



BIOCHEMICAL CHANGES IN *Tilapia guineensis* EXPOSED TO DIFFERENT CONCENTRATIONS OF PARAQUAT DICHLORIDE UNDER LABORATORY CONDITIONS

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Abstract

Biochemical changes in *Tilapia guineensis* exposed to Paraquat Dichloride at various concentrations of 0.00 control, 0.05, 0.10, 0.15, 0.20 and 0.25 mg/L was carried out to determine the levels of alterations in fish exposed to this chemical. A total of 180 *T.guineensis* were used for the study. Blood samples were collected from the exposed fish and were analyzed with Randox test kits. Results from the study indicated that the values of metabolites such as creatinine, total bilirubin and total protein significantly reduced ($P < 0.05$) in the exposed fish when compared to the control values, while significant elevation ($P < 0.05$) was observed in urea. The antioxidants analysis showed that the values of SOD and GSH reduced significantly ($P < 0.05$), while CAT and LPO increased in both sizes when compared to the control. Also, the enzymes analysis showed that all the enzymes under consideration were significantly ($P < 0.05$) elevated when compared to the control values. Moreover, the values of electrolytes such as Na^+ and K^+ ions significantly increased ($P < 0.05$) in the exposed fish when compared to the control values, while significant reduction ($P < 0.05$) of Ca^{2+} was equally observed in the treated fish and no significant difference ($P > 0.05$) in the values of Cl^- between the control and exposed fish. In conclusion the chemical caused some changes in the biochemical profiles of exposed fish.

Keywords: Paraquat, Aquatic Environment, Fish, Toxicology, Biochemical profiles.

INTRODUCTION

Increasing amount of industrial, agricultural and domestic wastes into the aquatic environment has led to various degrees of harmful effects on the aquatic organisms [1]. Pesticides applications have been on the rise in recent times to control pests on the farms. This ultimately, finds their way in to the aquatic environment [2]. Pesticide pollution in the aquatic environment has attracted the attention of researchers and policy makers across the globe [3]. Typically the use of pesticides has increased within the last few decades due to extensive use in agricultural and industrial processes; as such they are becoming threats to living organisms [4]. Contamination of aquatic environments by pesticides in turn leads to oxygen depletion; poisoning and resultant mass mortality of fishes. Fish has the tendency to accumulate toxicants from their environment using their various parts. Conversely, xenobiotics such as pesticides could lead to physiological dysfunction in various biological system, haematological index, behavioural response, biochemical, alterations in fish [5].

The final destination of a growing volume of pollutants released by the disposal of household, commercial, agricultural, and industrial wastes is the aquatic environment. This complex mixture of pollutants affects organ function, reproductive status, species survival, population size, and ultimately biodiversity [6]. These effects are caused at the organism level as well as at the level of humans and ecosystems. Throughout the world, herbicides are frequently employed to manage the negative impacts that weeds and pests have on fish farms and agricultural output [7]. Herbicides have positive impacts on agriculture, but when they are used in the environment, they typically have negative consequences on the environment and public health. Following application, the herbicide eventually finds its way into various aquatic habitats where it is discovered to be extremely hazardous to non-target animals, particularly aquatic life forms and their surroundings [8]. Furthermore, fish are frequently employed in biomonitoring and can enable the assessment of compounds that may be hazardous to people because they react to toxic agents similarly to higher vertebrates [9]. For many fish species, the main route of absorption begins in the water and continues through oral intake, the stomach, and dermal absorption, which is more effective [10].

Physiological changes in fish are aimed at maintaining equilibrium in the presence of toxicants, which are known to disrupt enzymes activities (Abbas, 1998). According to Celik [12] exposure of fish for a long time to most toxicants interferes with protein metabolism. Decrease in total protein in fish exposed to toxic levels of toxicant could be attributed to either a state of hydration and change in water equilibrium in the fish or a disturbance in liver protein synthesis or both [13]. All biological activities are regulated by biochemical profiles. Therefore, assessment of these biochemical activities can be considered as a diagnostic tool to determine the physiological status of cell or tissues [14]. Paraquat dichloride have become one of the most used herbicide due to its distinctive mode of action, and is one of few chemical options that can be used to prevent and mitigate problems with weeds that have become resistant to the very widely used non-selective herbicide [15]. Several authors have evaluated the effects of this chemical on fish, but the information on *T.guineensis* is limited, hence the need for this study. The present paper contributes to the assessment of toxicity and the effects of paraquat on some biochemical profiles of *T.guineensis*, an euryhaline specie commonly found in the brackish water zone in Nigeria.

MATERIALS AND METHODS

Experimental Location and Fish

The study was carried out in African Regional Aquaculture Center, an outstation of Nigerian Institute for Oceanography and Marine Research, Buguma, Rivers State, Nigeria. One hundred and eighty *T.guineensis* (mean length 19.87 ± 2.99 cm; mean weight 220.99 ± 3.11 g) were sourced from ponds during the low tide. The fishes were transported in six open 50l open plastic containers to the laboratory and acclimated for a period of seven days.

Preparation of Test Solutions and Exposure of Fish

Samples of paraquat dichloride used in this experiment were purchased from a commercial outlet in Port Harcourt, Nigeria. *T. guineensis* were exposed to each of the chemical at the concentrations of 0.00 control, 0.05, 0.10, 0.15, 0.20 and 0.25 mg/L in triplicates. Ten fish were randomly distributed into each test tank. The experiment lasted for a period of 15 days. The water in the tanks was renewed daily. The fish were fed twice daily at 3% body weight with a commercial feed.

Determination of Biochemical Profiles

At the end of each experimental period, 2ml of fresh blood sample was collected by making a caudal puncture with the help of fine needle and poured in heparinized sample bottles. Plasma was separated by centrifugation at 10,000rpm for 5-8 minutes in TG20-WS Tabletop High Speed Laboratory Centrifuge. Plasma electrolytes such as Na^+ , K^+ , Ca^{2+} and Cl^- were determined by using Hitachi 902 automatic analyzer (Japan), following the method described by Gabriel *et al* [16]. The metabolites such as Creatinine, Total Bilirubin, Total urea and Total protein were analyzed in conformity with the standard methods as described by APHA [17]. Enzymes and anti-oxidants were analyzed in the blood of the exposed *T.guineensis* using a universal microplate reader on a Jenway visible spectrophotometer (Model 6405). All the tests were performed in triplicates.

Statistical Analysis

All the data were expressed as mean and standard deviation of mean. The statistical package, SPSS Version 22 was used for the data analysis. The means were separated using two way ANOVA and the two means were considered significant at 5 % ($P < 0.05$).

RESULTS

The resultant effects of paraquat dichloride on the electrolytes in the plasma of *T. guineensis* are presented in Table 1. It was observed that sodium ion (Na⁺) and K⁺ increased with increasing concentrations of the pesticides. Also Ca²⁺ decreased significantly when compared to the control values. While Cl⁻ were within the same range of 15.0- 18.0 (Table 1). The effects of paraquat dichloride on the metabolites in the serum of *T. guineensis* are presented in Table 2. It was observed that creatinine, total protein and total bilirubin decreased with increasing concentrations of the herbicide. While urea increased significantly when compared to the control values. The effects of paraquat on the enzymes in the serum of *T. guineensis* are presented in Table 3. It was observed that the values of AST, ALT, ACP, ALP and LDH increased with increasing concentrations of the chemical. The effects of paraquat dichloride on the antioxidants in the serum of *T. guineensis* juveniles are presented in Table 4. It was observed that the values of SOD and GSH decreased with increasing concentrations of the herbicide. While CAT and LPO increased significantly when compared to the control values.

Table 1: Electrolytes Ions in the Plasma of *T. guineensis* Exposed to Paraquat Dichloride (Mean±SD)

Conc. (mg/L)	Electrolytes (mEq/L)			
	Na ⁺	Ca ²⁺	K ⁺	Cl ⁻
0.00	40.30 ± 1.01 ^a	38.88 ± 2.09 ^c	2.40 ± 0.99 ^a	1 3.00 ± 1.02 ^a
0.05	44.50 ± 2.02 ^a	25.02 ± 2.19 ^d	3.80 ± 1.03 ^a	1 4.00± 1.04 ^a
0.10	50.80 ± 1.20 ^b	22.77 ± 1.45 ^c	5.50 ± 0.02 ^b	1 5.00 ± 1.99 ^a
0.15	60.90 ± 1.02 ^c	18.04 ± 2.09 ^c	7.10 ± 0.01 ^b	1 5.00 ± 1.19 ^a
0.20	65.50 ± 1.54 ^c	14.50 ± 1.04 ^b	10.03 ± 0.04 ^c	1 5.00 ± 2.02 ^a
0.25	70.99 ± 2.01 ^d	8.56 ± 1.08 ^a	11.20 ± 0.18 ^c	1 5.00 ± 1.01 ^a

Means in the same column with different superscripts are significantly different (p<0.05)

Table 2: Metabolites Activities in the Plasma of *T. guineensis* Exposed to Paraquat Dichloride

Concentration (mg/l)	Metabolites (mmolL ⁻¹)			
	Creatinine	Urea	Total bilirubin	Total protein
0.00	90.00±5.00 ^c	5.00±0.00 ^a	21.33±1.15 ^b	35.10±1.00 ^c
0.05	83.33±5.77 ^b	5.00±0.00 ^a	20.00±1.00 ^b	26.00±5.56 ^b
0.10	76.60±5.77 ^a	6.33±0.57 ^b	16.66±1.52 ^a	27.66±6.65 ^b
0.15	65.00±5.00 ^b	5.33±1.15 ^a	17.66±0.57 ^a	23.66±3.21 ^b
0.20	60.00±10.00 ^a	7.33±2.08 ^c	14.66±0.57 ^a	19.00±1.00 ^a
0.25	48.30±2.88 ^a	11.00±0.00 ^c	18.33±1.15 ^a	28.00±1.00 ^b

Means within the same row with different super scripts are significantly different (P<0.05)

Table 3: Enzymes Activities in the Plasma of *T. guineensis* Exposed to Paraquat Dichloride

Conc. (mg/l)	Enzymes (IU/L)				
	AST	ALT	ACP	ALP	LDH
0.00	71.24±1.87 ^a	55.00±1.00 ^a	20.65±0.99 ^b	63.33±3.05 ^a	300.66±11.06 ^a
0.05	81.65±6.68 ^b	58.66±1.52 ^a	17.90±1.29 ^a	68.66±5.68 ^a	334.66±8.08 ^a
0.10	80.89±2.80 ^b	69.33±1.52 ^b	22.33±1.98 ^b	77.00±3.60 ^b	361.66±4.72 ^b
0.15	86.73±1.57 ^b	57.00±11.35 ^a	27.99±2.00 ^b	75.66±0.57 ^b	364.33±4.02 ^b
0.20	88.98±3.65 ^b	71.00±1.73 ^c	34.24±1.61 ^c	74.00±1.00 ^b	375.33±5.03 ^b
0.25	86.40±3.47 ^b	65.66±1.15 ^b	26.94±1.05 ^b	83.33±1.52 ^c	389.66±9.60 ^b

Means within the same row with different super scripts are significantly different (P<0.05)

Table 4: Antioxidants Levels in the Plasma of *T. guineensis* Exposed to Paraquat Dichloride

Concentrations (mg/l)	Antioxidants (U/mg)			
	CAT	GSH	SOD	LPO
0.00	80.00±2.98 ^a	7.66±0.57 ^a	15.33±0.57 ^a	12.66±1.15 ^a
0.05	85.00±7.05 ^a	7.33±1.15 ^a	14.33±0.44 ^a	13.66±0.57 ^a
0.10	91.68±7.93 ^a	5.33±9.57 ^a	11.33±0.51 ^a	18.66±1.52 ^a
0.15	97.87±6.90 ^a	5.00±1.00 ^a	11.33±0.57 ^a	17.00±1.00 ^a
0.20	99.00±5.87 ^a	5.00±0.00 ^a	10.33±0.52 ^a	19.66±0.57 ^a
0.25	112.85±9.98 ^a	5.66±0.57 ^a	11.33±0.09 ^a	14.33±1.54 ^a

Means within the same row with different super scripts are significantly different (P<0.05)

DISCUSSION

Electrolytes are needed for osmoregulatory purposes in the body system of living organisms, Therefore, alterations of the electrolyte balance of an organism would adversely affect the organism concerned. These functions can be compromise with stress due to the toxicant effect on the fish physiology. A slight variation of values in this present study is an indication that the pesticides affect the *T.guineensis* electrolytes system in the tissue. Additionally, this observation may be as a result of low concentration of the toxicant used in this experiment. This perhaps confirms the low toxicological effect of these chemicals on fish. Slight fluctuation in sodium (Na⁺) values was also reported by Awoyinka *et al.*[18] when they exposed *Clarias anguilaris* to crude oil. According to Nte and Akinrotimi [19] slight change in values of these electrolytes can disturb osmotic and ionic regulation in fishes as well as general physiology in the fish.

Assessment of different enzymes namely: ALP, AST, ALT, ACP and LDH are parts of standard laboratory tests to detect abnormalities in animals [20]. ALT, AST, ALP are non plasma specific enzymes that are localized in tissue cells of liver, heart, gills, kidneys, muscles and other organs and their presence in the blood may give specific information about organ dysfunction Variations in the activity of these enzymes resulting from toxicant or contaminant effects in various organs of fish have been observed in other species by different authors [21]. Such alterations in fish are aimed at maintaining equilibrium in the presence of these toxicants which are known to disrupt physiological and biochemical processes [22]. In this study, activities of these enzymes increased, as the concentration of paraquat increased in the serum of *T.guineensis*. These alterations were dose dependent. This assertion corroborated the findings of Gabriel *et al.* [23] in *C.gariepinus* exposed to cypermethrin . These authors opined that the increase of transferases is as a result of diversion of alpha-amino acids in the tricarboxylic acid (TCA) cycle as keto-acids to augment energy production.

Under stressful physiological conditions, the antioxidant defense systems including SOD, CAT and GSH can be induced by slight oxidative stress as a compensatory response, and thus the reactive oxygen species (ROS) can be removed to protect the organisms from oxidative damage [24]. The activity of antioxidants may be increased or inhibited under chemical stress depending on the intensity and duration of stress applied as well as susceptibility of exposure species. The plasma in fish performs various functions associated with the metabolism of toxicants [25]. Glutathione is a tripeptide and exists in reduced glutathione (GSH) and oxidized glutathione disulfide (GSSG) states. The decrease values of GSH observed in this study may be due to either direct scavenging of radicals or increased peroxidase activity [26]. In present study, SOD and GSH was suppressed by paraquat exposure in *T.guineensis* when compared to the control fish. Decreased SOD levels in the plasma of treated fish in this study indicate decreased ability of the tissues to handle O₂- free radicals. Similar findings on decreased SOD have been reported in the tissues of *Oreochromis niloticus* exposed to heavy metals intake [27]. The present study revealed that CAT activities in the plasma of *T.guineensis* exposed to paraquat increased significantly (P<0.05) after 15 days of exposure. The elevation of CAT in the present study may be physiological adaptation for the elimination of ROS generation. Similar results have been observed in Tilapia (*O.niloticus*) exposed to pesticides in the laboratory [28]. In the present study, lipid per oxidation increased significantly in the plasma of *T.guineensis* after 15 days of exposure. The elevation of lipid peroxidation in the study, suggested participation of free radical induced oxidative cell injury in mediating the toxicity of paraquat.

In this study, Total protein, creatinine and total bilirubin in the plasma of the exposed fish decreased with increased concentration of paraquat dichloride. While urea increased considerably when compared to the control values. Inyang *et al.* [29] reported similar results in plasma total protein, albumin, glucose and organ's total urea and creatinine of *Clarias gariepinus* exposed to diazinon. Similarly, Ben-Eledo *et al.* [30], observed that exposure of fish for a long time to most toxicants including pesticides interferes with protein metabolism, depletion of total protein in the plasma and serum of fish. The decrease in total protein and creatinine levels may be due to impaired synthesis of protein or enhanced loss of protein via excretion and is also suggestive of some problem in the kidney [31]. However, the very low levels of total bilirubin recorded in this study suggest that the liver may not have been affected by the toxicant. An increase in urea suggested that the kidney may have been affected by the toxicant. According to Kori-Siakpere *et al.* ([32], the ability of the kidney to excrete these products might further indicate an increase in glomerular filtration rate in the exposed fishes. Additionally, increase in values of these metabolites may suggest that the kidney is under stress to remove these metabolic wastes due to toxic effect of paraquat.

CONCLUSION

This study also showed that the toxicants caused significant alterations in biochemical profiles of the exposed *T.guineensis* , a clear indication that their usage in the fields and water environment may be a threat to aquatic biota. This is worrisome consequent to the contemporaneity of cypermethrin and dimethoate in the environment and some pesticide formulations. There is urgent need to regulate the use of pesticides containing these chemicals, especially in floodplains that serve as breeding sites for commercially and ecologically important fish species. Sustainable and environmental friendly farming systems must be advocated for in different parts of the country. Total changes to a new system of approach in crop protection procedures are paramount and urgent in order to resolve the economic and environmental consequences in application of pesticides. However, general environmental quality monitoring should be compulsory and the monitoring of the quality of water should be done on a regular basis and as a result, any abnormal changes in the physiology of the aquatic organisms can easily be detected and appropriate action taken before the outbreak of epidemics.

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