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ENZYMATIC RESPONSES IN *CLARIAS GARIEPINUS* JUVENILES EXPOSED TO ATRAZINE IN THE LABORATORY

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ABSTRACT
Degradation of aquatic environment due to herbicides pollution has become a menace in recent years. Biochemical parameter such as enzymes has become a valuable tool for assessment of pollution indices in fish. This study therefore assesses the enzymes response in juvenile of Clarias gariepinus exposed to Atrazine in the laboratory. One hundred and fifty (150) C.gariepinus of the same size range (mean length 11.74±2.64cm and mean weight 256.68±1.81g) were exposed to Atrazine in the laboratory. Ten fish were introduced into 15 aquaria tanks of 1.5m×1m×0.5 dimension, containing 0.00 (control), 0.05,0.10,0.5, and 0.20 of Atrazine for a period of 96 hours. Blood was extracted at 0hr, 24hrs, 48hrs, 72hrs and 96hrs, the collected blood samples were stored in heparinized bottles for enzymes analysis. The plasma was assayed for enzymes namely: aspartate transaminase (AST), alanine transaminase (ALT), acid phosphate (ACP), alkaline phosphates (ALP) and lactate dehydrogenase (LDH) in the exposed fish. Changes in enzymes response were concentration dependent, as it increased significantly ($p<0.05$) with increased concentration of Atrazine solution when compared to the control. The changes in enzyme activities observed in this study can be used as an indicator of the stress in C.gariepinius exposed to different levels of Atrazine solution. These changes if persistent can lead to death of fish and economic loss. There therefore the need for proper disposal of Atrazine when used as herbicides in agriculture in the field, prior to its discharge into aquatic environments so as to reduce eco-toxicological problems and health hazard to aquatic organisms.

***Keywords:** Enzymes, Clarias gariepinus, Contaminants, Eco-toxicology, Herbicides*

INTRODUCTION

Ecosystems on land and in water have been contaminated by the persistent and growing use of herbicides throughout the world to stop the spread of undesirable species in industrial plants or agricultural fields, which could jeopardize full production (both surface and ground waters). Such compounds, which lower water quality and have an effect on non-target species at all trophic levels, are especially harmful to aquatic systems and have the potential to upset the ecological balance of the surrounding areas [1]. Disturbances in the energy metabolism of organisms [2], impairment of neurotransmission, and oxidative stress are examples of how disruptions to the biochemical and metabolic processes of organisms can affect their regulatory mechanisms. The latter two are the toxicological mechanisms that are most frequently researched when organisms are exposed to pesticides. These factors become extremely important from a biological and ecological standpoint, especially in aquatic ecosystems where a variety of pollutants from multiple sources can build up and potentially cause oxidative stress in living things [3].

Many herbicides have been created over time to be used in various situations [4,5,6]. The majority of products consist of combinations of chemical compounds that serve as biologically active agents and distribute or bioaccumulate the active ingredient in conjunction with other compounds to create the desired result. Herbicides can be made to target different targets and have distinct action mechanisms by experimenting with different structures and features [7]. Triazine pesticides constitute a significant worry regarding their possible impact on non-target photosynthetic organisms. They are a type of pesticides that should be carefully considered, since they mostly comprise of herbicides that have the ability to selectively impede the electron transport during photosynthesis. Among the most widely used pesticides for weed control are herbicides based on atrazine, which works on multiple levels including oxidative stress induction, photosynthetic inhibition, mineral nutrition, and impairment of aromatic amino acid synthesis [8, 9, 10].

Many studies conducted over the past few decades have assessed the response of aquatic organisms to pesticide pollution by analyzing biomarkers [11]. Measurable biological measures known as biomarkers signal a change of any kind in a biological system under consideration [12]. Since herbicides were not intended to influence non-target species, it is highly possible that they will, but it is frequently unknown and difficult to forecast how these compounds will affect non-target species in terms of how they will respond to pollutants. A biochemical approach has been recommended to provide an early warning of potentially harmful changes in stressed fish [13]. Biochemical and physiological indicators, such as enzymes, could be used (as biomarkers) to identify possible environmental contaminations before the health of aquatic organisms is seriously affected and to develop water quality indices. Changes in the concentrations and activity of certain enzymes may indicate cell damage in particular organs in toxicological investigations after acute exposure. One of the main global environmental problems confronting humanity in the recent past is aquatic pollution. Numerous rivers in the nation are dealing with complex pollution issues as a result of industry and the unplanned urbanization that is common in the nation's largest cities [14]. Because of them, the ecosystem has been dangerously contaminated and degraded, especially the aquatic environment [15].

In a living thing, enzymes are crucial for metabolism and food utilization [16]. However, under the stress and effect of toxicants, this mechanism could change. This is due to the fact that organisms' cells contain enzymes that have several purposes [17]. On the other hand, enzymes escape into the bloodstream's plasma when toxicants interfere with the integrity of the cell from the outside, making it possible to evaluate the enzymes' activity as a helpful indicator of cell integrity. Aquatic species' response to pollution is manifested by a number of important biochemical processes involving enzymes related to the biotransformation system; these biomarkers serve as early warning indicators of aquatic contamination [17]. Worldwide, assessment of serum or plasma enzyme activity has been widely employed as an investigative tool in human medicine [18]. Furthermore, a thorough analysis of enzyme activity has been conducted to forecast the toxicity of chemicals, particularly pesticides, to aquatic creatures. Additionally, liver-specific transferases like alanine aminotransferase (ALT) and aspartate aminotransferase (AST) provide a quicker and more accurate assessment of liver and kidney impairment. Gabriel et al. [19] have found that changes in ALT and AST readings may indicate tissue injury in certain vital organs, including the kidney, liver, muscle, and gills. Similar to this, changes in the activities of phosphatases, such as acid phosphatase (ACP) and alkaline phosphatase (ALP), in tissues, organs, and plasma, have also been observed in fish exposed to toxicants at different concentrations. These phosphatases have been used as a good diagnostic tool in toxicological studies as well as a biomarker for tissue damage in fish [20].

A tissue's mild impairment can have a big impact. For example, mild cell inflammation will probably make the cell membrane more permeable, which will let cytoplasmic enzymes leak into the blood. On the other hand, cell necrosis will cause both mitochondrial and cytoplasmic enzymes to be found in the blood. There have been reports of changes in fish ALT (alanine transaminase), ALP (alkaline phosphatase), and AST (aspartate transaminase) activity as a result of toxicant or pollution effects in different fish organs [21]. Fish undergo these metabolic alterations in an effort to preserve balance in the face of these pollutants, which have the ability to interfere with physiological and biochemical functions. Long-term exposure of fish to most contaminants interferes with normal, functional metabolism at the cellular level, according to Das and Mukherjee [22]. A change in the fish's water homeostasis and/or a disruption in certain essential organs may be the cause of the decline in enzyme activity observed in fish exposed to toxicant levels.

Among the triazine herbicides are atrazine and simazine as well as promazine. It helps keep broadleaf weeds from emerging before and after they do in cultivated plants like corn (maize), sugarcane, etc. It is one of the most widely

used herbicides in Nigeria [23], and because of its mobility in soil, freshwater vertebrates are exposed to its hazardous effects, where it has been found in surface and ground waters [25]. Reduced plant condition, development, and reproduction as well as increased plant death are the most obvious effects of herbicide pollution. In addition to directly increasing fish mortality and altering fish behavior and reproduction, atrazine can have an indirect impact on catfish by changing their habitat and the availability of food. A lot of the information on the internet on how atrazine affects Clarias gariepinius enzyme activity is out of date and lacks specificity. Researchers like Aktar et al. [26], Lazartigues et al. [27], Nunes [28], Van and Pletschke [29], Gomes et al., [30], Gabriel et al., [31], and Nte et al., [32] are among those who have made a respectable effort. Nevertheless, within the restricted timeframe of 96 hours, no research has been conducted on the impact of atrazine on the enzyme activities of *Clarias gariepinus*. As a result, this research would help to provide light on the several ways that atrazine affects *Clarias gariepinus* enzyme activity.

MATERIALS AND METHOD

Experimental Location

The experiment was carried out at the Wet Laboratory in the Department of Fisheries and Aquaculture Management, Faculty of Agriculture, Nnamdi Azikiwe Unissversity, Awka, Anambra State, Nigeria.

Source of Experimental Fish

One Hundred and Fifty (150) *C. gariepinus* of equal size (mean length 11.74±2.64cm and mean weight 256.68±1.81g) were sourced from House Tully Fish Farms, Opunno, Awka, Anambra State, Nigeria. They were transferred in two 50 litre plastic tanks to the laboratory for acclimation process.

Acclimation and Feeding Of Fish

The experimental fish were acclimated in four 150L capacity circular plastic tanks containing 150L de-chlorinated water, for 7 days to experimental conditions at room temperature Netted materials with central slits was tied to the tops of the tanks to prevent escape of fish. Water renewal was done every two days. The fish were fed with a commercial feed at 5% body weight throughout this period.

Experimental Design

The experimental design was a completely randomized design (CRD) with four treatments levels and a control with each level having three replicates.

Procurement of Test Solution

A commonly used selective herbicide Vestrazine (Atrazine 100.0%) was purchased off shelf, from "Analytical" chemical shop, Eke-Awka Market, Awka, Anambra State, Nigeria.

Preparation of Test Solution

The solution of the chemical in water was prepared by serial dilution using the dilution formula of Grillo *et al* [33].

$N_1 V_1 = N_2 V_2$

Where N_1 = is the manufacture concentration of sodium bromide

V_1 = Volume of original solution added

N_2 = Concentration of the test solution desired

V_2 = Volume of test solution

Exposure of Fish to Atrazine

Ten *C. gariepinus* each were introduced individually into 15, aquaria tanks of 1.5m x 1m x 0.5m dimension, containing 0.00 (control), 0.05, 0.10, 0.15, and 0.20 of Atrazine. Each treatment(s) and control was replicated three times and the experimental duration lasted for a period of 96 Hours. The tank were covered with netted materials and supported with heavy objects to prevent the fish from escaping.

Evaluation of Physico-Chemical Parameters of Water

During the experiment, the following water quality parameters namely: Temperature, pH, Dissolved Oxygen, Nitrate and Ammonia levels of control and other treatment exposures were determined and the readings taken at 0, 24, 48, 72 and 96hr intervals in three replicates. Temperature was determined using the mercury-in-glass thermometer, which was inserted in water and the temperature (°C) reading was taken after four minutes.

pH was determined using a Jenway® type pH meter (Model 3015). The probe was first inserted in the buffer for 5 minutes to standardize the meter to pH 7, thereafter, it was dipped into the water and the static pH was read 60 seconds later. Dissolved oxygen was measured by Winkler's method described by APHA, [34]. Ammonia and nitrates were determined by automation using a multi-parameter photometer (Hanna instrument H183200).

Blood Sample Collection and Preservation

The blood was drawn from caudal vein known as *Vena cava* [35]. Fish were caught individually with a hand net. Blood samples were obtained with 5ml disposable syringes and 21-gauge hypodemic needle. During collection the head of each fish was covered with a piece of cloth for physical restriction with minimal stress [36]. The needle was inserted

perpendicularly into the vertical surface of the fish at a point slightly above the openings in the genital papilla. As the needle pierced the vein, blood flowed easily into the syringe and 3ml of blood was taken before the needle was withdrawn. The needle was then detached from the syringe and the 1.5ml blood was transferred into labelled heparinized bottles. The blood samples were analyzed at the Lively Stones Medical Laboratory, Rumukparali-Choba Road, Uniport, Choba, Port Harcourt.

Analytical procedure
Blood samples were frozen and were later defrozen and centrifuged for 15 minutes at 5000 rpm. Plasma specimens were separated, pipetted into eppendorf tubes and stored in a refrigerator at -20 °C until assayed [20]. The results were read using a universal microplate reader on a Jenway visible spectrophotometer (Model 6405).

Separation of plasma
The 8ml blood samples collected with heparin tubes were transferred into clean, dry centrifuge tubes and later centrifuged at 5000 rpm for 10 min at controlled temperature of 4 °C, to obtain plasma. Plasma was pipetted into Eppendorf tubes and later stored in refrigerator at -20 °C until analyzed [20]. All blood samples were analyzed in triplicates read using a universal microplate reader on a Jenway visible spectrophotometer (Model 6405).

Analysis of Enzymes in Juveniles of *C. gariepinus*
Commercial kits were purchased from Randox laboratories for the determination of the levels of the activities of the liver enzymes. These include Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Lactate dehydrogenase (LDH) and alkaline phosphatase (ALP).

Determination of Alkaline phosphatase (ALP)
The concentration of alkaline phosphatase in plasma was determined spectrophotometrically using RANDOX diagnostic kit on RX Monza analyzer made by Randox Laboratories limited, United Kingdom (model RX MONZA AP 542). This method was carried out according to [37]. Three cuvettes marked macro; semi micro and micro were arranged in a rack. 0.05 ml of plasma sample was pipetted into Macro cuvette, 0.02 ml sample was pipetted into semi-micro and 0.01 ml sample was pipetted into micro cuvette. 3.00 ml reagents were pipetted into macro cuvette, 1.00 ml of the reagents were pipetted into semi-micro cuvette and 0.50 ml of the reagents was pipetted into the micro cuvette. The solution was mixed and the initial absorbance was read at Hg 405 nm at a temperature of 37 ^{0}C. It was read again after 1, 2 and 3 minutes. (Timer was set to run simultaneously). ALP concentration was calculated using the following formula:

$$U/l = \frac{2760 \times \text{Absorbance 405 nm}}{\text{Minute}}$$

Determination of Aspartate Aminotransferase (AST)
The concentration of aspartate aminotransferase in plasma was determined spectrophotometrically using RANDOX diagnostic kit on RX Monza analyzer (Model RX MONZA AS 101). This method was carried out according to Reitman *et al*., [38] and Schmidt *et al*., [39]. AST was measured by monitoring the concentration of Oxaloacetate hydrazone formed with 2,4 dinitrophenylhydrazine. Two test tubes were labeled blank (B) and sample (S). In the reagent blank test tube was pipetted 0.5 ml of buffer (reagent 1) followed by 0.1 ml distilled water, while the sample test tube labeled (S) was pipetted 0.1 ml plasma sample and 0.5 ml of buffer. The mixture was incubated for exactly 30 minutes at 37 ^{0}C. Later 0.5 ml of 2,4-dinitrophenylhydrazine (reagent 2) was pipetted into the two test tubes, mixed and allowed to stand for exactly 20 minutes at 25 ^{0}C. Later, 5.0 ml of Sodium hydroxide was pipetted into the two test tubes. The solution was mixed and the absorbence of the sample was read at 546 nm against the reagent blank after 5 minutes.

Determination of Alkaline Aminotransferase (ALT)
Alanine amino transferase was measured spectrophotometrically using Randox diagnostic kit (Model AL100) according to the method of Reitman *et al*.[38] and Schmidt *et al.* [39]. This was done by monitoring the concentration of Pyruvate hydrazone formed with 2,4 dinitrophenylhydrazine. Two test tubes were labeled Reagent blank (B) and Sample (S). 0.5 ml of 100 mmol/l phosphate buffers and 0.5 ml of 200 mmol of L- alanine and 0.1 ml of distilled water was pipetted into reagent blank test tube. 0.1 ml of plasma sample, 0.5 ml of 100 mmol/l phosphate buffers and 0.5 ml of 200 mmol of L-alanine was pipetted into test tube (S). The solution in the three tubes was mixed, incubated for exactly 30 minutes at 37^{0}C. Later, 0.5 ml of 2.0 mmol/l of 2,4-dinitrophenylhydrazine was pipetted into reagent blank tube and sample test tubes. The solution was mixed, incubated for exactly 20 minutes at 25 ^{0}C. Lastly, 5.0 ml of Sodium hydroxide was pipetted into reagent test tube and sample test tube. The solution was mixed and the sample absorbance was read at 578 nm against the reagent after 5 minutes.

Determination of Acid Phosphatase (ACP)
Colorimetric method was used for the analysis, the reagent used were, citrate buffer (55mniol at pH of 4.8). p-nitrophenol phosphate (55mmol/L), sodium tartarate (200m1/L) and sodium hydroxide (200mlfL). The test tubes were labeled reagent blank, Sample 1 and sample 2 0.1ml of p-nitrophenol phosphate was dispensed into all the test tubes and 0.1ml of sodium tartarase was dispensed into sampled. These were incubated for 5 minutes at 39°C. At 30 seconds

interval, 0.lml of all the solution was added to sample 1 and sample 2. These were incubated for 30 minutes at 37°C before adding dilute sodium hydroxide (NaOH) to each of the test tubes and finally, 0.2m1 sample to the test tube. These were mixed thoroughly and the absorbance of the sample against the reagent blank was done at wavelength of 405nm.

Determination of Lactate Dehydrogenase (LDH)
The analysis of LDH was done by method prescribed by Huckabec [40]. Homegenate (50mg/ml) was prepared in cold TLA and centrifuge at 100mg for 5 minutes. After the centrifugation, sodium lactate was then added and the lactate content were then read and expressed as rig/mg tissues.

Statistical Analysis
Date obtained from the experiments were collated and subjected to ANOVA using Statistical Package for the social Sciences, (SPSS) version 22, differences among means were separated by Turkeys Comparative Test at 0.05%.

RESULTS
The effect of Atrazine on the Physico-Chemical Parameter of Water in the Experimental Tanks
Table 1 shows the results for the physiochemical parameters of water in tanks of *C.gariepinus* exposed to different concentration of vestrazine (0.00, 0.05, 0.10, 0.15, and 0.20mg/l) respectively for 96hrs. The results indicates significant reduction ($P<0.05$) in the values of dissolved oxygen from 6.67±0.25 in the control to 4.03±0.99 at 0.20mg/l concentration of the chemical. Also, significant ($P<0.05$) increase with increasing concentration of the chemical were however recorded in the values of nitrite and ammonia. While other parameters such as temperature and pH were within the same range comparable to the control in all concentrations of the chemical.

Changes in Enzymes Levels in the Plasma of *C. gariepinus* Exposed to Different Concentration of Atrazine for 96hrs
The enzymes in the plasma of *C. gariepinus* exposed to acute concentrations of Atrazine for 0 hours are presented in Table 2. Generally the values of the enzymes (AST, ALT, ALP, ACP, LDH) in the plasma of the exposed *C. gariepinus* were within the same range with no significant differences in all concentration. At 24hours of exposure (Table 3), slight increase was observed in the AST, ALT, and ALP, while there was significant increase in the value of LDH. However the values of ACP were within the same range with no significant difference ($p>0.05$) in all concentration. At 48hours of exposure of *C.gariepinius* to varying concentrations of Atrazine (Table .4) there was significant increase in AST, ALT, ALP, and LDH while the values of ACP was within the same range. At 72 and 96hours (Table 5 and 6), there was significant increase in the values of AST, ALP, ALP, ACP, LDH, with increasing concentrations.

Comparative Value of Concentrations of Atrazine for 96 Hours
Comparative values of Aspartate transaminase (AST) into the plasma of *C.gariepinus* exposed to Atrazine for 96hours is shown in Figure 1. The values of Aspartate transaminase increased as the experimental period increased with the value of (21.37 ± 5.09) observed at the control, and (29.12 ± 7.89) in 0.20mg/L at 24hours. Comparatively, the value of alanine transaminase (ALT) as shown in (Figure 2) indicated that the values of alanine transaminase in *C.gariepinus* exposed to varying concentrations of Atrazine were elevated progressively as the experimental period increased and peaked at 96hours for all concentrations. The highest value of (75.33 ± 9.65) was recorded in the fish exposed to 0.20mg/L of the chemical at 96hour, while the lowest value of (47.50 ± 7.88) was observed at the control (Figure 2). The values of alkaline phosphate (ALP) (Figure 3) increased considerably as the experimental period increased; this was more pronounced at the concentration of 0.10, 015, and 0.20mg/L concentration of the chemical. The values of acid phosphate (ACP) (Figure 4) were within the same range with no much significant difference in all concentrations of exposure with the value of (10.25 ± 1.65) observed at the control, and (10.99 ± 0.83) in 0.20mg/L at 48hours. The value of lactate dehydrogenase (LDH) is shown in (Figure 5) the value increase significantly as the experimental period progressed from 24 to 96hours.However the LDH records its highest value in the concentration of 0.20mg/L at 96hours with a value of (477.02 ± 39.45).

Table 1: Physicochemical Parameters of Water in Tanks of *C. gariepinus* exposed to acute concentrations of Atrazine for 96 Hours

Concentrations (mg/L)	Physico- Chemical Parameters of Water				
	Temperature	**pH**	**DO**	**Nitrite**	**Ammonia**
0.00	28.33±0.77[a]	6.53±0.06[a]	6.67±0.25[a]	0.00±0.00[a]	0.09±0.02[a]
0.05	28.34±0.40[a]	6.63±0.06[a]	6.17±0.21[a]	0.05±0.00[b]	0.24±0.06[b]
0.10	28.30±0.92[a]	6.70±0.10[a]	5.03±0.51[b]	0.05±0.00[b]	0.31±0.01[c]
0.15	28.29±0.51[a]	6.77±0.06[a]	5.00±0.78[b]	0.07±0.00[c]	0.32±0.05[c]
0.20	28.45±0.99[a]	6.80±0.10[a]	4.03±0.99[c]	0.07±0.00[c]	0.36±0.017[c]

Means within the same column with different superscript are significantly different ($P<0.05$)

Table .2: Enzymes in the Plasma of *C. gariepinus* Exposed to Atrazine for 0 Hours (Mean ± S.D)

	Enzymes (IU/L)				
Conc. (mg/l)	**AST**	**ALT**	**ALP**	**ACP**	**LDH**
0.00	21.35 ± 4.34[a]	46.23 ± 2.20 [a]	12.00 ± 0.88 [a]	10.21 ± 0.02 [a]	311.14 ± 21.11 [a]
0.05	21.26 ± 0.77[a]	46.12 ± 2.12 [a]	12.22 ± 0.02 [a]	10.42 ± 0.02 [a]	312.41 ± 12.78 [a]
0.10	21.37 ± 0.39 [a]	45.89 ± 2.88 [a]	12.11 ± 0.19 [a]	10.61 ± 0.05 [a]	311.78 ± 15.09 [a]
0.15	22.04 ± 0.77 [a]	46.12 ± 6.12 [a]	12.12 ± 0.11 [a]	10.73 ± 0.04 [a]	312.97 ± 16.99 [a]
0.20	22.09 ± 0.54 [a]	46.02 ± 3.66 [a]	12.82 ± 0.77 [a]	10.88 ± 0.02 [a]	311.98 ± 12.99 [a]

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

Table 3: Enzymes in the Plasma of *C. gariepinus* Exposed to Atrazine for 24 Hours (Mean ± S.D)

	Enzymes (IU/L)				
Conc. (mg/l)	**AST**	**ALT**	**ALP**	**ACP**	**LDH**
0.00	21.37 ± 5.09[a]	46.25 ± 4.77 [a]	12.02 ± 0.99 [a]	10.24 ± 1.89 [a]	315.88 ± 20.80 [a]
0.05	22.05 ± 2.67[a]	48.99 ± 5.90 [a]	12.89 ± 2.77 [a]	10.77 ± 1.74 [b]	336.88 ± 24.78 [a]
0.10	24.89 ± 4.77 [b]	51.00 ± 2.90 [b]	13.08 ± 0.88 [b]	10.83 ± 1.73 [b]	349.65 ± 23.72 [a]
0.15	26.77 ± 5,99 [b]	53.77 ± 4.44 [b]	13.52 ± 2.04 [b]	10.89 ± 0.84 [a]	382.04 ± 17.54 [a]
0.20	29.12 ± 7.89 [b]	58.12 ± 7.08 [b]	13.99 ± 3.82 [b]	10.98 ± 0.77 [a]	390.52 ± 15.88 [b]

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

Table 4: Enzymes in the Plasma of *C. gariepinus* Exposed to Atrazine for 48 Hours (Mean ± S.D)

	Enzymes (IU/L)				
Conc. (mg/l)	**AST**	**ALT**	**ALP**	**ACP**	**LDH**
0.00	21.39 ± 3.04[a]	46.98 ± 7.05 [a]	12.14 ± 3.04 [a]	10.25 ± 1.65 [a]	316.09 ± 21.07 [a]
0.05	23.99 ± 2.83[a]	49.85 ± 9.04 [a]	13.96 ± 2.83 [b]	10.88 ± 1.55 [b]	340.09 ± 27.03 [b]
0.10	27.56 ± 5.05 [b]	58.88 ± 4.53 [b]	14.03 ± 3.77 [c]	10.95 ± 1.82 [b]	368.77 ± 25.04 [b]
0.15	29.04 ± 7,04 [b]	63.05 ± 5.90 [c]	14.65 ± 2.88 [c]	10.97 ± 0.31 [a]	390.88 ± 34.66 [b]
0.20	34.99 ± 8.03 [c]	68.05 ± 9.44 [c]	15.08 ± 5.90 [d]	10.99 ± 0.83 [a]	400.06 ± 28.05 [c]

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

Table 5: Enzymes in the Plasma of *C. gariepinus* Exposed to Atrazine for 72 Hours (Mean ± S.D)

	Enzymes (IU/L)				
Conc. (mg/l)	**AST**	**ALT**	**ALP**	**ACP**	**LDH**
0.00	21.40 ± 3.99[a]	47.00 ± 7.12 [a]	12.18 ± 5.77 [a]	10.29 ± 1.77 [a]	317.58 ± 29.54 [a]
0.05	26.03 ± 2.71[a]	52.07 ± 8.68 [a]	14.04 ± 2.77 [b]	10.98 ± 1.04 [b]	351.11 ± 25.67 [b]
0.10	30.99± 7.12 [b]	59.66 ± 7.98 [b]	14.88 ± 5.03 [c]	11.05 ± 1.71[b]	379.05 ± 27.12 [b]
0.15	34.67 ± 9.62[b]	68.18 ± 5.01 [c]	15.05 ± 2.09 [c]	11.37 ± 0.89 [a]	399.02 ± 35.91 [b]
0.20	39.09 ± 6.11 [b]	69.99 ± 9.03 [c]	15.99 ± 9.05 [d]	11.99 ± 1.62 [a]	418.77 ± 30.17 [c]

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

sTable 6: Enzymes in the Plasma of *C. gariepinus* Exposed to Atrazine for 96 Hours (Mean ± S.D)

	Enzymes (IU/L)				
Conc. (mg/l)	**AST**	**ALT**	**ALP**	**ACP**	**LDH**
0.00	21.45 ± 3.07[a]	47.50 ± 7.88 [a]	12.45 ± 8.05 [a]	10.32 ± 1.05 [a]	318.04 ± 31.08 [a]
0.05	29.99 ± 2.88[a]	55.88 ± 8.90 [b]	14.83 ± 2.04 [b]	11.08 ± 3.06 [b]	368.99 ± 29.88 [b]
0.10	36.02± 7.05 [b]	63.88 ± 9.05 [b]	14.94 ± 7.44 [b]	11.66 ± 1.75[b]	385.11 ± 29.80 [b]
0.15	40.12 ± 9.05[b]	72.66 ± 7.77 [c]	15.89 ± 2.78 [c]	11.72 ± 0.94 [b]	423.78 ± 45.00 [c]
0.20	49.55 ± 9.03 [b]	75.33 ± 9.65 [c]	16.05 ± 9.66 [d]	12.05 ± 1.88 [c]	477.02 ± 39.45 [c]

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

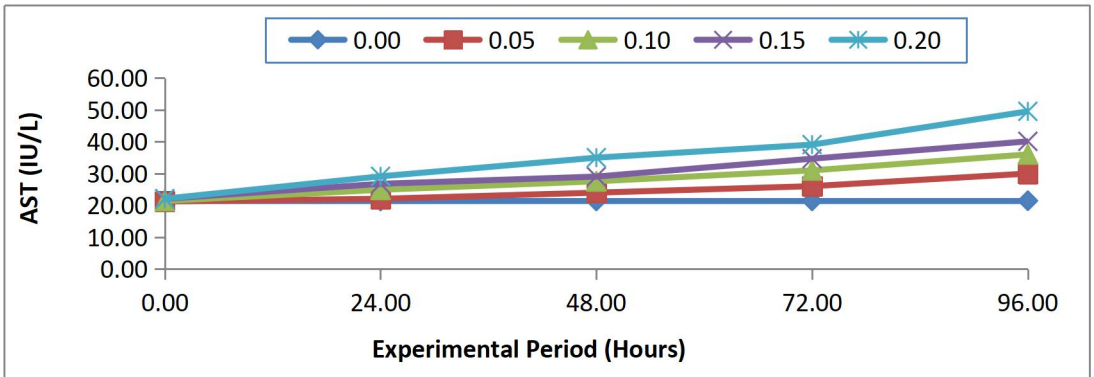

Figure 1: Changes in Aspartase Transaminase (AST) in the plasma of *C.gariepinus* exposed to Atrazine for 96 Hours

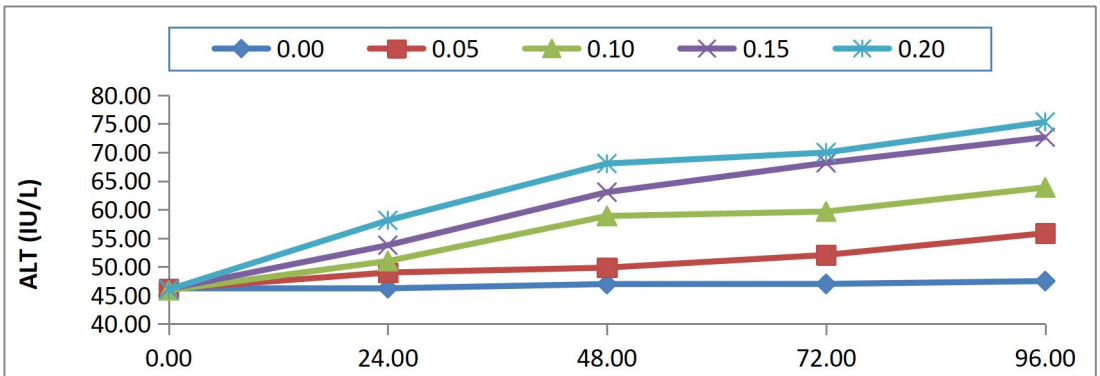

Figure 2: Changes in Alanine Transaminase (ALT) in the plasma of *C.gariepinus* exposed to Atrazine for 96 Hours

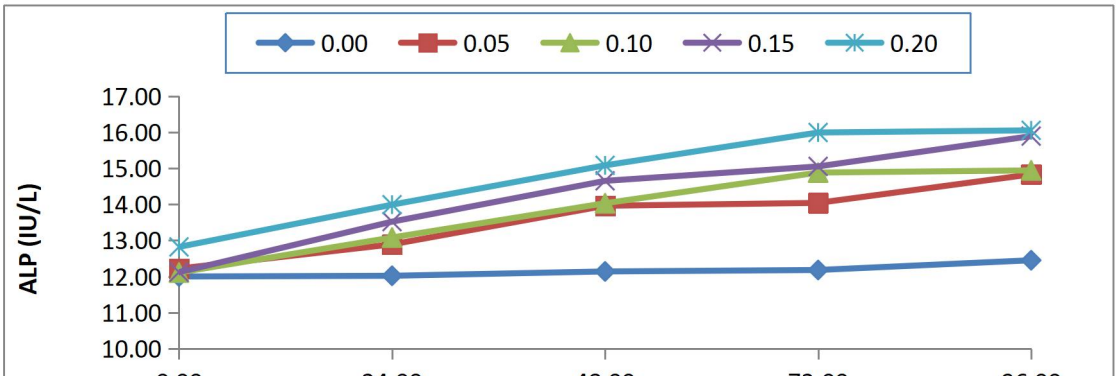

Figure 3: Changes in Alkaline Phosphatase (ALP) in the plasma of *C.gariepinus* exposed to Atrazine for 96 Hours

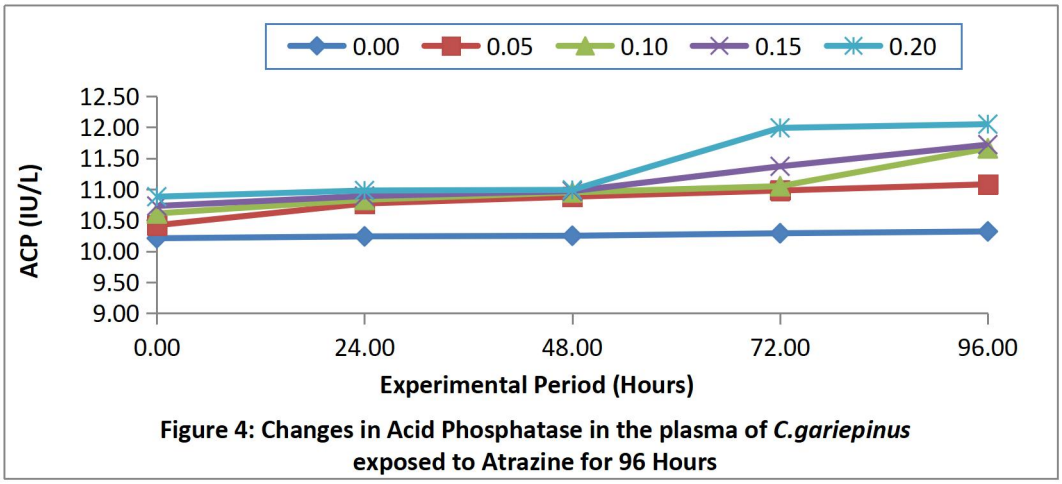

Figure 4: Changes in Acid Phosphatase in the plasma of *C.gariepinus* exposed to Atrazine for 96 Hours

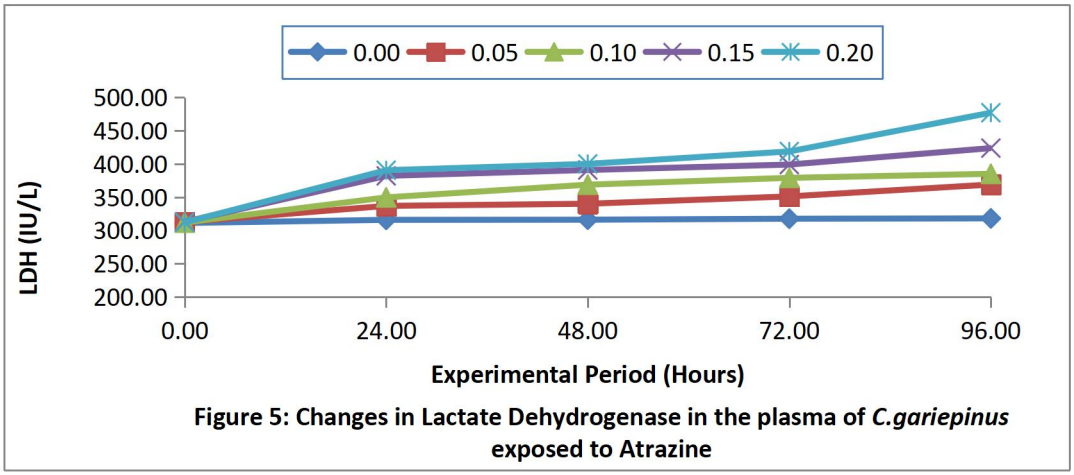

Figure 5: Changes in Lactate Dehydrogenase in the plasma of *C.gariepinus* exposed to Atrazine

DISCUSSION

Critical water quality parameters that affect fish health, growth, and reproduction in the aquatic medium include temperature, dissolved oxygen, pH, ammonia, and nitrate, all of which were assessed in this study. All the parameter values fell within the same range, with the exception of the dissolved oxygen value, which decreased as the chemical concentration increased. Enzyme assays including ALP, AST, ALT, and ACP are used in this study as standard laboratory analysis tests to find abnormalities in the physiological status of aquatic animals. Several writers have documented changes in any of these enzymes as a result of contaminants in fish plasma [41, 42, 43]. Fish undergo these changes in an effort to preserve balance in the face of these toxins, which are known to interfere with physiological and metabolic functions. Additionally, ACP and ALP activity are useful markers of toxicant stress in fish. Furthermore, any harm or malfunction in test organs is a sign of a change in phosphatase activity [41]. The decreased activity of these enzymes may result from an increased need for protein to offset the higher energy expenditure of detoxification, tissue repair, and homeostasis in times of stress [42]. ACP's involvement in cellular activity and damage, such as autolytic breakdown or cellular necrosis, which results in insufficient enzyme synthesis or leakage of enzyme into the extracellular compartment as a result of pesticide treatment, is correlated with an increase in ACP activity [43]. Phosphatase activity may rise or decrease in response to necrotizing alterations in various organs. The factors that affect the rate at which an enzyme leaves the cell and enters the circulation, the rate at which a certain cell type produces an enzyme, or the proliferation of a specific type of cell that produces an enzyme, can all contribute to the changing activities of the enzymes. It is widely acknowledged that changes in the extracellular fluid's or plasma's enzyme activity can serve as sensitive indicators of mild cellular injury [43].

In this investigation, the activity of these enzymes rose in proportion to the rise in atrazine levels in the fish plasma that were exposed. This outcome is consistent with Das et al.'s research [44] on *Labeo rohita* exposed to cypermethrin in a lab setting. They proposed that the diversion of alpha-amino acids in the tricarboxylic acid cycle (TCA) as keto acids to enhance energy generation in the fish cell is the cause of the increase in transferases. Furthermore, a rise in ACP and ALP seen in the plasma of *Sarotherodon melanotheron* exposed to industrial effluents has been linked to fish cellular toxicity [45]. The fish in this study had higher levels of AST activity in their plasma, most likely to help them meet their energy needs under stressful conditions. Similar results imply that amino acids may be able to provide this energy need. ALP activity [46]. ALP and ACP activity rose in this study after *Clarias gariepinus* was exposed to atrazine; an increase in these enzymes is a sign that the fish's essential organs have been compromised. After *C. gariepinus* was exposed to cypermethrin in a lab setting, Gabriel et al.'s findings [47] corroborated this theory. ALTP activity is known to occur in the cell membrane and may be implicated in metabolic processes. It represents a change in the mass of the endoplasmic reticulum. This rise could indicate an increase in metabolic transport, which could ultimately cause the

exposed organism's biosynthesis and energy metabolism route to change [48]. However, an increase in lysosomal mobilization and cell necrosis as a result of effluent toxicity is suggested by an elevation in ACP, as seen in this work. This study's reduced ALP activity demonstrated that oxidative deamination and inactive transamination had taken place.

Hepatic enzyme activity levels were significantly elevated ($P < 0.05$) by atrazine toxicity. The results of the current investigation showed that C. gariepinus blood plasma treated with various dosages of atrazine had increased levels of AST and ALT. After 96 hours, the high amount of atrazine exposure was associated with an increase in serum AST and ALT activity when compared to the control. Numerous plasma enzymes have been examined as suitable stress markers. As a result, the activities of numerous serum enzymes, such as AST and ALT, have been widely used in the diagnosis of some fish diseases as well as the identification of impairment in fish tissues brought on by environmental contamination. Consequently, an increase in the activity of enzymes in the extracellular fluid, or plasma, is thought to be a reasonable signal of mild cellular impairment, which leads to tissue damage and stress [49]. As a result of sub-acute exposure to atrazine, the plasma ALT enzyme was elevated in this investigation. 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 compounds resulted in a decrease in cell membrane permeability, which in turn led to an accumulation of enzymes in the hepatocytes, or an increase in permeability that allowed liver enzymes to escape into the bloodstream [50, 51].

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 [52] study on goldfish (Carassius auratus) underexposure to nickel provides support for this theory. He found that the release of these transaminases into the bloodstream under metal stress leads to deterioration of the hepatic tissue, heart, and kidney. Furthermore, he suggested using serum enzymes as indicators for environmental toxicity. Thus, the primary cause of these enzymes' activity in C. gingipinus plasma is the liver damage caused by atrazine concentrations, which releases these enzymes into the bloodstream from the aqueous part of the cytoplasm of the liver cells. The results of the current study are consistent with those of Naveed et al. [53] 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. Furthermore, supported by earlier research by Li et al. [54], who examined freshwater rainbow trout (Oncorhynchus mykiss) exposed to toxic fungal pesticides, it was demonstrated that elevated plasma AST and ALT activity and that this increase in enzyme activity could be caused by abnormalities in the physiological and anatomical features of tissues. Additionally, studies by Qiu et al. [55] demonstrated that increased ALT activity in the blood indicated hepatocyte membrane deterioration, while elevated AST activities in bighead carp (Aristichthys nobilis) and silver carp (Hypophthalmichthys molitrix) were linked to mitochondrial disruption brought on by severe hepatitis.

Anaerobic and cytoplasmic, LDH is an enzyme that helps produce glucose by converting pyruvate to lactate. It is typically connected to a metabolic activity within cells. It is an essential enzyme in the tricarboxylic acid cycle and glycolytic pathway. When engaging in intense muscle exercise, the enzyme exhibits increased activity [56]. One of the greatest producers of LDH is the liver, and even a tiny amount of injured liver tissue can release an enzyme that significantly raises the level that is seen. The breakdown of liver cells and increased cell permeability, which causes the enzymes from the damaged liver cells to escape into the serum, were the reasons given for the increase in enzyme activity following exposure to some pollutants [57].
In the current investigation, longer exposure times and higher atrazine concentrations were associated with higher blood LDH levels. A higher concentration of atrazine puts the gills under stress and damages their structure, which lowers their ability to take in the oxygen needed for aerobic respiration. As a result, reduced oxygen levels in tissues carry out anaerobic activity to meet energy needs, which are met by the exposed fish's plasma's elevated level of LDH. Oreochromis mosambicus subjected to various Zn and Hg concentrations showed similar results[58]. On the other hand, Saha et al. [59] also observed that a stressed-out fish (O. massambicus) exposed to phenol might constantly move its opercula when it is in need of oxygen. This could be caused by increased LDH activity, which in turn triggers anaerobic oxidation to generate energy. Oxygen stress induces the production of LDH, an essential glycolytic enzyme in biological systems [60]. Amin et al. [61] reported that $K_2Cr_2O_7$ markedly increased serum LDH activity, which is thought to be a potential indicator of necrotic lesions. As a result of cell membrane disintegration and enzyme leakage, cell necrosis increases the concentration of the LDH enzyme in serum and tissue, serves as a marker of cell death, and increases the activity of the enzyme in the blood.

An alternate aerobic glycolytic pathway that converts lactate to pyruvate for the synthesis of glucose—a primary source of energy during stress generated by heavy metals—is the cause of the elevated level of lactate dehydrogenase [62]. Thus, another sensitive indicator for determining the toxicity of heavy metals is the fluctuation in lactate dehydrogenase activity. Increased liver dehydrogenase (LDH) and serum activity were observed by Oluah et al. [63] in C. punctactus subjected to escalating dosages of sub-lethal Gammalin 20 and Acetellic 25 EC. Several body cells have cytoplasms that contain LDH. On the other hand, a rise in the circulation may indicate harm to the muscles or liver [64]. By keeping an eye on the value of LDH, the degree of cellular damage caused by atrazine toxicity was determined [65]. It follows that an increase in serum LDH activity corresponds with a greater manifestation of Cr's degenerative effects. Elevation

of LDH activity in the serum of rats treated with K2Cr2O7 [66]. One indicator of tissue damage is enhanced lactate dehydrogenase (LDH) activity, which is elevated in liver necrosis.

CONCLUSION AND RECOMMENDATIONS

The physiological makeup of the fish underwent notable alterations as a result of the exposure of Clarias gariepinius to sublethal amounts of atrazine, as demonstrated by changes in the study's enzyme activity. Clarias gariepinius may die with continuous exposure to herbicide pollution in aquatic habitats, which can also cause financial loss. Therefore, proper pesticide disposal is necessary to avoid ecological issues in the aquatic medium. Additionally, it's important to routinely check the aquatic environment to stop toxins from bioaccumulating in fish systems. The study's conclusion was that fish underwent stress following exposure to various atrazine concentrations. The chemical caused cellular damage in fish, which changed the activity of the enzyme LDH substantially. Thus, the outcome suggests that it will be a useful instrument for aquatic pollution biological monitoring.

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