



Full Length Article

Toxicity of Sublethal Concentrations of Glyphosate and Paraquat Herbicide in the African Catfish (*Clarias gariepinus*)

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Abstract

The effects of exposing juveniles of African catfish (*Clarias gariepinus*) to two commonly used herbicides were studied over 8 weeks. Some fish biochemical parameters were evaluated over the period of the experiment. Fractionated concentrations of glyphosate (0.0265, 0.053 and 0.106 mg L⁻¹) and paraquat (0.0035, 0.007 and 0.014 mg L⁻¹) were administered to fish juveniles for eight weeks. These concentrations represent fractions of the 96 h LC₅₀ which was determined in an earlier study. Antioxidant enzymes were analysed in the liver of the fish every fortnight. Comparing with the control, significant increases ($p < 0.05$) in enzyme activities of superoxide dismutase (SOD), catalase (CAT), lipid peroxidation (LPO) and in glutathione peroxidase (GPx) were observed. These parameters were however not all concentration and time dependent. The result revealed that the two herbicides caused changes in fish antioxidant enzymes, as such could be useful in forestalling the ecotoxic effects of similar chemicals in the environmental xenobiotics. © 2018 Friends Science Publishers

Keywords: Fish, Antioxidant, Herbicides, Sub-lethal, Enzymes

Introduction

The environment is under constant threat largely due to anthropogenic activities. Expanding human population, agricultural mechanization, urbanization and industrialization have resulted in tremendous stress on the environment and as a consequence, have negatively impacted our environment. The neglect of nature laws and the over-exploitation of resources have worsened these environmental problems. Recently though, humans have become better informed of the need to avoid mistakes of the past and are now taking conscious steps to stop further environmental degradation. The level of human population and other organisms in the environment has put the survival of man and other life forms in danger, hence, preservation of the environment is *sine-qua non* for the existence of human beings (Bhatia, 2002).

Drainage and irrigation systems may become polluted through the application of pesticides during agricultural and pest control activities, and this may negatively impact the living and non-living members of the contaminated water course (Kreuger *et al.*, 1999; Mohamed *et al.*, 2012). The persistence in the environment of some pesticides may contaminate any of the terrestrial or aquatic species (Bakry *et al.*, 2011). The improper use of chemicals to kill fish is a common practice in the present day world especially in developing countries. As such, it may become hazardous to the health of man when these

aquatic organisms are harvested and consumed (Williams, 2011).

Glyphosate is an herbicide, commonly applied to kill a wide range of unwanted plants in agriculture, forestry and in canals, lakes, fish ponds and slow running water. It has been suggested that this herbicide may disrupt the balance of the ecosystem, thereby damaging non-target species as a result of changes in haematological, oxidative and metabolic parameters (Neskovic *et al.*, 1996). Paraquat, also a non-selective and contact herbicide is used for killing vegetative pests. Paraquat is ordinarily redundant in the soil. However, if there is a reaction between the cations in the herbicide and anions in the soil, paraquat may escape into the surface layers of the soil, becoming more insoluble (Eizadi-Mood *et al.*, 2011; Ada *et al.*, 2012). Paraquat has a fast action. There has been report of its toxicity in humans if ingested (Ogamba *et al.*, 2011).

Reactive oxygen species (ROS) are produced as a result of an imbalance in ratio of pro and anti-oxidants and consequently, oxidative stress develops. Contaminants in the environment like heavy metals and herbicides have been implicated in modulating antioxidant defense systems thereby causing oxidative damage in aquatic life forms through the generation of ROS (Achuba and Osakwe, 2003; Liu *et al.*, 2006; Monteiro *et al.*, 2006). Fishes are equipped with a defensive system to counter the influence of ROS arising from the breakdown of chemicals and other xenobiotics. The essential enzymes involved in

detoxification of ROS in an organism. These enzymes are catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD). Under oxidative stress, these enzymes become easily inducible, indicating an ability to adapt to stress conditions. Antioxidant mediated toxicities may occur if these systems are inhibited (Di Giulio *et al.*, 1989).

Previously, there have been reports on some aspects of the toxicity of these chemicals in fish. Histopathology, behavioral changes and acute toxicity were reported by Ayoola (2008) after exposing African catfish (*Clarias gariepinus*) to glyphosate. A similar study was reported on *Oreochromis niloticus* by Ayoola (2008). Oxidative parameters were reported by Gluszczak *et al.* (2011) to be impacted in Piava (*Leporinus obtusidens*) on exposure to acute concentrations of glyphosate. Genotoxicity and cytotoxicity arising from glyphosate exposure have also been reported in the blood of fish by some other researchers (Cavalcante *et al.*, 2008; Moreno *et al.*, 2014). Paraquat toxicity in fish are not as much as reported for glyphosate. Ogamba *et al.* (2011) studied the toxicological impact of paraquat on fish metabolism including total bilirubin, albumin, creatinine, total protein and total urea in the gills and muscle of *C. gariepinus* for four days and reported that Paraquat modulated the activities of these enzymes.

Clarias gariepinus is a popular species in tropical aquaculture. It is widely distributed and accepted by many farmers in Africa because of its fast growth, large size, low bone content, hardiness, high yield, tolerance to poor water quality, omnivorous feeding habit, fine flavour, adaptability to overcrowding, high market value and has been successfully propagated artificially thereby making its fry and fingerlings easily available (Osman *et al.*, 2006). It may therefore be imperative to monitor the health of fish as whatever negatively affects it can also affect man's quest for alternative source of protein.

Hence, this research was aimed at investigating the long term toxicity of glyphosate and paraquat in the African catfish *Clarias gariepinus* viz-a-viz changes in fish antioxidant enzymes as the toxic endpoint.

Materials and Methods

Fish Collection and Maintenance

The *C. gariepinus* juveniles used in this study averaged 3.94 ± 1.51g in body weight and standard body length of 7.35 ± 2.33 cm. They were bought from a fish farm in Ota, Ogun State, Nigeria. The fish samples were taken in aerated jerry cans containing water from the fish farm into fish tanks in the laboratory. Ten fish were held in tanks, each with a capacity of 25 L and allowed to go through a two week acclimatization period in dechlorinated water. Fishes were fed at 4% body weight, twice daily, with 35% crude protein of pelleted diet during this period. Adapted to laboratory conditions by the fishes was believed to have been achieved

when death at less than 5% was recorded and feeding was discontinued 24 h prior to the onset of the experiment.

Preparation of Test Solutions and Exposure of Fish

Commercial formulations of glyphosate and paraquat were purchased from an outlet in Lagos, Nigeria. Three concentrations each, 0.0265, 0.053 and 0.106 mg L⁻¹ glyphosate; 0.0035, 0.007 and 0.014 mg L⁻¹ paraquat, were used. These concentrations represents respectively, 20%, 10% and 5% of the LC₅₀ of the herbicides determined in an earlier study by Ayanda *et al.* (2015b) according to OECD guidelines No 203. The concentrations were dispensed into 150 L tanks holding dechlorinated water. This was further connected to three 25 L tanks and control. A renewal bioassay was employed. Ten fishes were distributed randomly into each test tanks and this was replicated three times, giving thirty fishes for each concentration of the two herbicides. Water physicochemical parameters were monitored in the course of the experiment to ensure they were within permissible range. Dechlorinated tap water alone was used for the control experiment.

Tissue Sampling

Every two weeks, two fishes from each 25 L tank were removed from each of the three concentrations for each herbicide treatment and control (six fishes in all for each concentration and control). The fishes were then sacrificed, their livers were removed, washed in ice-cold 1.15% KCl solution, weighed and homogenized in a homogenizing buffer using a Teflon homogenizer. Centrifugation of the homogenate obtained was at 4°C, 12,000 g and for 10 min. Clear supernatants were recovered and used for estimation of protein and assaying of the enzymes' activity.

Biochemical Profiling

After the fish livers have been processed, enzyme activity was measured. Glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) activities were assayed according to Paglia and Valentine (1967), Clairborne (1985) and Misra and Fridovich (1972) respectively. The malondialdehyde (MDA) content, which measures lipid peroxidation, was assayed in the form of thiobarbituric reactive substances (TBARS) as described by Buege and Aust (1978).

Statistical Analysis

One-way analysis of variance (ANOVA) was adopted for analyzing data from enzyme activity using SPSS software (Standard Version 10.0) to test for differences between levels of treatment; and means were separated using Duncan Multiple Range Test (DMRT) where applicable (Duncan, 1955). Test of significance was at 5% level.

Results

Activities of Enzymes in Fish Liver Exposed to Sublethal Doses of Herbicides

Glyphosate: Antioxidant enzymes (SOD, CAT and GPx) activity and MDA content in fish liver after 8 weeks of exposure to glyphosate are summarised in Table 1 to 4.

Table 1 shows that at the end of the eight weeks exposure period, the activity of SOD in the liver increased in concentration and with time. Compared with the control, each of the concentrations showed significant increase ($p < 0.05$) in SOD levels with time i.e., between week two and week eight. Significant increase in SOD levels within concentration was also observed in each of

the exposure period.

Activity of CAT increased within concentration and with time during the exposure period. There was a significant increase ($p < 0.05$) in CAT levels in all of the exposure concentrations between week two and week eight. However, significant increase in CAT levels, within concentration, was noticed only in weeks six and eight (Table 2).

All exposure concentrations increased MDA levels significantly ($p < 0.05$) in the liver between week two and week eight. However, as observed in CAT, significant increase was noticed within concentration only starting at week six (Table 3).

The increase in GPx levels in fish liver was similar with other antioxidant enzymes. Table 4 shows that apart

Table 1: Activities of SOD (Unit/mg protein) in fish exposed to sub-lethal concentrations of glyphosate over eight weeks

Conc. (mg L ⁻¹)	Week 2	Week 4	Week 6	Week 8
0.00	21.11±0.15 ^{aA}	21.05±0.14 ^{aA}	20.97±0.26 ^{aA}	21.01±0.11 ^{aA}
0.0265	24.21±0.26 ^{aAB}	26.32±0.26 ^{aB}	30.29±0.62 ^{bB}	32.46±1.04 ^{bB}
0.053	25.43±0.11 ^{aB}	27.01±0.21 ^{abB}	30.99±0.94 ^{bB}	33.12±1.78 ^{bB}
0.106	26.64±0.12 ^{aB}	29.25±0.22 ^{bB}	31.54±0.90 ^{bB}	34.92±0.87 ^{bB}
% Fold Increase	1.26	1.39	1.50	1.66

Table 2: Activities of CAT (Unit/mg protein) in fish exposed to sub-lethal concentrations of glyphosate over eight weeks

Conc. (mg L ⁻¹)	Week 2	Week 4	Week 6	Week 8
0.00	117.295±3.349 ^{aA}	115.266±3.883 ^{aA}	111.531±3.106 ^{aA}	116.988±3.878 ^{aA}
0.0265	121.083±3.263 ^{aA}	127.523±4.226 ^{abA}	135.013±4.068 ^{abB}	141.345±5.031 ^{bB}
0.053	125.513±3.920 ^{aA}	131.113±4.376 ^{abA}	137.048±4.588 ^{abB}	143.93±5.076 ^{bB}
0.106	126.06±3.281 ^{aA}	132.473±4.588 ^{abA}	138.728±4.158 ^{abB}	146.085±5.108 ^{bB}
% Fold Increase	1.07	1.15	1.24	1.25

Table 3: MDA (nmole/mg protein) in fish exposed to sub-lethal concentrations of glyphosate at eight weeks

Conc. (mg L ⁻¹)	Week 2	Week 4	Week 6	Week 8
0.00	1.29±0.013 ^{aA}	1.30±0.095 ^{aA}	1.20±0.024 ^{aA}	1.20±0.024 ^{aA}
0.0265	1.46±0.056 ^{aA}	1.76±0.059 ^{bA}	1.78±0.014 ^{bB}	1.79±0.013 ^{bB}
0.053	1.49±0.018 ^{aA}	1.74±0.019 ^{bA}	1.81±0.025 ^{bB}	1.79±0.032 ^{baB}
0.106	1.51±0.006 ^{aA}	1.73±0.01 ^{abA}	1.79±0.027 ^{bB}	1.80±0.026 ^{bB}
% Fold Increase	1.17	1.33	1.49	1.5

Table 4: Activities of GPx (mmol/min/mg protein) in fish exposed to sub-lethal concentrations of glyphosate at eight weeks

Conc. (mg L ⁻¹)	Week 2	Week 4	Week 6	Week 8
0.00	1.892±0.081 ^{aA}	2.147±0.101 ^{aA}	2.136±0.141 ^{aA}	1.911±0.085 ^{aA}
0.0265	2.569±0.136 ^{aB}	2.811±0.169 ^{abB}	3.258±0.139 ^{cB}	3.151±0.164 ^{bcB}
0.053	2.968±0.16 ^{aC}	2.985±0.187 ^{aB}	3.039±0.167 ^{abB}	3.145±0.143 ^{abB}
0.106	2.592±0.167 ^{aB}	3.093±0.176 ^{bB}	3.100±0.188 ^{bB}	3.008±0.184 ^{bB}
% Fold Increase	1.37	1.44	1.45	1.57

Table 5: Activities of SOD (Unit/mg protein) in fish exposed to sub-lethal concentrations of paraquat at eight weeks

Conc. (mg L ⁻¹)	Week 2	Week 4	Week 6	Week 8
0.00	20.5±0.84 ^{aA}	19.96±0.56 ^{aA}	20.56±0.33 ^{aA}	20.23±0.31 ^{aA}
0.0035	21.06±1.20 ^{aA}	21.98±0.82 ^{aA}	23.89±0.53 ^{abB}	24.48±1.22 ^{bB}
0.007	22.13±0.77 ^{aA}	23.87±1.12 ^{aB}	26.57±0.35 ^{bC}	29.21±1.51 ^{cC}
0.014	23.45±0.89 ^{aB}	25.41±0.67 ^{aB}	28.65±1.39 ^{bC}	30.32±0.98 ^{bc}
% Fold Increase	1.14	1.27	1.39	1.49

Values with the same small letter superscript in the same row, and values with the same capital letter superscript in the same column are not significant ($p \geq 0.05$). (Mean values ± SE are for 6 fishes)

from the control and concentration 0.053 mg L^{-1} , the other concentrations showed significant increases ($p < 0.05$) in GPx levels with time i.e. from week two to week eight. Furthermore, there was a significant increase in GPx levels within concentration in each of the period of exposure (Table 4).

Paraquat

Antioxidant enzymes (SOD, CAT and GPx) activity and MDA content in the liver tissues of *C. gariepinus* after eight weeks of exposure to paraquat are summarised in Table 5 to 8. Similar to what was observed in glyphosate, Table 5 shows that SOD activities in fish liver increased as concentration increased and time progressed. However, significant increase ($p < 0.05$) in SOD levels was not observed until week six in all concentrations. Furthermore, there was significant increase ($p < 0.05$) in SOD levels within concentration in each of the period of exposure (Table 5).

CAT levels increased significantly ($p < 0.05$) within concentration and with time. There was significant increase in CAT levels in all exposure concentrations as time progressed when compared with the control. However, significant increase in CAT levels was observed beginning only from weeks six in each of the period under observation (Table 6).

Table 7 shows that GPx levels significantly increased ($p < 0.05$) only in concentration; and this increase was observed starting from week four and then week eight. There was no significant increase ($p > 0.05$) with time in all

concentrations.

MDA levels also significantly increased with time and concentration ($p < 0.05$). However, concentration 0.007 mg L^{-1} did not show a significant increase ($p > 0.05$) during the eight weeks period. Also, significant increase was observed within concentration only in weeks six and eight (Table 8).

Discussion

Antioxidant is any substance that does not allow or retards deterioration or destruction by oxidation. It is an important contributor to the stabilization of lipid sample by preventing free radical formation. There is a natural balance between the quantity of free-radicals that the body generates and the antioxidants needed to scavenge them so as to protect the body cells against their damaging effects. Nevertheless, under normal physiological conditions, these protective antioxidant enzymes are present in quantities that are only enough to deal successfully with the physiological rate at which free-radicals are generated (Agarwal and Prabakaran, 2005). Hence, whenever there is an external influence responsible for generation of free radicals, there may be an imbalance in the free radical/antioxidant ratio, a situation where the antioxidants produced will not be enough to clear the free radicals generated.

Antioxidant enzymes, glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation which was measured in form of thiobarbituric reactive substances (TBARS or MDA) all increased significantly ($p \leq 0.05$) during the eight week exposure

Table 6: Activities of CAT (Unit/mg protein) in fish exposed to sub-lethal concentrations of paraquat at eight weeks

Conc. (mg L^{-1})	Week 2	Week 4	Week 6	Week 8
0.00	91.77±3.656 ^{aA}	92.003±3.408 ^{aA}	89.220±3.188 ^{aA}	88.340±2.099 ^{aA}
0.0035	100.355±4.395 ^{aA}	105.613±3.731 ^{abA}	110.45±3.251 ^{abB}	118.66±3.075 ^{bbB}
0.007	101.410±4.778 ^{aA}	106.775±3.394 ^{aA}	114.263±3.542 ^{abB}	120.648±3.248 ^{bbB}
0.014	104.613±4.151 ^{aA}	108.09±4.618 ^{aA}	116.97±3.637 ^{abB}	122.133±3.130 ^{bbB}
% Fold Increase	1.14	1.17	1.31	1.38

Table 7: Activities of GPx (mmol/min/mg protein) in fish exposed to sub-lethal concentrations of paraquat at eight weeks

Conc. (mg L^{-1})	Week 2	Week 4	Week 6	Week 8
0.00	1.608±0.106 ^{aA}	1.602±0.107 ^{aA}	1.616±0.139 ^{aA}	1.602±0.119 ^{aA}
0.0035	1.795±0.133 ^{aA}	2.194±0.139 ^{aAB}	2.100±0.137 ^{aAB}	2.238±0.159 ^{aAB}
0.007	1.95±0.156 ^{aA}	2.249±0.163 ^{aAB}	2.361±0.186 ^{aB}	2.594±0.196 ^{aB}
0.014	2.006±0.176 ^{aA}	2.563±0.178 ^{aB}	1.754±0.168 ^{aAB}	2.521±0.188 ^{aB}
% Fold Increase	1.25	1.59	1.08	1.57

Table 8: Activities of MDA (nmole/mg protein) in fish exposed to sub-lethal concentrations of paraquat at eight weeks

Conc. (mg L^{-1})	Week 2	Week 4	Week 6	Week 8
0.00	1.29±0.009 ^{aA}	1.28±0.005 ^{aA}	1.28±0.007 ^{aA}	1.28±0.007 ^{aA}
0.0035	1.28±0.013 ^{aA}	1.29±0.011 ^{abA}	1.32±0.016 ^{bbB}	1.31±0.010 ^{abAB}
0.007	1.29±0.005 ^{aA}	1.30±0.012 ^{aA}	1.32±0.013 ^{aB}	1.32±0.014 ^{aB}
0.014	1.29±0.014 ^{aA}	1.31±0.011 ^{abA}	1.33±0.011 ^{bbB}	1.34±0.010 ^{bbB}
% Fold Increase	1.00	1.02	1.04	1.05

Values with the same small letter superscript in the same row, and values with the same capital letter superscript in the same column are not significant ($p \geq 0.05$). (Mean values ± SE are for 6 fishes)

period. The increased levels of lipid peroxidation in fish liver in response to the exposure to the herbicides in this study suggest there is increase in the production of reactive oxygen species (ROS). Reactive Oxygen Species induction enhances the oxidation of polyunsaturated fatty acids which can end up in peroxidation of lipids (Valavanidis *et al.*, 2006; Liu *et al.*, 2008). Significant differences were noticed in malondialdehyde (MDA) levels in both concentration and time (Table 8).

According to Cossu *et al.* (2000), high levels of MDA content can be a reflection of lowered antioxidant status, making such organisms more sensitive to lipid peroxidation. This may have been the reason for the elevated levels of MDA observed in this study. Thus reactive oxygen species are generated in fish by these herbicides leading to peroxidation of lipid. The destruction of the lipid bilayer of cells is a very serious threat to their continued existence as cells become weak and unprotected from other substances injurious to them.

Fishes treated with paraquat at concentration 0.007 mg L^{-1} showed no significant difference in MDA levels throughout the eight week period (Table 8).

The percentage fold increase in MDA levels, between the control and the highest concentration is just about 1.05%, and this is even lower in fish treated with 0.007 mg/L of paraquat. Probably, the fishes in this group just about coped very well with the changes in MDA levels resulting in no significant increase. Interplay or any one of factors such as genetic makeup, health status, strength of immune system and physiology of the fishes might have played a key role in making them cope within the eight weeks period.

Activities of SOD, CAT and GPx also significantly increased throughout the exposure period. Similar observations were reported by Vasanth *et al.* (2012) after exposure of *Labeo rohita* to sub-lethal concentrations of anthracene. According to Nwani *et al.* (2013), organisms are equipped with a group of enzymes that work in tandem to reduce oxidative stress and to repair macro-molecules that have been damaged as a result of exposure to xenobiotics or during normal metabolism.

Catalase and Superoxide Dismutase are the main enzymes in this group for the elimination of reactive oxygen species (ROS), providing a first line of defense against ROS. Catalase converts hydrogen peroxide to water and molecular oxygen while SOD converts the superoxide anion radical to oxygen and hydrogen peroxide respectively (Shao *et al.*, 2012). It seems from the present study that the fishes have responded very well to the oxidative stress induced on them by the herbicides as evident in the increased levels of these antioxidant enzymes (Tables 1 and 2). Furthermore, the elevated levels of these antioxidants enzymes did not decrease throughout the period under study. This implies that the antioxidant enzymes were constantly clearing the ROSs generated. Part of the functions of these antioxidant enzymes is repair of damage and adaptive response. Either

or both of these functions appear to have come into play in the present study. This also support the claims of Livingstone (2001) that ROS generated in living systems are detoxified and held back in check by antioxidant defence system, which are generally found in different tissue types in animal species. The increase in the activity of SOD, and CAT in this study reflects the development of a compensatory mechanism in response to increased oxidative stress.

The elevated level of GPx in the liver of fish in the present study was found to be significant ($p \leq 0.05$) during the eight weeks exposure period (Table 7). This suggests that the enzyme is protecting the cells against lipid peroxidation. This may be a reflection of an adaptation to the oxidative conditions to which the fish have been exposed (Lenartova *et al.*, 1997). Abdel-Tawwab and Wafeek (2010) reported similar findings in *Oreochromis niloticus* after exposure to cadmium. Parthasarathy and Joseph (2011) reported a significant ($p \leq 0.05$) decline in the activities of GSH-dependent antioxidant enzymes, GPx and GST, in the liver tissue of *Oreochromis mossambicus* after exposure to lambda cyhalothrin and concluded that it reflected an increased oxidative stress in λ cyhalothrin induced fishes.

The elevated activities of SOD, CAT, LPO and GPx in fish liver in this study lend credence to the hypothesis that sublethal concentrations of glyphosate and paraquat induced oxidative stress in *C. gariepinus* and are probably an adaptive response geared towards protecting the fish from the herbicide-induced free radical toxicity. The overall increases between the control and the highest concentrations in the activities of all of these enzymes were more in paraquat than glyphosate. It may mean that paraquat was able to induce more toxicity in the fishes than glyphosate. This is supported by the lower LC_{50} value of paraquat (0.07 mg L^{-1}) as against 0.530 mg L^{-1} for glyphosate which consequently means lower sublethal concentrations.

In an earlier study, Ayanda *et al.* (2015a), reported several pathological changes were observed in the liver of fish on exposure to paraquat. The result in the present study further confirms the toxic effects of these commonly used herbicides in fish. The cumulative effects of these different toxic endpoints can overcome the fish's ability to 'fight back' which can lead to the death of fish.

Conclusion

Sublethal concentrations of glyphosate and paraquat modulated antioxidant enzymes in fish liver. Hence, their continuous use to kill unwanted plants means that our aquatic environment remains at the mercy of these chemicals. Ultimately, there will be a constant threat to life of the aquatic population not to mention the end users of these aquatic lives. To prevent this, it may be imperative to take adequate precautions when the application of these chemicals becomes unavoidable.

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