EFFECTS OF DIMETHOATE ON ENZYME PROFILES IN BLACK JAW TILAPIA (*Sarotherodon melanotheron*)

Nwankwo, A.K[1*] ., Okeke P.A[2] ., Ohaturuonye, S.O., Onwujiariri C. A[3] and Onwujiariri C. E[4]

[1] Department of Mariculture, Nigerian Institute for Oceanography and Marine Research, Buguma, Rivers State, Nigeria
[,2]Department of Fisheries and Aquaculture, Faculty of Agriculture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
[3,4] African Regional Aquaculture Centre/Nigerian Institute for Oceanography and Marine Research, Aluu. P.M.B. 5122, Port Harcourt, Rivers State, Nigeria

Corresponding author:
anayochukwukelechi@gmail.com

ABSTRACT

Changes in the activity of enzymes are useful markers of stress brought by any toxins that fish have been exposed to. The current study examined the effects of various dimethoate concentrations (0.00-control, 0.50, 1.00, 1.50, and 2.00 mg/l) on the activities of some enzymes, such as lactate dehydrogenase (LDH), acid phosphatase (ACP), lactate transaminase (AST), alanine transaminase (ALT), and alkaline phosphates (ALP), in the plasma of Sarotherodon melanotheron exposed for a duration of 15 days. The analysis of the enzyme results showed that every enzyme significantly (P <0.05) exceeded the control values. The juvenile fish exposed to the chemical, had a greater degree of severity than the adult fish, depending on the concentration. In conclusion, changes in plasma enzyme parameters can be used as rapid and reliable indicators of monitoring the effects of toxicants on aquatic organisms and ultimately the ecosystem as a whole. These parameters may be attributed to target tissue damage and dysfunction brought by the toxicants.

Keywords: *Contaminants, Aquatic ecosystem, Pollution, Tilapia, Enzymes,*

INTRODUCTION

Pesticides are among the pollutants that have been recognized as one of the main contaminants of aquatic ecosystems, having harmful effects on the living things that can be either acute or long-term. Numerous biochemical alterations in fish have been documented, often at sublethal as well as lethal levels. Fish species are susceptible to hormonal and enzymatic disruption brought on by stress and pesticide effects. Fish populations are more significantly impacted by low-level chronic exposure than by acute poisoning. Pesticide doses that are not high enough to kill fish have been linked to subtle alterations in physiology and behavior that hinder fish survival and reproduction [1]. Pesticides have been linked to changes in fish ion concentrations, organic components, enzyme activity, endocrine activity, and chemo-regulators [2, 3]. When fish are exposed to toxins, which are known to interfere with the activities of enzymes, they go through physiological modifications to preserve equilibrium [4]. Long-term exposure to the majority of toxicants alters the metabolism of proteins in fish [5]. The reduction in total protein in fish exposed to dangerous levels of toxicant may be caused by either a disruption in liver protein synthesis or a change in the fish's water equilibrium and state of hydration, or maybe both [6]. Every biological activity is governed by proteins called hormones and enzymes.Protein and enzyme activities can be used as a diagnostic tool to assess the physiological state of cells or tissues [7]. Dimethoate is a potent organophosphate pesticide and acaricide. Like other organophosphates, pesticides block the enzyme acetylcholinesterase, which is essential to the healthy functioning of the central nervous system.

When used as pollution indicators, biochemical measures in organisms provide information on the beneficial or harmful reactions in organisms exposed to specific chemical concentrations. Prior to the occurrence of other toxicological points, such as death, such analysis provides early warning signs [8]. The phosphatases ACP and ALP, also known as phosphomonoesterases, are active at particular pH values. Fish suffering from pesticide toxicity exhibit increased ACP and ALP activity [9]. Since the ACP is a lysosomal enzyme, the cellular damage is most likely the cause of the increase in activity. However, it is challenging to link necrosis with the decline in ACP activity. An emphasis shift from the normal ATPase system, which includes phosphorylation, to the energy breakdown pathway can be explained by an increase in the activities of acid phosphatase and alkaline phosphatase. Velisek *et al.* [10] claim that pesticides raise phosphorylase activity, which includes the release of inorganic phosphates from phosphate esters, and decrease glycogen content, which causes glucogenesis. Reduced ATPase system activity results in less inorganic phosphates being released from phosphate esters.

Furthermore, a number of scientific investigations have discovered that the toxic material, fish species, exposure duration, and water quality all affect the rise or fall in serum enzyme activities, such as aminotransferases ALT and AST, which are intracellular enzymes released by cell damage or death [11,12,13]. When heavy metals build up in fish tissues, they can disrupt numerous biochemical and histological pathways, making fish extremely vulnerable to pollution [14]. In freshwater fish exposed to harmful substances including heavy metals, zinc ions, and other pollutants, blood enzyme activity can increase [15]. Additional research, like that of Raja et al. [16], examines how the concentrations of two enzymes, ALT and AST, change when an adult Carassius auratus is exposed to different salt concentrations. Furthermore, Edori *et al.*'s study [17] in the catfish *Clarias gariepinus* revealed significant biochemical and enzymatic alterations following sub-acute exposure to agricultural pollutants, which may be detrimental to the fish. An analysis of Filippov *et al.*'s [18] study on the activity of fish digestive enzymes reveals that the effects of organic pollutants on fish activity can vary based on the kind of fish, the hydrolyzed substratum type, toxicant concentrations, and experimental settings. Few research on the effects of pollutants have looked at the enzymatic activity in fish blood, particularly S. melanotheron, which makes the current study necessary. The majority of studies on the effects of pollutants have focused on physiological and biochemical alterations. Thus, this work evaluates the enzymatic reactions of lab-exposed black jaw tilapia (*Sarotherodon melanotheron) to dimethoate.*

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, 360 *S. melanotheron* , of which 120 were juveniles, 120 were sub adults and 120 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, Dimethoate was utilized, and it was acquired from a store in Port Harcourt, Nigeria. *S. melanotheron* 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 eight 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 [19]. A Jenway visible spectrophotometer (Model 6405) with a universal microplate reader was used to read the data. The blood of the exposed *S.melanotheron* 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 et al [20] approach since it may be done manually using a colorimetric end-point technique. While ALP, ACP, and LDH were performed using the Huang *et al*. [21] 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 the values of dissolved oxygen, where a lesser values were obtained at higher concentration of the chemical. The effects of dimethoate on the enzymes in the plasma of *S.melanotheron* juveniles are presented in Table 2. It was observed that the values of aspartate transaminase (AST), alanine transaminase (ALT), acid phosphatase (ACP), alkaline phosphates (ALP), and lactate dehydrogenase (LDH) in the plasma of *S. melanotheron* juveniles SOD and GSH decreased with increasing concentrations of the herbicide. These values increased significantly ($P<0.05$) when compared to the control values. IThe same trends were equally in the enzymatic activities in the sub-Ault and adult fish exposed to the chemical (Table 3 and 4).

Table1: Physico-Chemical Parameters of Water in Experimental Tanks of *S.melanotheron* Exposed to Dimethoate

Concentrations (mg/L)	DO (mg/L)	Temperature (ºC)	pH	NH3 (mg/L)	Salinity (ppt)
0.00	5.91±0.45 ^{b}	29.02±2.66^{a}	6.66±0.44 ^{a}	0.01±0.00 ^{a}	11.88±0.41 ^{a}
0.50	5.60±0.77^{b}	29.12±3.44^{a}	6.62±0.55 ^{a}	0.02±0.00 ^{a}	11.82±0.98 ^{b}
1.00	5.01±0.81 ^{b}	29.38±1.22 ^{a}	6.60±0.77 ^{a}	0.02±0.00 ^{a}	11.85±1.88 ^{a}
1.50	4.17±0.48 ^{a}	29.02±5.01 ^{a}	6.60±0.54 ^{a}	0.03±0.00 ^{a}	11.85±0.61 ^{a}
2.00	4.00±0.44^{a}	29.78±4.81 ^{a}	6.60±0.51 ^{a}	0.03±0.00 ^{a}	11.89±1.44 ^{a}

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

Table 2: Enzymes Activities in *S.melanotheron* Juveniles Exposed to Dimethoate

Concentrations (mg/L)		Enzymes (IU/L)			
	AST	ALT	ACP	ALP	LDH
0.00	57.12±1.03^{a}	40.98±1.65 ^{a}	14.03±2.05 ^{a}	50.03±2.33 ^{a}	215.22±9.02 ^{a}
0.50	61.02±1.02 ^{a}	47.99±1.52 ^{a}	18.02±1.77 ^{a}	56.88±1.88 ^{a}	225.02±9.88 ^{a}
1.00	70.43±4.87 ^{b}	55.02±1.82 ^{b}	23.75±1.01 ^{b}	64.33±2.44 ^{b}	250.33±7.02 ^{b}
1.50	78.02±3.11 ^{b}	70.77±1.02 ^{c}	25.03±1.89 ^{b}	69.56±1.01 ^{b}	260.11±3.03 ^{b}
2.00	80.01±5.02 ^{c}	75.02±1.43 ^{c}	32.01±2.22 ^{b}	81.02±2.11 ^{c}	280.33±9.01 ^{c}

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

Table 3: Enzymes Activities in *S.melanotheron* Sub-Adults Exposed to Dimethoate

Concentrations (mg/L)		Enzymes (IU/L)			
	AST	ALT	ACP	ALP	LDH
0.00	60.44±1.02 ^{a}	50.70±1.02 ^{a}	15.77±1.99 ^{a}	60.01±7.01 ^{a}	320.44±9.02 ^{a}
0.50	80.22±6.01 ^{b}	55.12±1.22 ^{a}	18.02±1.79 ^{a}	62.33±8.01 ^{a}	337.02±9.77 ^{a}
1.00	83.54±2.01 ^{b}	60.33±1.64 ^{b}	20.88±1.03 ^{b}	70.32±9.44 ^{b}	350.01±9.44 ^{b}
1.50	85.32±3.33 ^{b}	70.04±1.98 ^{c}	30.77±1.56 ^{c}	72.44±8.21 ^{b}	362.88±7.77 ^{b}
2.00	90.54±3.65 ^{c}	75.33±1.43 ^{c}	35.02±1.77 ^{c}	82.01±1.11 ^{c}	371.03±9.41 ^{b}

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

Table 4: Enzymes Activities in *S.melanotheron* Adults Exposed to Dimethoate

Concentrations (mg/L)		Enzymes (IU/L)			
	AST	ALT	ACP	ALP	LDH
0.00	70.03±1.32 ^{a}	53.44±1.61 ^{a}	16.01±1.02 ^{b}	60.04±7.77 ^{a}	320.01±9.02 ^{a}
0.50	81.99±6.68 ^{b}	56.41±1.02 ^{a}	18.99±1.06 ^{a}	65.55±8.99 ^{a}	340.33±9.33 ^{a}
1.00	84.99±2.98 ^{b}	65.03±1.11 ^{b}	22.92±1.02 ^{b}	71.77±9.88 ^{b}	370.42±9.66 ^{b}
1.50	86.88±3.70 ^{b}	70.44±1.33 ^{c}	33.99±1.41 ^{c}	74.88±8.02 ^{b}	381.77±7.91 ^{b}
2.00	95.90±3.32 ^{c}	74.88±1.02 ^{c}	38.02±1.51 ^{c}	84.03±1.12 ^{c}	392.01±5.01 ^{b}

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

DISCUSSION

An increase in the activity of acid and alkaline phosphatases can be explained by the tissue shifting its focus from the regular ATPase system to the phosphatase system for energy breakdown. Pesticides raise phosphorylase activity and

lower glycogen levels [22]. Activated phosphatases can catalyze the release of inorganic phosphatases from phosphate esters, which can lead to phosphorylation in the case of a reduced ATPase system. In this study, pesticide poisoning changed how ACP and ALP functioned. The plasma of S.melanotheron treated to dimethoate had greater levels of ALP and ACP. The lysosomal enzyme ACP exhibits an increase in activity in response to cellular injury [23]. An accelerated rate of enzyme turnover under pesticide stress may be the cause of the elevated ACP and ALP activity. These phosphatases, also known as phosphomonoesterases, are active at particular pH values. The duration, pesticide value, and concentration all affect how ACP and ALP alter. The findings of this investigation are consistent with the findings reported by Das and Mukherjee [24], who noted a similar pattern in *Labeo rohita* exposed to cypermethrin in a lab setting. The pesticide cypermethrin caused modifications in the *Labeo rohita* fish, which led to an increase in the two enzymes, ACP and ALP. However, the increase in ACP is much smaller than that in ALP.

Additionally, Sastry and Sharma [25] examined the impact of endrin on the levels of alkaline and acid phosphatase in the plasma of the teleost fish *Ophiocephalus (Channa) punctatus* after a ten-day exposure period. While there was no discernible change in the activity of alkaline phosphatase in the kidney or liver, the enzyme was suppressed, they observed an increase in acid phosphatase activity in the plasma, liver, and kidney. This aligns with the current research.Oruc and Uner [26] exposed the fish *Cyprinus carpio* to the acute and long-term impacts of 2, 4-Diamin (herbicide), but they saw no change in the activity of alkaline phosphatase and acid phosphatase. The freshwater edible catfish Clarias batracus (Linn.) was subjected, for 24, 74, 120, and 168 hours, to sublethal concentrations of two distinct pesticide groups: carbaryl, a carbamate, and phorate, an organophosphorus pesticide, by Jyothi and Narayan [27]. They informed them of the elevated alkaline phosphatase level. In contrast, Verma et al. [28] found that *Mystus vittatus* serum contained elevated levels of three phosphatases: acid, alkaline, and glucose-6-phosphatase. These levels were observed after the pesticides thiotox, dichlorvos, and carbofuran, as well as their combinations, were applied. Notably, the elevation in alkaline phosphatase was greater than that in acid and glucose-6-phosphatases.

The study observed an increase in LDH enzymes when *S.melanotheron* specimens of varying sizes were exposed to a pesticidal action. This results from membrane damage brought on by lipid peroxidation, which lowers biotransformation capabilities [29]. Therefore, pesticide intoxication can be viewed as a measure of toxicity stress due to the triochemical alterations as demonstrated by the enzymes ACP and ALP. It is useful as an index for keeping track of pesticide contamination.Numerous serum enzymes have been examined as suitable stress markers. Therefore, the diagnosis of various fish illness cases has often involved the application of several blood enzyme activities, such as AST and ALT.Additionally, the damage caused by environmental pollution in fish tissues has been discovered. Consequently, an increase in the activity of enzymes in the serum or extracellular fluid is thought to be a reasonable sign of mild cellular impairment, which is followed by tissue damage and stress [30]. In this investigation, the plasma ALT enzyme was elevated following subacute dimethoate administration. The cellular membrane appears to be affected by this increase that is linked to the liver, which appears to be the greatest. The presence of hazardous chemicals resulted in a decrease in cell membrane permeability, which in turn led to a buildup of enzymes in the hepatocytes. Alternatively, the permeability was increased, causing liver-to-blood enzyme leaching [31].

Since the effects of toxicants on hepatocytes resulted in liver necrosis and subsequent leaking of these cellular enzymes into the bloodstream, an increase in AST and ALT levels may generally be indicative of liver dysfunction and deterioration. Rahimikia's work [32] on goldfish (Carassius auratus) underexposure to nickel supports this. He found that the release of these transaminases into the bloodstream causes damage to the kidney, heart, and liver tissue under metal stress. Furthermore, he suggested using serum enzymes as indicators for environmental toxicity. Therefore, the primary cause of the enzyme activity in *C. gibelio's* serum is the enzymes' release into the bloodstream from the aqueous part of the liver cells' cytoplasm as a result of Zn metal ions-induced liver damage. The results of the current study are consistent with those of Naveed et al. [33] in *Channa punctatus*, who observed that increased exposure to heavy elements resulted to both aminotransferases' recommended activities being higher than they were before to transamination.

CONCLUSION AND RECOMMENDATIONS

The information indicates that dimethoate could potentially endanger *S. melanotheron's* life even at extremely low concentrations. The fish plasma's levels of enzymes were disturbed by the sub-lethal amounts of dimethoate. Enzyme perturbations may serve as early warning indicators for determining the harmful effects of pesticides in aquatic systems. The findings of this study could help legislators and pesticide manufacturers formulate more effective solutions for maintaining aquatic animals' environmental and ecophysiological health. These findings might also provide a solid grasp of the aquatic life's toxicological endpoint. To completely comprehend lindane's effects on fish and other creatures, as well as any potential ramifications for the ecosystem and public health, more research is required.

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