CHANGES IN SOME METABOLITES IN THE PLASMA OF *Clarias gariepinus* EXPOSED TO ATRAZINE

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Abstract

Atrazine, a commonly used herbicide, has been implicated in adverse effects on aquatic organisms. This study aimed to investigate the changes in specific metabolites in the plasma of Clarias gariepinus exposed to atrazine. A total of 150 juvenile fish were randomly assigned in a completely randomized design (CRD) with four treatment levels and a control, each with three replicates. The results indicated a significant reduction ($p<0.05$) in the values of dissolved oxygen from 6.67±0.25 in the control to 4.03±0.88 at 0.20mg/l concentration of the chemical. Also, significant ($p<0.05$) increase with increasing concentrations of the chemical were however recorded in the values of nitrites 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. At zero hour (before the commencement of the experiment) the values of all the metabolites (Urea, Creatinine, Total bilurubin, Albumin and Total protein) in the plasma of the exposed C. gariepinus were within the same range with no significant differences in all concentrations. At 24 hours of exposure, slight reductions were observed in the values Total bilurubin, creatinine and Total protein, while the values of urea were slightly elevated. However, the values of albumin were within the same range with no significant difference ($p>0.05$) in all concentrations. At 48. 72, and 96 hours of exposure of C.gariepinus to varying concentrations of Atrazine, there was significant reduction ($p<0.05$) in the values of Total bilurubin, creatinine, albumin and total protein. While the values of Urea increased significantly ($p<0.05$) with increasing concentrations of the chemical. The findings of this study highlight disruptions in amino acid metabolism, lipid homeostasis, carbohydrate metabolism, and oxidative stress response. This study contributes to a better understanding of the metabolic consequences of atrazine exposure on aquatic organisms. Further investigations are needed to elucidate the underlying mechanisms and potential long-term effects on fish health and ecosystem integrity, emphasizing the importance of proactive measures in herbicide management and environmental conservation.

Keywords: *Chemical, Metabolites, Aquatic pollution, Contaminants, Catfish*

INTRODUCTION

Chemical contamination of the aquatic environment is a result of the growing use of herbicides and pesticides in agriculture (including the production of vegetables for home and commercial use) to control pests and weeds. Over the past few decades, freshwater contamination with a wide range of pollutants has become a serious concern [1]. The potential risks connected with freshwater contamination are becoming more widely recognized [2]. Toxic substances linked to mining, industrial, and agricultural activities are particularly of concern. The uncontrolled use of pesticides poses a threat to biodiversity and the genetic base of the aquatic ecosystem [3]. Domestic and wild animals are exposed to possible health risks due to chemical contamination, particularly fish, amphibians, arthropods, birds, and even mammals. The organisms' age, concentrations, and exposure route all affect the toxicity level. The organisms in aquatic ecosystems are exposed to environmental stresses brought on by the various contaminants that enter them. These pressures may be harmful to individual organism, a population, or a community, and ultimately affect the structure of natural ecosystems [4]. Herbicide use in agricultural fields and urban settings has expanded in modern agriculture [5].

Pollutants entered into the environment by humans and natural processes ultimately end up in aquatic systems. Worldwide, the number of aquatic species has decreased due to the release of hazardous compounds. Fish populations may suffer from toxicant concentrations that are much less deadly [6]. One of the primary causes of the rising levels of pollutants in aquatic ecosystems is the increase in human activity. In many developing nations, environmental concerns have been made worse by the sharp rise in coastal pollution in recent years. Aquatic habitats have become contaminated as a result of the discharge of waters from cities, public areas, and industries [7]. It is well recognized that a variety of pollutants from industrial, municipal, and agricultural sources can affect an animal's physiological and haematological processes [8]. Certain chemicals, like pesticides, are expressly made to interfere with an organism's normal endocrine function; nevertheless, most of the time, these chemicals unintentionally affect non-target creatures, like fish and other aquatic animals, as a byproduct of other processes [9].Pollutants are introduced to fish through the water and through their eating habits. They get into their bodies by the gills, the skin, or even the mother in utero [10]. Pollutants' tendency to accumulate in animal body tissues due to their lipid solubility is one of their most pernicious traits.

At certain concentrations, the majority of herbicides tend to jeopardize creatures that are not their target. This usually happens when runoff from rainfall causes soil erosion, which allows pesticides to enter the aquatic ecosystem [11]. Herbicide runoff into natural water bodies from agricultural fields has become a global occurrence. Herbicides may disintegrate in regular runoff and floods, moving them into freshwater and saltwater. Different pathways may be used by aquatic fish to absorb pesticide from agricultural regions. The long-term effects of these sub-lethal concentrations of herbicides, even at extremely low concentrations, can be fatal to organisms by changing their behavior, feeding habits, rates of reproduction, school group dynamics, and in extreme situations, even cause death [12]. Aquatic species' bodily tissues collect herbicides found in the environment, and these species eventually incorporate these herbicides into the food chain [13]. Organs such the liver, kidney, gills, brain, muscles, and sexual organs are destroyed as a result of lingering effects, and they are also prevented from carrying out their biochemical duties as best they can [14]. An estimated three million individuals worldwide are poisoned by pesticides every year, and 200,000 of them pass away from it; most of them are from developing nations [15]. It is also thought that underreporting, a lack of data, and incorrect diagnosis could make pesticide poisoning more common in developing nations than is currently recognized. Many current pesticides are designed to be as selective as possible against the creatures they are intended to control, yet it is rare to achieve absolute control over one organism without exposing the surrounding environment and affecting sensitive non-target species [16]. Fish growth can be directly impacted by toxic compounds in the aquatic environment by altering their metabolic processes, or indirectly by limiting the availability of food [17]. Because of their vulnerability to water pollutants, fish are among the creatures employed in eco-toxicological investigations. Because aquatic ecosystems are so diverse, it is challenging to determine the degree of toxicity that herbicides pose to aquatic animals. Toxicological techniques are therefore thought to be a suitable means of assessing the effects of toxicants on aquatic populations. The histopathological and biochemical effects of paraquat poisoning on C. gariepinus have been examined by Ayoola [18] and Omitoyin et al. [19]. The histological alterations in the liver and gills of C. gariepinus following exposure to paraquat were also examined by Doherty et al. [20]. Nevertheless, given the widespread usage of these herbicides, it is critical to consider other negative impacts they may have on fish. This is the context in which the current investigation was conducted. This study aims to use some of the biomarkers used to identify stressed situations in fish to examine the acute toxicity effects of atrazine in catfish, Clarias gariepinus. The purpose of this study is to evaluate the herbicide atrazine's toxicity on Clarias gariepinus metabolites.

Material and Methods

Experimental Location

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

Source of Experimental Fish

One Hundred and Fifty (150) *Clarias 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-Akwa Market, Akwa, Anambra State, Nigeria.

Preparation of Test Solution
The solution of the chemical in water was prepared by serial dilution using the dilution formula of Grim Shaw (1978)
$N_1 V_1 = N_2 V_2$
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 were 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, [21]. Ammonia and nitrates were determined by automation using a multi-parameter photometer (Hanna instrument H183200).

Blood Sample Collection and Preservation
After the syringe's needle was removed, 1.5 ml of blood was put into bottles that had been labeled with heparin. At the Lively Stones Medical Laboratory, located at Rumukparali-Choba Road, Uniport, Choba, Port Harcourt, the blood samples were examined.

Analytical procedure
A caudal vein called the Vena cava was used to extract the blood. Using a hand net, fish were captured one by one. 5 ml disposable syringes and a 21-gauge hypodermic needle were used to draw blood samples. Each fish was physically restrained with as little stress as possible during collection by having its head covered with a piece of fabric [36]. A position just above the vaginal papilla apertures was where the needle was put perpendicularly into the fish's vertical surface. Three millilitres of blood were obtained before the needle was removed as the vein was easily punctured. After being frozen, blood samples were defrosted and centrifuged at 5000 rpm for 15 minutes. Before being tested, plasma specimens were pipetted into eppendorf tubes, divided, and kept in a refrigerator at -20 °C [20]. A Jenway visible spectrophotometer (Model 6405) with a universal microplate reader was used to read the data.

Separation of plasma
The 2ml 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 analysed [22]. All blood samples were analyzed in triplicates read using a universal microplate reader on a Jenway visible spectrophotometer (Model 6405). Then the resultant supernatant was transferred into sample bottles for analysis.

Determination of Metabolites
Fish exposed to atrazine had some of their plasma's metabolites examined. Multiple analytical techniques were used in the metabolites study. Lipids were analysed using the methodology previously published by Sujatha *et al.* [23],

Cheesborough [24], and Amunanddson and Zhou [25] for estimating total bilurubin, albumin, and urea concentration. The Lowry *et al.* technique [26] was used to estimate the concentrations of creatinine and total protein.

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

Physico-chemical Parameters of Water in the Experimental Tanks

The results of the physico-chemical properties of the water in exposure tanks in *C.gariepinus* exposed to Atrazine are presented in Table 1. The results indicated a significant reduction ($p<0.05$) in the values of dissolve oxygen from 6.67±0.25 in the control to 4.03±0.88 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 y were within the same range comparable to the control in all concentrations of the chemical.

Changes in Metabolite Levels *in* the Plasma of *C. gariepinus* Exposed to Different Concentrations of Atrazine for 96 hours.

The metabolites in the plasma of *C. gariepinus* exposed to acute concentrations of Atrazine for 0 Hours are presented in Table 2. Generally, the values of all the metabolites (Urea, Creatinine, Total bilurubin, Albumin and Total protein) in the plasma of the exposed C. *gariepinus* were within the same range with no significant differences in all concentrations. At 24 hours of exposure (Table 3), slight reductions were observed in the values Total bilurubin, creatinine and Total protein, while the values of urea were slightly elevated. However, the values of albumin were within the same range with no significant difference ($p>0.05$) in all concentrations. At 48. 72, and 96 hours of exposure of *C.gariepinus* to varying concentrations of Atrazine (Table 4, 5, and 6), there was significant reduction in the values of Total bilurubin, creatinine, albumin and total protein. While the values of Urea increased significantly with increasing concentrations of the chemical.

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: Metabolites in the Plasma of *C. gariepinus* exposed to acute concentrations of Atrazine for 0 Hours

Concentrations (mg/L)	Metabolites (mmol/l)				
	Total Bilurubin	**Urea**	**Creatinine**	**Albumin**	**Total Protein**
0.00	10.22 ± 1.27^{a}	6.19 ± 2.61^{a}	156.61 ± 10.21^{d}	3.81 ± 0.02^{a}	10.69 ± 0.81^{a}
0.05	10.11 ± 1.01^{a}	6.18 ± 1.18^{a}	155.71 ± 11.61^{a}	3.82 ± 0.03^{a}	10.71 ± 0.11^{a}
0.10	10.18 ± 0.81^{a}	6.21 ± 2.61^{a}	155.61 ± 11.12^{a}	3.81 ± 0.01^{a}	10.76 ± 0.44^{a}
0.15	10.14 ± 0.21^{a}	6.19 ± 2.42^{a}	155.21 ± 9.81^{a}	3.80 ± 0.04^{a}	10.68 ± 0.31^{a}
0.20	10.21 ± 0.26^{a}	6.21 ± 1.11^{a}	155.21 ± 2.11^{a}	3.83 ± 0.04^{a}	10.64 ± 0.14^{a}

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

Table 3: Metabolites in the Plasma of *C. gariepinus* exposed to acute concentrations of Atrazine for 24 Hours

Concentrations (mg/L)	Metabolites (mmol/l)				
	Total Bilurubin	**Urea**	**Creatinine**	**Albumin**	**Total Protein**
0.00	10.23 ± 1.15^{a}	6.18 ± 2.05^{a}	156.61 ± 10.77^{a}	3.83 ± 0.02^{a}	10.68 ± 0.99^{a}
0.05	10.10 ± 1.77^{a}	9.99 ± 1.74^{a}	152.43 ± 11.82^{a}	3.55 ± 0.03^{a}	9.98 ± 0.11^{b}
0.10	9.73 ± 0.99^{b}	11.87 ± 2.02^{b}	153.43 ± 11.32^{b}	3.35 ± 0.01^{a}	9.06 ± 0.32^{b}
0.15	9.03 ± 0.81^{b}	13.06 ± 2.11^{b}	151.05 ± 9.77^{b}	3.30 ± 0.04^{a}	8.68 ± 0.88^{c}
0.20	8.77 ± 0.87^{b}	15.77 ± 1.99^{b}	148.99 ± 2.77^{b}	3.11 ± 0.04^{a}	8.44 ± 0.77^{c}

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

Table.4: Metabolites in the Plasma of *C. gariepinus* exposed to acute concentrations of Atrazine for 48 Hours

Concentrations (mg/L)	Metabolites (mmol/l)				
	Total Bilurubin	**Urea**	**Creatinine**	**Albumin**	**Total Protein**
0.00	10.24 ± 1.77^{a}	6.19 ± 2.73^{a}	158.09 ± 10.04^{a}	3.84 ± 0.03^{a}	10.69 ± 0.77^{a}
0.05	9.00 ± 1.94^{b}	12.05 ± 1.75^{b}	150.77 ± 11.55^{a}	3.04 ± 0.04^{a}	9.04 ± 0.11^{b}
0.10	8.03 ± 0.55^{b}	14.04 ± 2.02^{b}	145.01 ± 11.88^{b}	3.00 ± 0.01^{a}	8.16 ± 0.32^{b}
0.15	7.99 ± 0.72^{c}	15.06 ± 2.88^{b}	141.05 ± 9.41^{b}	2.90 ± 0.04^{a}	7.98 ± 0.34^{c}
0.20	7.03 ± 0.99^{c}	18.04 ± 1.41^{b}	137.01 ± 9.05^{c}	2.71 ± 0.04^{a}	7.05 ± 0.61^{c}

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

Table 5: Metabolites in the Plasma of *C. gariepinus* exposed to acute concentrations of Atrazine for 72 Hours

Concentrations (mg/L)	Metabolites (mmol/l)				
	Total Bilurubin	**Urea**	**Creatinine**	**Albumin**	**Total Protein**
0.00	10.25 ± 1.89^{a}	6.20 ± 2.88^{a}	158.10 ± 10.99^{a}	3.85 ± 0.12^{a}	10.68 ± 0.99^{a}
0.05	7.04 ± 1.05^{b}	15.05 ± 1.78^{b}	148.04 ± 11.03^{a}	3.00 ± 0.07^{a}	8.04 ± 0.11^{b}
0.10	6.01 ± 0.88^{b}	16.03 ± 2.11^{b}	142.01 ± 11.62^{b}	2.84 ± 0.09^{a}	7.04 ± 0.77^{b}
0.15	5.99 ± 0.66^{c}	18.99 ± 2.72^{b}	140.05 ± 9.40^{b}	2.60 ± 0.07^{a}	6.97 ± 0.77^{c}
0.20	5.07 ± 0.73^{c}	20.07 ± 1.99^{b}	138.01 ± 9.11^{c}	2.45 ± 0.08^{a}	6.09 ± 0.05^{c}

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

Table 6: Metabolites in the Plasma of *C. gariepinus* exposed to acute concentrations of Atrazine for 96 Hours

Concentrations (mg/L)	Metabolites (mmol/l)				
	Total Bilurubin	**Urea**	**Creatinine**	**Albumin**	**Total Protein**
0.00	10.26 ± 1.02^{a}	6.21 ± 2.99^{a}	158.12 ± 10.77^{a}	3.86 ± 0.99^{a}	10.69 ± 0.55^{a}
0.05	6.11 ± 1.22^{b}	17.02 ± 1.66^{b}	145.89 ± 11.55^{a}	2.88 ± 0.07^{a}	7.11 ± 0.33^{b}
0.10	5.72 ± 0.63^{b}	19.01 ± 2.33^{b}	140.01 ± 11.77^{b}	2.80 ± 0.22^{a}	6.55 ± 0.88^{b}
0.15	4.92 ± 0.61^{c}	20.09 ± 2.72^{b}	138.05 ± 9.44^{b}	2.50 ± 0.11^{a}	6.09 ± 0.43^{c}
0.20	4.65 ± 0.89^{c}	21.07 ± 3.03^{b}	135.01 ± 9.02^{c}	2.40 ± 0.04^{a}	5.08 ± 0.01^{c}

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

Comparative Values of Metabolites in the Plasma of *C. gariepinus* Exposed to Acute Concentrations Atrazine for 96 Hours

Comparative values of Total bilurubin in the plasma of *C.gariepinus* exposed to Atrazine for 96 hours is shown in Figure 1. The values of Total bilurubin reduced as the experimental period increased, with the highest value of 10.26 observed at the control, while the lowest (4.65) at 96 hours. Comparatively, the values of urea as shown in Figure 2, indicated that the values of urea in *C. gariepinus* exposed to varying concentrations of Atrazine were elevated progressively as the experimental period increased and peaked at 96 hours for all concentrations. The highest value of 21.07 was recorded in the fish exposed to 0.20mg/L of the chemical at 96 hour, while the lowest value of 6.21 was observed in the control (Figure 2). The values of creatinine reduced considerably as the experimental period increased, this was more pronounced at the concentration of 0.10, 0.15 and 0.20mg/l concentrations of the chemical (Figure 3).The values of Albumin (Figure 4) slightly reduced when compared to the control value in all concentrations of exposure. Comparative the value of Total protein is shown in Figure 5. The values of Total protein reduced as the experimental period progressed from 24 to 96 hours. However, a sharp decline was observed in the concentration of 0.20mg/l at 96 hours.

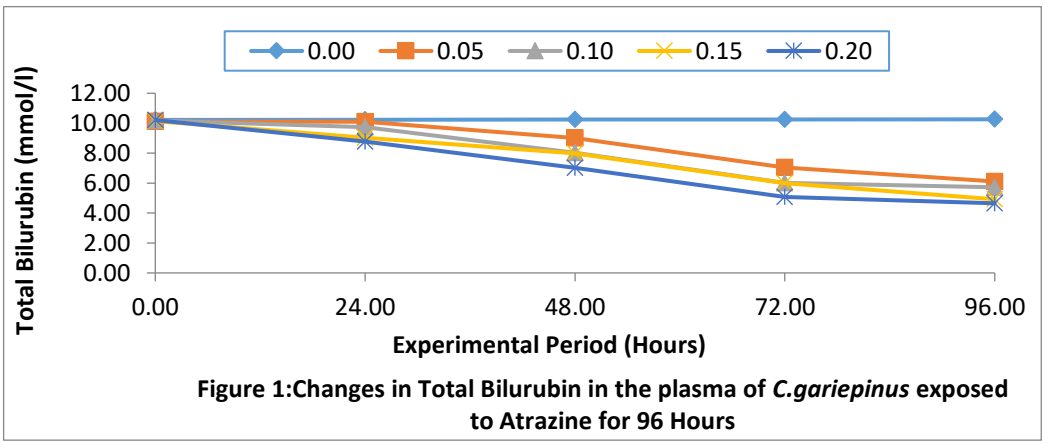

Figure 1:Changes in Total Bilurubin in the plasma of *C.gariepinus* exposed to Atrazine for 96 Hours

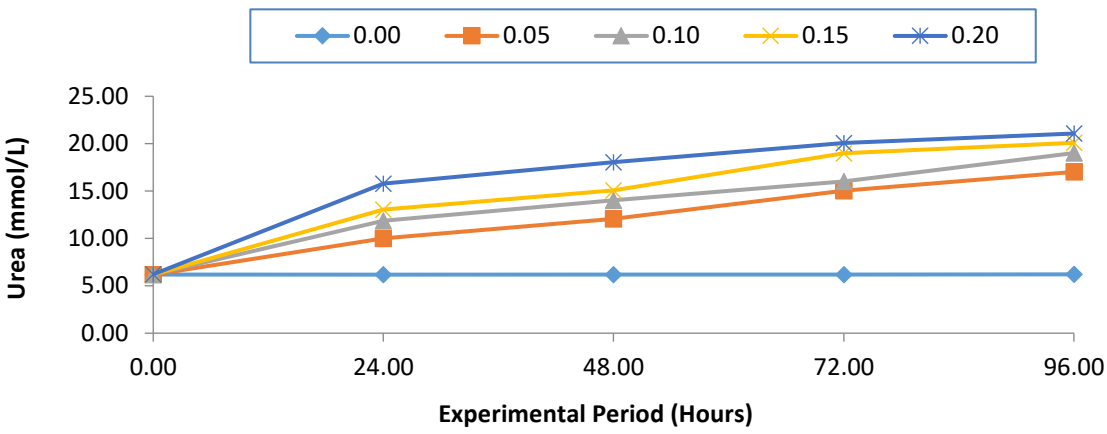

Figure 2: Changes in Urea in the plasma of *C.gariepinus* exposed to Atrazine for 96 Hours

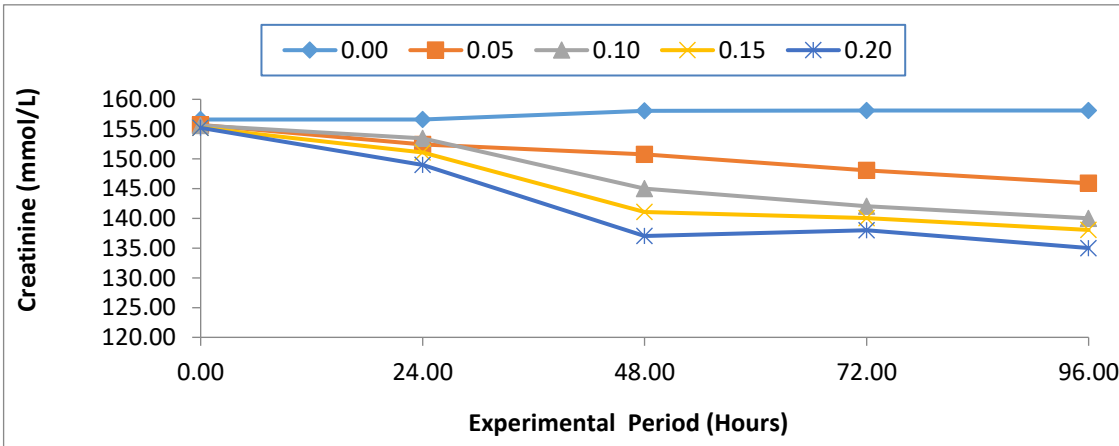

Figure 3: Changes in the values of Creatinine in the plasma *C.gariepinus* exposed to Atrazine for 96 Hours

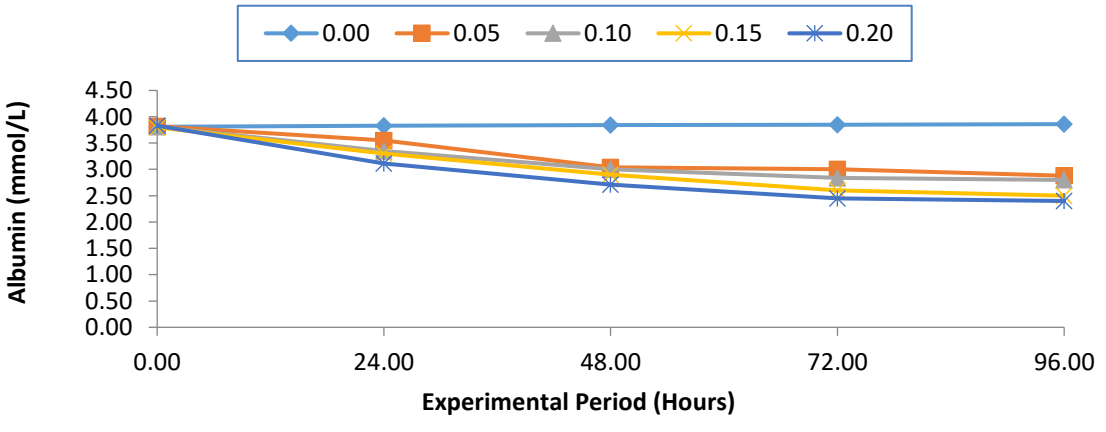

Figure 4: Changes in the values of Albumin in the plasma of *C.gariepinus* exposed to Atrazine for 96 hours

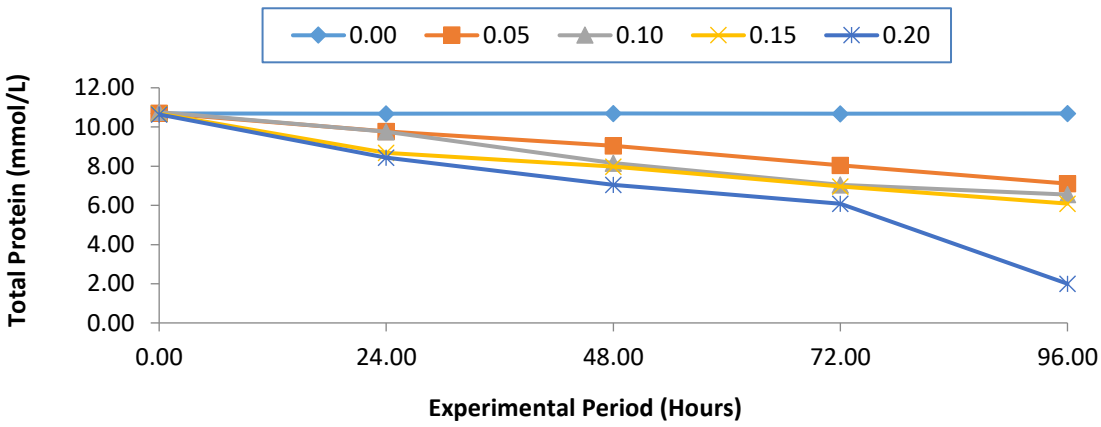

Figure 5: Changes in the values of Total protein in the plasma of *C.gariepinus* exposed to Atrazine for 96 hours

DISCUSSION

Physiochemical Parameters in *C. gariepinus* Exposed to Atrazine

The observed increases in nitrite, ammonia, and dissolved oxygen after 96 hours of toxicant exposure, as well as the variations across different concentrations and times, may be caused by the extreme instability of these parameters. The results of this study show that atrazine application does not significantly alter the physicochemical parameter to the point where it can negatively impact fish visually, even though herbicides alter the quality of the water in and around sprayed areas, decrease the amount of dissolved oxygen in the water, and increase temperature, all of which may be threats to fish species' ability to survive. The study's water quality measurements fall within the range intended for aquaculture. This study validates the findings of Adedeji [27], who found that fish life is supported by pH values between 6.5 and 9.0. Catfish and other air-breathing fish can withstand low dissolved oxygen concentrations of 4 mg/l, according to Bhatnagar and Devi's [28] research. As a result, the dissolved oxygen content is within what is required for fish to survive. Adigun [29] and Kolo *et al*. [30] also reported findings that were comparable.

Fundamentally, fish survival through metabolism depends heavily on the temperature, pH, and other physiochemical characteristics of the water in an aquatic ecosystem. Therefore, a fish's incapacity to adjust to its surroundings may result in a shift in its physiological reactions, ultimately leading to its demise. Nigerian surface water resources and the ambient values of the area are met by the temperature, pH, dissolved oxygen, and other characteristics [31, 32]. Therefore, it's possible that neither the temperature nor any other physiochemical parameter found in this study caused the fish to perish. It has been noted that the values found in this study fall within the tolerance ranges of fish species found in warm water. Thus, it's possible that the water quality characteristics had nothing to do with the metabolite alterations that were seen in this study's *Clarias gariepinus* sample.

Changes in Metabolites in *C. gariepinus Exposed to Atrazine*

Albumins, fibrinogens, and globulins are examples of the plasma proteins that are essential for moving materials throughout the fish's circulatory system. They serve as nutrients, carriers, protectors, buffers, and sources of energy [33]. Five metabolites were detected in the exposed fish's plasma in this investigation. As the concentration of atrazine in the plasma of the exposed fish grew, there were notable changes in the levels of bilirubin and creatinine, two of the breakdown products of haemoglobin in the blood. As has been noted with pesticide exposure, differentiating creatinine and bilirubin D may be predicted in the plasma of the subjected fish [34]. Tilapia mosssabica treated with phosphamidon showed a comparable change in blood bilirubin levels [35], whereas C. gariepinus exposed to potassium permanganate showed just a modest increase in serum bilirubin levels [36]. However, because there is a link between exposure to toxicants and kidney disease, alterations in the amounts of creatinine and bilirubin in plasma have often been utilized in fish as an indicator of gill and kidney dysfunction [37, 38]. This is a reference to both accelerated muscle tissue catabolism and renal failure. According to the available information, fish exposed to pesticides may have exhibited glomerular dysfunction as opposed to tubular insufficiency. This result is consistent with the observation that uric acid showed the greatest increase in serum nitrogenous substances in these fish. One theory is that uric acid accumulated in blood due to inhibition of uric acid and other nitrogenous compound branchial excretion [39].

Another crucial biochemical metric for comprehending the overall health of fish and the molecular processes behind their metabolism under pollution stress is protein [40]. The levels of total protein in the plasma of C. gingipinus exposed to atrazine were shown to have decreased in this study. Under stressful situations, fish primarily use protein and carbohydrates as energy sources. Variations in each of these blood components have been used to identify stress in teleosts in a broad way. In the current investigation, the total protein concentration begins to decrease on the first day of exposure to atrazine for acute toxicity. These continuing decreases of total protein were also noted on the other days of exposure. It has been noted that *Oreochromis niloticus* and *Chrysichthyis auratus* exhibit a decrease in total protein concentration when exposed to atrazine herbicide [41]. Plasma protein concentrations in carp were considerably lower after being treated to 10 µl/L atrazine for 72 hours [42].

Additionally, there was agreement between our results and those of other researchers [43–47].The fish's stress response and subsequent adaptation to its new surroundings may be the cause of the protein decrease [48]. Fish fulfill their additional energy needs from body proteins, which are used to create glucose for fish through the process of gluconeogenesis, as opposed to mammals, which store protein in body tissue for muscular energy in the event of a carbohydrate supply being lacking [49, 50]. Stress-mediated immobilization of these molecules to satisfy an increased energy requirement by the fish to adapt to the toxicant-exposed environmental conditions may be the cause of a lower protein level [51]. Thus, we deduced that in the current investigation, fish exposed to acutely hazardous dosages of atrazine showed lower protein concentrations than the control group due to the conversion of protein to glucose through the process of gluconeogenesis. However, our findings are at odds with those of Rhamdia quelen, which was found to have higher protein levels in silver catfish [52].

The osmotic balance between the tissue membrane and the blood in circulation is crucially maintained by serum albumin [53]. The current study's observation of a considerable drop in serum albumin was consistent with Ravichandran's research [54]. This is further corroborated by a study that found that serum albumin continued to decline when exposure to Nuvan toxicants increased [55]. Mystus vittatus, an Indian catfish, similarly showed a similar pattern of serum albumin degradation following exposure to Nuvan toxicant [56]. Similar outcomes were observed in Cyprinus carpio following exposure to monocrotphos insecticides [57]. Albumin marginally decreased as atrazine concentration increased, which is consistent with research by Ogundiran et al. [58] that showed albumin in the liver decreased due to necrosis that happened as detergent concentration increased. Fish are not able to produce new liver cells, which could be the cause of this.

It was also noted that metabolic issues may have resulted from the physiological alterations in the exposed fish's plasma. When compared to the control, albumin values likewise dropped in the plasma and all other examined organs save the liver. This observation was in line with Das et al.'s [59] confirmation that a drop in albumin levels is indicative of a renal issue. The author added that albumin and total protein were impacted by the kidney's incapacity to hold onto large molecular weight proteins that were lost through urine. Additionally, urea has proven a valuable indicator for assessing how chemicals affect the kidney, muscle, gills, liver, and plasma [60]. In this investigation, as the toxicant concentrations rose, so did the values of urea in the plasma. This is consistent with the findings of Rao's study [61], which suggested that an organ's susceptibility to toxins depends on their potency. One could argue that the urea's erratic reaction to concentration increases suggests that the kidney's glomerular filtration rate was slightly stressed.

Conclusion and Recommendations

Herbicides are used to control plants, and they are often directed towards plant-specific processes and locations. Consequently, fish are not particularly acutely toxic to the active components in the majority of herbicides. This generalization is not always true, though. Since both plants and animals share the target system, some herbicides, including antrazine, are somewhat hazardous to fish. The information from this study indicates that atrazine causes the test organism, *C. gariepinus*, to have a variety of abnormalities and health issues. The pesticide atrazine is hazardous to fish organs and alters their physiological state, according to the study's findings. The findings demonstrated a detrimental influence on the levels of protein, albumin, total bilurubin, urea, and creatinine in the plasma of *Clarias gariepinus*. The study's observations about the metabolite levels in the plasma of Clarias gariepinus, including protein, albumin, total bilurubin urea, and creatinine, suggest that atrazine may have an impact on the organisms' susceptibility to stresses in the aquatic environment. Atrazine use near rivers and other coastal areas needs to be strictly regulated and closely observed to prevent exposure to aquatic habitats.

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