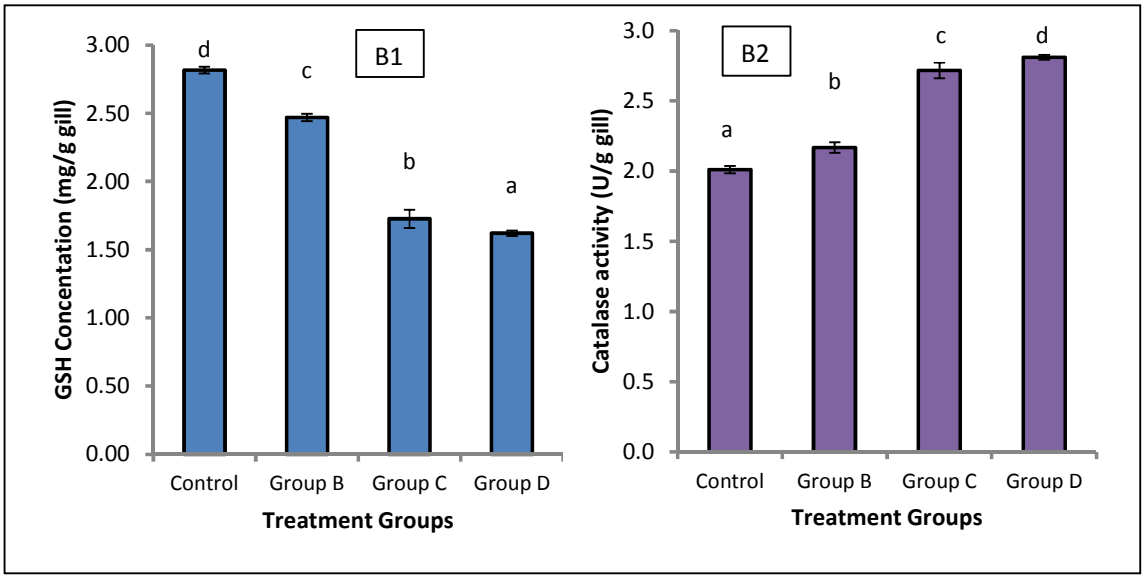

Fig. 6. GSH concentration and GPx activity of gills of cat fish exposed to graded doses of Roundup herbicide

Bars represent mean±standard deviation of triplicate determinations.
Bars with different alphabets are significantly different ($p<0.05$)

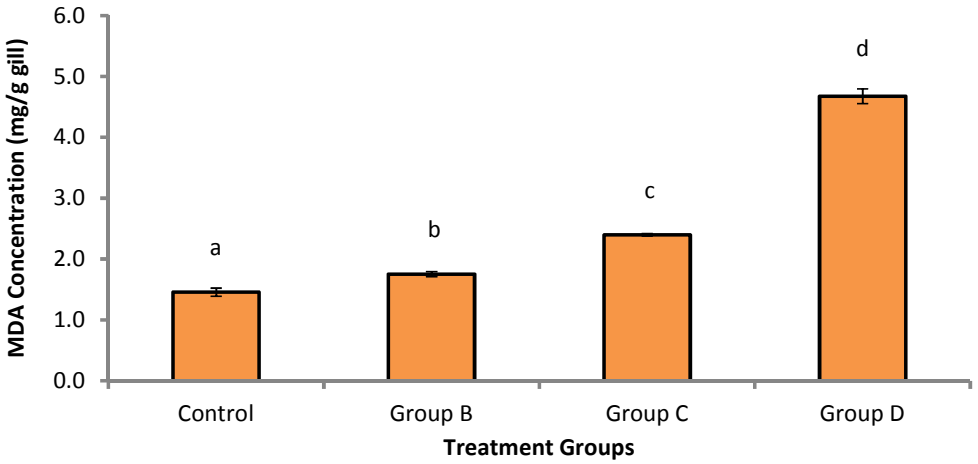

Fig. 7. Malondialdehyde activity of gills of cat fish exposed to graded doses of Roundup herbicide

Bars represent mean±standard deviation of triplicate determination.
Bars with different alphabets are significantly different (p <0.05)

4. DISCUSSION

Fishes are exposed directly to toxicants as they take in water and diet and so are used extensively for environmental monitoring [37]. Fish and some aquatic invertebrates are effective determinants for toxic, mutagenic and carcinogenic potential of pollutants because of the capacity to metabolize, concentrate and store water-borne pollutants [38]. The accumulation of pollutants in fish and other aquatic organisms is indicative of the state of pollution in the surrounding environment [39]. The sub-lethal concentrations of Roundup herbicide (with corresponding concentration of 10 mg/100L, 60 mg/100L and 300mg/100L of glyphosate) used in this study could be environmentally relevant considering that glyphosate has been detected at sub-lethal level up to 328 µg/L in some water bodies [40] and between 0.36 and 2.16 mg/L [41]. The concentration of glyphosate used in this study did not exceed the freshwater aquatic life standard of 65 mg/L [42], but in view of the repeated applications of the pesticide, the concentration in aquatic ecosystem may be higher, thus, suggesting the relevance of the concentrations. It is also important to note that, there are no safe levels of endocrine disruptors [43,44,45]. In cognizance of these facts, it therefore raises concern to monitor and assess pesticides impacts in the environment.

The result of glyphosate concentration in tissue showed significant increase as the dose increased. This result supports the work of Brändli and Reinacher, [46] who detected glyphosate in the urine of the farmers and in urine of those who had no direct contact with agricultural activities at values ranging from 0.5 to 2 ng glyphosate per ml urine (drinking water limit: 0.1 ng/ml). However, this result contradicts the report by Malik et al. [47] which stated that glyphosate has no significant potential to accumulate in animal tissue. Other reports indicate that the bioconcentration factors for glyphosate to be very low because of its high solubility and photodegradation in water and low octane-water partition coefficient (kow), making glyphosate readily excreted by the fishes [48,49]. If the above assertions are tenable, aaccumulation of glyphosate in the fish samples (juveniles), therefore, could be as a result of the fishes not having well developed excretory organs capable of effectively removing ingested herbicide. The FDA/EPA tolerance level for glyphosate in fish is set at 0.25 ppm [50]. When glyphosate is ingested via diet or water by humans, it interferes directly and inhibits cytochrome- P_{450} enzymes, responsible for modulating the synthesis of sex steroid hormones [51]. This inhibition may result in the synergistic disruptions of biosynthesis of aromatic amino acids by gut bacteria, resulting in variety of health condition including gastrointestinal damage, autism, infertility, dementia and cancer [51]. Glyphosate has been detected in the urine, faeces, milk and feed of the animals.

Furthermore, Roundup treated and control groups showed the presence of pesticides such as p-p^{1}DDT, p-p^{1}DDE, HCB, α-HCH, γ-HCH, t-nonachlor and γ-chlordane in fish tissues. The borehole water used in this study may be the source of these organochlorine pesticides. The underground water source may have been polluted by illegal use of banned organochlorine pesticides [52]. These pesticides are used in homes, industries and agricultural farms and their residues percolate into underground water or runoff during storms into rivers, streams and eventually oceans. Pesticides may become part of the water column overtime and fish ingest it through their gills or fish scales and are absorbed into tissues and organs [53]. Bioconcentration of pesticides residues in animal tissues could cause death of the organism at high concentration or disruption of normal metabolic and physiologic functions. The accumulation of pesticides in edible portions of fish is a problem for humans because when these fishes are eaten, the pollutants which accumulate may cause diseases such as cancer, tumours, reproductive inhibition or failure, suppression of immune system, disruption of endocrine system, birth defects, cellular and deoxyribonucleic acid damage, teratogenic effects, intergenerational effects amongst others [53,54,55,56].

Trans-nonachlor (t-nonachlor) is a component of chlordane. The recommended guideline values of 0.2 µg/L was given for chlordane and all isomers in drinking water [57]. However, the provisional tolerable daily intake (PTDI) value was 0.0005mg/kg body weight [58]. The results of this study showed the highest concentration of t-nonachlor as 0.005ug/g, and this is within the range of acceptable limit. The wide distribution of α-HCH in fish tissues may be explained by the photochemical isomerisation of γ-HCH to α-HCH [59]. Fish age, size and weight are determinant factors for presence and levels of organochlorine pesticides [60]. Symptoms of γ-HCH intoxication include seizures, convulsions, vomiting and dizziness [61]. Gamma-HCH have anti-estrogenic properties and increase incidences of adenomas and carcinomas over the liver in mice [62]. Other organochlorine detected in tissues of *C. gariepinus* include p-p^{1}DDT and p-p^{1}DDE. However, p-p^{1}DDD and p^1-p^{1}DDD were not detected. It is important to note that p-p^{1}DDT has lower half life than p-p^{1}DDD and p-p^{1}DDE, and lower persistence when compared to DDD and DDE [63]. Firstly, the non-accumulation of p-p^{1}DDD and p^1-p^{1}DDD in fish tissues may be due to low persistence of the pesticides in soil sediments, low octane-water partition coefficient (kow) which makes them water soluble and readily excreted from the system, thus leaving very insignificant residuals that amount to no threat in humans when fishes with such residuals are consumed. Secondly p-p^{1}DDD were not detected in the tissues which may have been as a result of reduced presence of the parent form of the compound p-p^{1}DDT which normally undergo chemical changes to p-p^{1}DDD in the presence of water [64]. Due to environmental regulation DDT has been banned in Nigeria and has been classified by National Agency for Food and Drug Administration and Control (NAFDAC) as probable human carcinogens but is still in use.

The accumulation of these pesticides in tissues of aquatic organisms could induce the production of reactive oxygen species (ROS) and exert oxidative stress in target organisms living in the exposed aquatic environment. In the present study, the gill of *C. garienpinus* exposed to graded glyphosate concentration showed important changes in the activity of the various oxidative stress parameters. The observed decrease in reduced Glutathione (GSH) concentration in fish groups exposed to graded doses of Roundup pesticide may be related to its increased utilization and conversion to oxidized glutathione (GSSG) by the increased activities of GPx as observed in this study. It may as well result from inefficient GSH regeneration [65], by the actions of glutathione reductase or poor delivery of reducing equivalents by NADPH systems. This does not corroborate with the work done by Di Giulio et al. [66], which reported high levels of GSH in catfish exposed to polluted waters compared with control. Also, Pandey et al. [67] observed increase in GSH activity in Wallagoattu fish from the Panipal River in India. A higher hepatic glutathione concentration (GSH) in *Cyprinus caprio* inhabiting the polluted Ceyhan River in Turkey was reported by Sahan et al. [68].

The observed increase in SOD and CAT activities may be a response to the increased superoxide anions ($^{\cdot}O_2^-$) and H_2O_2 levels [69]. Superoxide anion, are formed during hydroxylations of xenobiotics, by cytochrome P_{450} system and these hydroxylation reaction obtains hydrogen from NADH (or NADPH) via flavoprotein and Cyt P_{450}. [70]. Glutathione peroxidase activity may be increased due to the increased production of H_2O_2 derived from $^{\cdot}O_2^-$ [71]. The dose dependent increase in the antioxidant enzyme activities (such as SOD) may

be due to the excessive generation of free radicals induced by glyphosate metabolism. Superoxide dismutase catalyzes the conversion of superoxide radical to H_2O_2. Therefore, elevated CAT activity may indicate increased presence of H_2O_2 substrate as a result of increased conversion of superoxide radical by SOD. Catalase catalyses rapidly, tissue-toxic hydrogen peroxides to water and molecular oxygen. Furthermore, increase in redox reaction may increase oxygen consumption.

Therefore, the significant increase in MDA concentration in the gills could be attributed to excess free radicals generation, beyond the capacity of tissue's antioxidant system to quench [72]. This assertion corroborates the increased activities of antioxidant enzymes (SOD, CAT, and GPx) and depletion of antioxidant molecule (GSH) recorded in this study. This is also in line with reports [73,74,75], that when antioxidant defences are impaired or overcome, oxidative stress may exert effects on biomolecules like proteins, lipids and DNA. Thus, explaining the significant increase in concentration of MDA – a product of lipid peroxidation recorded in this study.

The consumption of fish that bears residues of Roundup by human could lead to occurrence similar to metabolic changes in human subjects. A study by Chaufan et al. [23] indicated that a combination of glyphosate, aminomethyl-phosphonic acid (AMPA) and surfactants/ adjuvants induced toxic metabolic and oxidative changes in human HepG2 cell line. Pure glyphosate without surfactants was found to exert proliferative effects only in human hormone-dependent breast cancer, T47D cells, but not in hormone-independent breast cancer, MDA-MB231 cells [76]. Furthermore, Glyphosate in soil inhibits bacteria and kills off algae and can cause manganese, to be unavailable for plant absorption. A similar process is suspected to take place in the digestive tract of humans and animals when they consume food containing glyphosate residues and this may affect adversely the microflora of the gastrointestinal tract of humans and animals [46].

5. CONCLUSION

The accumulation of pesticides in fish can result in chronic illness and cause potential damage to the consuming population. Pesticides affect vital organs such as kidney, liver, gills etc eliciting oxidative stress. This study revealed that glyphosate induced adverse oxidative changes in juvenile *C. garienpinus.* Therefore, the use of glyphosate on/near fish farm or in area close to aquatic environment should be discouraged or properly regulated. The changes in concentration or levels of biomarkers of oxidative stress in fish may be helpful in assessing the risk of environmental contaminants and the safety of fish meat for human nutrition.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the ethics committee of the Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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