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Enzymatic Responses in *Tilapia guineensis* Exposed to Sodium Bromide in the Laboratory

Cookey A* and Kalada I

Department of Aquaculture, Nigerian Institute for Oceanography and Marine Research, Nigeria

***Corresponding author:** Ayaobu-Cookey, Department of Aquaculture, Nigerian Institute for Oceanography and Marine Research, Victoria Island, Lagos, Nigeria, Email: ibfcookey@gmail.com

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Abstract

To evaluate the systemic effect of the chemical on the fish's enzyme profile, changes in the enzyme activities aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP), alkaline phosphates (ALP), and lactate dehydrogenase (LDH) in the plasma of *Tilapia guineensis* exposed to sodium bromide were found. From *T. guineensis* juveniles and adults, blood samples were taken and examined. The results of the enzyme study revealed that all of the enzymes under analysis were considerably (P 0.05) higher than the control values. The severity of these changes depended on the concentration and was greater in the exposed fish's juvenile than adult. In conclusion, alterations in plasma enzyme parameters may be caused by target tissue damage and dysfunction brought on by toxicants, and these parameters can be used as quick and accurate indicators of monitoring toward the impact of toxicants on aquatic organisms and ultimately the entire ecosystem.

Keywords: Enzymes; Tilapia; Fish; Toxicity; Biochemistry

Abbreviations: AST: Aspartate Transaminase; ALT: Alanine Transaminase; ACP: Acid Phosphatase; ALP; Alkaline Phosphates; LDH: Lactate Dehydrogenase.

Introduction

One of the main pollutants of aquatic ecosystems that can have either short-term or long-term negative effects on living things has been identified as pesticides. They have apparently triggered a number of metabolic changes in fish, both at lethal and more frequently at sublethal levels. Fish species are vulnerable to the hormonal and enzymatic disruption that stress and the impacts of pesticides cause. Chronic low level exposure has a more significant effect on fish populations than acute poisoning. Inconspicuous physiologic and behavioral alterations that impair reproduction and survival are associated with pesticide concentrations that are not high enough to kill fish [1]. According to Raja M, et al. [2], pesticides have been connected to changes in fish's ion concentrations, organic components, enzyme activity, endocrine activity, and chemo-regulators.

In human medicine, assessments of serum enzyme activity are frequently employed as a diagnostic tool [3]. Recent years have seen a significant increase in the use of enzyme activity to forecast the toxicity of pesticides in aquatic creatures, particularly fish [4]. The most significant collection of enzymes in lower animals, particularly in telost fish, includes several enzymes such amino transferases, phosphatases, and lactases [5]. A set of enzymes known as transferases, which also includes alanine transaminase (ALT) and aspartate transaminase (AST), catalyzes the inter conversion of a keto acid into an amino acid. While the phosphatases are hydrolase enzymes that remove phosphate groups from a variety of compounds, including nucleotides, proteins, and alkaloids Tamas L, et al. [6], acid phosphatase

(ACP) and alkaline phosphatase (ALP) are responsible for this. Additionally, there is the lactase, or lactate dehydrogenase, which is in charge of converting lactic acid from the muscles into pyruvic acid, which is a necessary step in the creation of cellular energy [7].

Fish undergo physiological adjustments to maintain balance when exposed to toxins, which are known to interfere with the activities of enzymes [8]. Fish that have been exposed to most toxicants for a long time have altered protein metabolism [9]. The change in the fish's water equilibrium and state of hydration or a disruption in liver protein synthesis, or maybe both, may be responsible for the decrease in total protein in fish exposed to hazardous amounts of toxicant [10]. Enzymes and hormones, which are both proteins, control all biological processes. In order to measure the physiological status of cells or tissues, protein and enzyme activities can be thought of as a diagnostic tool [11]. An effective organophosphate insecticide and acaricide is dimethoate. Pesticides, like other organophosphates, inhibit acetyl cholinesterase, a crucial enzyme for the proper operation of the central nervous system.

Given the wide range of adverse effects that chemical exposure in fish can have, many biomarkers may be used to gauge how organisms react to such toxins. Existing biomarkers are better suited to certain groups of contaminants based on their sensitivity and specificity, which differ depending on the contaminant or group of contaminants. The presence of specific contaminants may be detected by biomarkers that are employed as endpoints in ecotoxicology, and they may exhibit high sensitivity to the compound(s) (enable early identification, responding to low contaminant concentration) [10]. Alkaline phosphatase (ALP), lactic dehydrogenase (LDH), Alanine aminotransferase (ALT), and Aspartate aminotransferase (AST) are examples of enzymes that are included in standard or comprehensive blood panels. Due to their quick reaction to testing of sublethal contamination concentrations at the lowest levels of biological organization, i.e. biochemical/cellular responses, they are regarded as having significant toxicological importance. They are a developing method for evaluating the severity of the harm caused to organisms by contaminants, figuring out how these affect the survival of organisms, and learning about consequences at higher levels of biological structure [11].

Fish are the main aquatic species that get contaminated by toxicants. Fish are frequently employed to evaluate the condition of the aquatic environment, and as a result, they can act as bio indicators of environmental pollution. A teleost with widespread distribution and commercial value for fisheries and aquaculture is *Tilapia guineensis.* Due to a variety of traits, including high growth rates, efficiency in adapting to different diets, great resistance to diseases and handling practices, ease of reproduction in captivity and prolific rate, and finally, good tolerance to a wide range of environmental conditions, it is a good biological model for toxicological studies [12]. Although the effects of this substance on fish have been examined by a number of authors, there is little known about *T. guineensis*, necessitating the present investigation.

Materials and Methods

Experimental Location and Fish

The study was conducted at the African Regional Aquaculture Center in Buguma, Rivers State, Nigeria, which is a branch office of the Nigerian Institute for Oceanography and Marine Research. During low tide, ponds yielded 360 *T. guineensis*, of which 180 were juvenile and 180 were adults. The fish were brought to the lab in six open, 50-liter plastic containers, where they acclimated for seven days.

Preparation of Test Solutions and Exposure of Fish

In this experiment, sodium bromide was utilized, and it was acquired from a store in Port Harcourt, Nigeria. *T. guineemsis* were subjected to the substance in triplicates at concentrations of 0.00 control, 0.50, 1.00, 1.50, and 2.00 mg/L. Each test tank had ten fish, placed there at random. The test was conducted for fifteen days. Every day, fresh water was added to the tanks. The fish were given commercial feed twice daily at 3% body weight.

Analytical Procedure

A 2ml sample of fresh blood was taken at the conclusion of each experimental period by puncturing the caudal artery with a tiny needle and pouring the sample into heparinized sample vials. Blood samples were immediately centrifuged at 5000 rpm for 15 minutes. Separated plasma samples were pipetted into eppendorf tubes and kept in a freezer at -20°C until they were analyzed [13]. A Jenway visible spectrophotometer (Model 6405) with a universal micro plate reader was used to read the data. The blood of the exposed *T. guineensis* was examined for five enzymes: aspartate amino transaminase (AST), alanine amino transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), and lactate dehydrogenase (LDH). AST was examined using the Bessey MM, et al. [14] approach since it may be done manually using a colorimetric end-point technique. While ALP, ACP, and LDH were performed using the Huang C, et al. [15] technique.

Statistical Analysis

The mean and standard deviation of the mean were used to express all the data. The data analysis was done using SPSS Version 22, a statistical program. Using two-way ANOVA, the means were split, and the two means were deemed significant at 5% ($P < 0.05$).

Results

The water quality parameters Table 1 were within the same range except in DO, where a lesser values were obtained at higher concentration of the chemical. The effects of sodium bromide on the enzymes in the plasma of *T.guineensis* juveniles are presented in (Table 2). 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. The same trend was observed in the antioxidants of adult fish exposed to the chemical (Table 3).

Concentrations (mg/L)	DO (mg/L)	Temperature (°C)	pH	NH_3 (mg/L)	Salinity (ppt)
0	5.90±0.65^{b}	29.88±2.82^{a}	6.68±0.77^{a}	0.01±0.00^{a}	11.09±0.77^{a}
0.5	5.69±0.80^{b}	29.76±3.89^{a}	6.63±0.90^{a}	0.02±0.00^{b}	11.07±0.98^{b}
1	5.01±0.21^{b}	29.48±1.88^{a}	6.62±0.82^{a}	0.02±0.00^{a}	11.04±1.66^{a}
1.5	4.12±0.81^{a}	29.66±5.83^{b}	6.61±0.67^{a}	0.03±0.00^{a}	11.05±0.99^{a}
2	4.01±0.32^{a}	29.72±4.82^{b}	6.67±0.88^{a}	0.03±0.00^{a}	11.09±1.08^{a}

Means within the same column with different super scripts are significantly different ($P<0.05$).
Table1: Physico-Chemical Parameters of Water in Experimental Tanks of *T. guineensis* Exposed to Sodium Bromide.

Concentrations(mg/L)		Enzymes (IU/L)			
	AST	ALT	ACP	ALP	LDH
0	59.08±1.71^{a}	46.03±1.08^{a}	15.77±0.88^{a}	55.99±2.05^{a}	218.88±9.64^{a}
0.5	63.99±1.87^{a}	49.08±1.77^{a}	17.88±1.88^{a}	58.88±1.88^{a}	230.90±9.72^{a}
1	71.59±3.09^{b}	59.88±1.77^{b}	22.88±1.08^{b}	68.09±2.54^{b}	268.88±4.88^{b}
1.5	79.88±3.04^{b}	74.77±1.88^{c}	26.99±1.51^{b}	76.08±1.90^{c}	272.88±3.88^{b}
2	89.93±7.77^{b}	78.99±1.99^{c}	34.88±2.88^{b}	89.77±2.88^{a}	297.88±9.88^{a}

Means within the same column with different super scripts are significantly different ($P<0.05$).
Table 2: Enzymes Activities in *T. guineensis* Juveniles Exposed to Sodium Bromide.

Concentrations(mg/L)		Enzymes (IU/L)			
	AST	ALT	ACP	ALP	LDH
0	70.99±1.87^{a}	55.07±1.88^{a}	16.65±1.88^{b}	60.04±7.77^{a}	322.99±9.99^{a}
0.5	81.55±6.68^{b}	59.62±1.88^{a}	18.90±1.29^{a}	65.55±8.99^{a}	348.88±9.12^{a}
1	84.77±2.43^{b}	69.88±1.09^{b}	22.33±1.98^{b}	71.77±9.88^{b}	377.98±9.98^{b}
1.5	88.07±3.88^{b}	71.04±1.07^{c}	34.24±1.61^{c}	74.88±8.89^{b}	388.33±7.45^{b}
2	97.99±3.99^{c}	77.88±1.99^{c}	36.94±1.05^{c}	83.78±1.98^{c}	399.66±9.88^{b}

Means within the same column with different super scripts are significantly different ($P<0.05$).
Table 3: Enzymes Activities in *T. guineensis* Adults Exposed to Sodium Bromide.

Discussion

Standard laboratory procedures to find abnormalities in animals include measuring various enzymes, including ALP, AST, ALT, ACP, and LDH [16]. The presence of the non-plasma specific enzymes ALT, AST, and ALP in the blood may provide specific information regarding organ dysfunction because they are localized in the tissue cells of the liver, heart, gills, kidneys, muscles, and other organs. Different publications have found variations in these enzymes' activities due to toxicant or pollutant effects in various fish organs in other species [17,18]. Numerous blood serum soluble enzymes have been proposed as useful stress indicators. In order to diagnose fish diseases and to find tissue damage brought on by environmental contamination, serum ALT, AST, ALP, and LDH activities have been widely used. According to Palanivelu V, et al. [19], an increase in these enzyme activities in the serum or extracellular fluid is a sensitive sign of even slight cellular damage and denotes stress-related tissue deterioration.

All of the enzymes that were measured in this investigation had higher values in the plasma of *T. guineensis* that had been exposed to sodium bromide. This observation is consistent with the findings of Vaglio A, et al. [20], who found that when heavy metals were exposed to *Sparus aurata*, it increased the fish's plasma ALT, AST, and ALP activity. The current findings concur with those of Jee JH, et al. [21] who discovered that Korean rockfish (*Sebastes schlegeli*) treated to cypermethrin had increased serum ALT, AST, and LDH activities. The plasma ALP and LDH activity in the fish *Rhamdia quelen* Borges A, et al. [22] and *Labeo rohita* Das BK, et al. [23] significantly increased after exposure to cypermethrin. Nile tilapias were exposed to sub-acute doses of pyrethroid and deltamethrin for 28 days, which similarly resulted in a rise in serum ALP [24]. The researchers came to the conclusion that the rise in this enzyme in the blood could be caused by liver necrosis and subsequent leaking of this enzyme into the bloodstream.

The results of elevated ALT, AST, ALP, and LDH levels typically point to liver degeneration and hypofunction because toxicants' effects on hepatocytes typically manifest as tissue destruction, which leads in the release of cellular enzymes into the blood plasma. As a result, elevations in these enzyme activities in *T. guineensis* serum are mostly caused by the leakage of these enzymes from the liver cytosol into the blood stream as a result of liver damage caused by metals and pesticides, which provides evidence of the hepatotoxic effect of toxicants. According to Harvey RB, et al. [25], elevated serum levels of these enzymes are typically a sign of disease and necrosis in an animal's liver. Blood levels of ALT, AST, and ALP may rise as a result of cellular damage in the liver.

Conclusion

The exposure of *T.guineensis* juveniles and adults sizes to sub-lethal concentrations of sodium bromide can induce various toxicological effects in the form of enzymatic degradation. Given that the blood is the first organ to be affected by unfavorable changes in the environment, blood biochemical profiles can reveal vital information about the internal environment of an organism. The results of the current investigation demonstrated that alterations in the plasma enzyme activities of fish exposed to pesticides were thought to be a biochemical manifestation of the harmful effects of toxicants. All plasma enzyme parameters showed higher increases in fish treated with pesticides than in the control fish without exposure. In conclusion, alterations in plasma enzyme parameters may be caused by target tissue damage and dysfunction brought on by toxicants, and these parameters can be used as quick and accurate indicators of monitoring toward the impact of toxicants on aquatic organisms and ultimately the entire ecosystem.

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