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## Haematological studies of African catfish (*Clarias gariepinus* Burchell, 1822) exposed to sublethal concentrations of atrazine

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### Abstract

The effects of atrazine on haematological parameters, physicochemical parameters and behavioural responses of *Clarias gariepinus* juveniles were examined under laboratory conditions after exposure to sublethal concentrations for 8 weeks. A sublethal examination was conducted on blood parameters of *Clarias gariepinus* exposed to atrazine using concentrations 0.15mg/L, 0.29mg/L, 0.44mg/L, 0.59mg/L, 0.74mg/L and 0.00mg/L as control. An indication of subtle but rapid deterioration of life due to the effects atrazine exerts on fish was evident in the blood parameters. Haemoglobin, Red Blood Cells, White Blood Cells, Packed Cell Volume, Mean Corpuscular Volume, Mean Corpuscular Haemoglobin and Mean Corpuscular Haemoglobin Concentrations in all the treatments varied significantly ( $P < 0.05$ ) from the control. It is however imperative to note that the life of an organism is in the blood, so the enormous impact of atrazine on the blood characteristics posed an adverse damage to the treated fish.

**Keywords:** Contaminants, toxic, aquatic, living organisms

### 1. Introduction

Aquatic ecosystems are generally known to be altered from their pristine state due to anthropogenic activities carried out in nearby areas. The use of agrochemicals to either boost food production or for the control of pests results in the contamination of the environment<sup>[1]</sup>. Despite the roles they play in yielding satisfactory results by being harmful to target organisms, agrochemicals are washed from farmlands through surface runoffs into aquatic environment and may cause adverse effects on organisms in water bodies<sup>[2, 3, 4]</sup>. This may threaten the wellbeing of man through the food chain who consumes the affected aquatic organisms directly<sup>[5, 6]</sup>. The agricultural sector is the major cause of concern for aquatic environment because of the use of pesticides which are toxic, persistent and have the tendency to accumulate in organisms<sup>[7]</sup>. Reports have estimated that 1 to 6% of applied herbicides used contaminated pristine water resources when released to the aquatic environment<sup>[8]</sup>.

Atrazine was first registered as an herbicide by the US EPA in 1958; it is a type of selective herbicide used for controlling broadleaf and some grassy weeds and is mostly sprayed on farmlands where crops such as corn, sorghum, yams, cassava and sugarcane are grown<sup>[3, 9]</sup>. It gets in to the aquatic environments as run-off, directly during aerial spray or while washing used containers thereby resulting in poisoning of the bio-system<sup>[1]</sup>. In a like manner, it has been reported that atrazine introduced into aquatic environments may cause hormone dependent diseases such as reproductive disorders by affecting the normal functioning of testosterone, prolactin, progesterone, estrogen and luteinizing hormones or cause cancers due to its effects, its penetrability into mammalian tissues and its endocrine disrupting potential<sup>[8]</sup>. Results from several researches involving the use of various fish species support that pesticides at high concentrations are known to reduce the survival, growth and reproduction rate of fish and produce many visible effects on fish<sup>[10]</sup>. This is because fish are very sensitive to foreign substances<sup>[11]</sup>. Aquatic systems serve as the final destination to most contaminants released in the environment<sup>[7]</sup> and run-offs of chemical and synthetic fertilizers and pesticides to aquatic ecosystems may indirectly expose man to pesticides as a result of consumption of fish contaminated by these toxins<sup>[5, 6]</sup>.

Fish, a non-target organism for atrazine herbicide may serve as a channel for human exposure to this toxicant. *C. gariepinus* is one of the most available freshwater fish species mostly eaten

in Nigeria <sup>[12]</sup>. They are usually found in streams, rivers, lakes, swamps as well as floodplains which may be located close to farmlands or downstream and they feed on macro invertebrates, small fishes, as well as dead animal matter in their habitat <sup>[13]</sup>. The toxicity study of atrazine on juveniles of *C. gariepinus* is essential in order to determine the extent of its effects on them as this toxicant can be carried to consumers along the food chain causing harmful effects on both fish and man. The study aimed at assessing the effects of atrazine on haematological parameters of juveniles of *C. gariepinus* after exposure to sublethal concentrations, examining the physicochemical parameters of the test solutions during the exposure period and to observe and record behavioural responses of *C. gariepinus* juveniles in the varying atrazine concentrations.

## 2. Materials and Methods

### 2.1 Study area

The study was carried out at Department of Fisheries and Aquaculture general purpose laboratory, Federal University of Agriculture Makurdi. Makurdi, where the study area is situated is the capital of Benue State. Benue is a rich agricultural region that grows crops such as rice, yams, maize, millet, guinea corn, soya beans, potatoes, groundnuts, cassava, etcetera and the State also has many rivers.

### 2.2 Sublethal test

A total of 18 plastic bowls were stocked each with ten (10) surviving, randomly selected, acclimatised juvenile *Clarias gariepinus* and exposed to sublethal concentrations of atrazine. Juvenile *Clarias gariepinus* measuring 16-18g and 14-16cm range of weight and length respectively were used. Using a 96hr LC<sub>50</sub> of 8.84mg/L, lower concentrations of atrazine were prepared for the sublethal test by taking fractions of 1/12, 1/15, 1/20, 1/30 and 1/60 as suggested by <sup>[14]</sup>. The bowls represented five treatments 0.15mg/L, 0.29mg/L, 0.44mg/L, 0.59mg/L and 0.74mg/L in triplicates for each concentration and 0.00mg/L of atrazine served as the control. The study lasted 8 weeks; all bowls were well aerated using aerators. The physicochemical parameters of test solutions which include temperature, pH, dissolved oxygen (DO), electrical conductivity (EC) and total dissolved solids (TDS) were recorded during the study period using digital multi-parameters water tester. Fish were fed at 5% body weight during the study period.

### 2.3 Haematological studies

Eight weeks after exposing the *C. gariepinus* to atrazine solutions, blood samples were collected by randomly selecting fish from various treatments using 2ml syringe and needle. The blood samples were analysed at Federal Medical Centre Makurdi, Benue State for the following: Haemoglobin (Hb), Packed Cell Volume (PCV), Red Blood Cell (RBC), and White Blood Cell (WBC) while Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were determined using the formulae stated by <sup>[15]</sup>.

### 2.4 Data analysis

The data obtained were subjected to Analysis of Variance

(ANOVA) using GENSTAT (2014) and means were separated using Least Significant Difference (LSD) at 5% level of significance.

## 3. Results

Table 1 shows the blood parameters of juveniles of *C. gariepinus* exposed to sublethal concentrations of atrazine. Hb, RBC, WBC, PCV, MCV, MCH and MCHC in all treatments varied significantly ( $P < 0.05$ ) from that of control. The control (0.00mg/L) had the highest Hb mean value of  $10.05 \pm 0.05$ g/dL while the concentration 0.44mg/L had the lowest Hb of  $7.41 \pm 0.01$ g/dL. Highest mean RBC value of  $3.06 \pm 0.10$ fL was recorded from concentration 0.59mg/L and lowest RBC of  $2.15 \pm 0.05$ fL was recorded from 0.44mg/L. WBC had the highest value recorded in 0.15mg/L concentration with  $58.45 \pm 0.05$ fL whereas the lowest WBC value of  $35.90 \pm 0.10$ fL was obtained in concentration 0.59mg/L. The control (0.00mg/L) recorded the highest PCV value of  $30.00 \pm 0.10\%$  while 0.44mg/L had the lowest PCV of  $23.00 \pm 0.01\%$ . Mean value of MCV in concentration 0.15mg/L was recorded highest with  $112.55 \pm 0.05$ fL while the lowest mean MCV value of  $83.35 \pm 0.05$ fL was obtained in concentration 0.59mg/L. Highest mean MCH of  $37.55 \pm 0.05$ pg was recorded from the concentration 0.15mg/L while the lowest MCH of  $27.35 \pm 0.05$ pg was recorded from 0.59mg/L concentration. MCHC varied slightly from the highest mean value of  $33.70 \pm 0.10$ g/dL recorded in both concentrations 0.44mg/L and 0.74mg/L to the mean value of  $32.85 \pm 0.05$ g/dL obtained in concentration 0.59mg/L.

The results of the physicochemical parameters of atrazine solutions during the study are presented in Table 2. DO, pH, TDS and EC varied significantly ( $p < 0.05$ ) from the control. Highest temperature ( $26.98 \pm 0.26^\circ\text{C}$ ) was recorded from the control (0.00mg/L) and lowest temperature of  $26.51 \pm 0.39^\circ\text{C}$  was recorded from 0.59mg/L solution. Highest mean pH value of  $7.72 \pm 0.03$  was recorded from the concentration 0.74mg/L while the lowest pH of  $7.26 \pm 0.20$  was recorded from the control (0.00mg/L) solution. Mean value of dissolved oxygen in the control  $6.51 \pm 0.22$ mg/L was the highest recorded and it varied significantly from the lowest DO value of  $2.72 \pm 0.21$ mg/L obtained from concentration 0.74mg/L. The highest mean value of total dissolved solids  $832.42 \pm 9.82$ mg/L was recorded from the concentration 0.74mg/L whereas the lowest TDS mean value of  $607.75 \pm 27.37$ mg/L recorded in the control test solution. Highest mean EC of  $1653.80 \pm 23.37$  $\mu\text{S/cm}$  was recorded from the concentration 0.74mg/L test solution while the lowest EC of  $956.58 \pm 19.00$  $\mu\text{S/cm}$  was recorded from the control solution.

During the study, there were no behavioural changes observed on fish in the control treatment; their colour was normal, whereas, abnormal behaviours such as restlessness, torpidity, being motionless, erratic swimming and uncoordinated movements were recorded. In addition, most treated fish showed refusal to food administered to them or fed on a little quantity in comparison to those in the control group before they eventually adapted to their new environments (atrazine-treated test solutions), therefore, significant levels of weight loss were recorded in treated fish (Figure 1).

**Table 1:** Effects of sublethal concentrations of atrazine on haematological parameters of juveniles of *Clarias gariepinus*

Conc. of atrazine (mg/L)	Hb (g/dL)	RBC (fL)	WBC (fL)	PCV (%)	MCV (fL)	MCH (pg)	MCHC(g/dL)
0.00 (Control)	10.05±0.05 <sup>e</sup>	2.85±0.05 <sup>c</sup>	51.60±0.40 <sup>c</sup>	30.00±0.10 <sup>c</sup>	107.15±0.05 <sup>d</sup>	35.75±0.05 <sup>e</sup>	33.35±0.05 <sup>b</sup>
0.15	9.00±0.00 <sup>d</sup>	2.45±0.05 <sup>b</sup>	58.45±0.05 <sup>e</sup>	28.00±0.01 <sup>b</sup>	112.55±0.05 <sup>e</sup>	37.55±0.05 <sup>f</sup>	33.35±0.05 <sup>b</sup>
0.29	8.35±0.05 <sup>c</sup>	2.55±0.05 <sup>b</sup>	49.55±0.45 <sup>b</sup>	26.00±0.01 <sup>ab</sup>	100.01±0.05 <sup>c</sup>	33.25±0.05 <sup>c</sup>	33.25±0.05 <sup>b</sup>
0.44	7.41±0.01 <sup>a</sup>	2.15±0.05 <sup>a</sup>	53.64±0.04 <sup>d</sup>	23.00±0.01 <sup>a</sup>	104.20±0.01 <sup>c</sup>	35.25±0.05 <sup>d</sup>	33.70±0.10 <sup>c</sup>
0.59	8.20±0.01 <sup>b</sup>	3.06±0.06 <sup>d</sup>	35.90±0.10 <sup>a</sup>	26.00±0.01 <sup>ab</sup>	83.35±0.05 <sup>a</sup>	27.35±0.05 <sup>a</sup>	32.85±0.05 <sup>a</sup>
0.74	8.41±0.01 <sup>c</sup>	2.61±0.01 <sup>b</sup>	57.75±0.25 <sup>e</sup>	26.00±0.01 <sup>ab</sup>	96.25±0.05 <sup>b</sup>	32.35±0.05 <sup>b</sup>	33.70±0.10 <sup>c</sup>
p-value	0.00	0.00	0.00	0.00	0.00	0.00	0.03

Means in the same column with different superscripts differ significantly (P<0.05)

KEY

Hb = Haemoglobin

RBC = Red Blood Cells

PCV = Packed Cell Volume

MCH = Mean Corpuscular Haemoglobin

WBC = White Blood Cells

MCV = Mean Corpuscular Volume

MCHC = Mean Corpuscular Haemoglobin Concentration

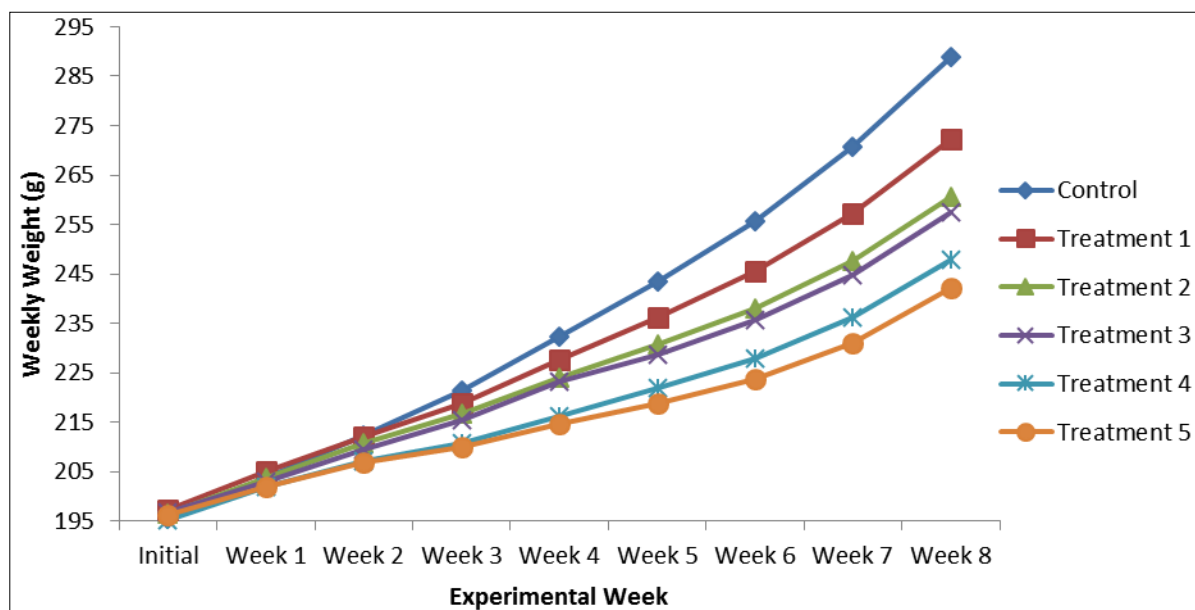
**Table 2:** Physicochemical parameters of test solutions obtained during exposure of juveniles of *Clarias gariepinus* to sublethal concentrations of atrazine

Conc. of Atrazine (mg/L)	Temp (°C)	pH	DO (mg/L)	TDS (mg/L)	EC (µS/cm)
0.00 (Control)	26.98±0.26	7.26±0.20 <sup>a</sup>	6.51±0.22 <sup>d</sup>	607.75±27.37 <sup>a</sup>	956.58±19.00 <sup>a</sup>
0.15	26.53±0.40	7.52±0.11 <sup>ab</sup>	4.91±0.34 <sup>c</sup>	695.08±12.47 <sup>b</sup>	1292.10±55.07 <sup>b</sup>
0.29	26.55±0.41	7.57±0.05 <sup>b</sup>	4.67±0.32 <sup>bc</sup>	701.75±1.58 <sup>b</sup>	1403.80±3.11 <sup>c</sup>
0.44	26.58±0.41	7.62±0.05 <sup>b</sup>	3.96±0.31 <sup>b</sup>	706.75±1.66 <sup>b</sup>	1401.40±7.42 <sup>c</sup>
0.59	26.51±0.39	7.66±0.06 <sup>b</sup>	3.14±0.18 <sup>a</sup>	724.17±1.76 <sup>b</sup>	1428.40±16.40 <sup>c</sup>
0.74	26.57±0.40	7.72±0.03 <sup>b</sup>	2.72±0.21 <sup>a</sup>	832.42±9.82 <sup>c</sup>	1653.80±23.37 <sup>d</sup>
p-value	0.95	0.04	0.00	0.00	0.00

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

KEY

Temp = Temperature, DO = Dissolved Oxygen, TDS = Total Dissolved Solids, EC = Electrical Conductivity



**Fig 1:** Mean Weekly Weight of *Clarias gariepinus* juveniles exposed to Sublethal Concentrations of Atrazine

KEY

Control = 0.00mg/L of Atrazine

Treatment 3 = 0.44mg/L

Treatment 1 = 0.15mg/L

Treatment 4 = 0.59mg/L

Treatment 2 = 0.29mg/L

Treatment 5 = 0.74mg/L

#### 4. Discussion

After exposure to sublethal concentrations of atrazine, the result for haematological indices showed significant differences (p<0.05) in Hb, RBC, WBC, PCV, MCV, MCH and MCHC between the control and the various treatments. The health status of blood in living organisms is imperative because of its numerous roles including oxygen and nutrient transportation. Its major function of regulation of internal environment of a system (homeostasis) as stated by Ada *et al.* [16] usually makes any intense attack on it severe or outrightly leads to death of an organism. Reduction in Hb concentration

with increased concentration of atrazine observed conforms with report of Edori *et al.* [17] in which *C. gariepinus* was exposed to sublethal concentrations of paraquat. The decrease in Hb possibly led to a condition in which the capacity of the blood to transport oxygen to tissues is reduced; consequently, decline in red blood cells may depict iron decrease in body cells which supports oxygen carrying potential of blood. Ada *et al.* [6] opined that Hb reduction invariably contributes to the stress and anaemic state of organisms which further alters respiration, metabolism and triggers morbidity and death. Hence, Hb reduction in treated fish might have affected the

oxygen available to body tissues, resulting to slow metabolic rate and low energy production which also explains the changes in their behaviours that included restlessness, gasping for air, low food consumption and subsequently loss of body weight amongst others. The RBC decreased with increase in concentration of atrazine (except in 0.59mg/L), although the difference was not significant; likewise, Ugwuene <sup>[18]</sup> revealed that reduced RBC count implies a reduction in the level of oxygen carried to the tissues. Lack of significant changes in RBC count in exposed fish in some concentrations reported could be attributed to two factors; that the different treated concentrations were below the threshold limit of fish reaction or that blood samples were collected much sooner or later than the point fish were stressed; this assertion conforms to the report of Gabriel and Erondu <sup>[19]</sup> who revealed that this occurs when blood sampling is done either before reactions in blood have not been evoked or after fish must have adapted to the toxicant. WBC showed significant difference ( $p < 0.05$ ), at first, it increased then decreased with increase in concentration of atrazine and continuously fluctuated; this is contrary to the findings of Zubair <sup>[20]</sup> who reported a continuous decrease with increase in sublethal concentration of malathion. The trend in WBC with increased concentration of atrazine is similar to that observed in *C. gariepinus* exposed to paraquat dichloride by Seiyaboh *et al.* <sup>[21]</sup>. The WBC showed a marked increase in the least treated concentration (0.15mg/L) followed by the highest concentration (0.74mg/L); this could be due to attempts made by the fish to fight against the effects of the pollutant which could have led to its increment in order to ameliorate healthy state of fish; Ada *et al.* <sup>[16]</sup> stated that a rise in WBC count is viewed as adaptation of organisms and their efforts to combat invaders from cells. Baker *et al.* <sup>[22]</sup> suggests that sharp increase in exposed blood cells above that of the control group could be resistance to prevalent unwanted change and adaptability to the environment. PCV decreased in this study and that implied poor transportation of oxygen and absorbed nutrients which could possibly have resulted to a decreased status of fish condition. The fluctuations in MCV, MCH and MCHC in this study indicates that the concentration of Hb in RBC were lower in the exposed fish than in the control over the exposure period, Ada *et al.* <sup>[16]</sup> reported that herbicides may trigger the multiplication of the blood cells to make up for the low load of haemoglobin per cell whereas Edori *et al.* <sup>[17]</sup> explained that their decrease imply the malfunctioning of organs responsible for producing blood in fish. MCH, which indicates blood level conditions, fluctuated, thus indicating of anaemic condition in the test fish exposed to atrazine. A probable anaemic response in test fish was perhaps as a result of destruction of intestinal cells, which may have affected food intake and body weight, Frakes *et al.* <sup>[23]</sup>, Zubair <sup>[20]</sup> and Edori *et al.* <sup>[17]</sup> reported similar trends in fish exposed to different toxicants.

Water physicochemical parameters usually serve as indicators of the quality of water bodies, revealing the toxic levels and effects of chemicals present in aquatic media and on aquatic organisms. During this study, only temperature showed no significant difference ( $p > 0.05$ ) between the mean values of the treated fish and that of the control. Significant changes were however recorded in the mean values of pH, DO, TDS and EC between the control and various treatments. Generally, means of temperature and pH fluctuated slightly between the control and the treated solutions; this is line with studies of Ayoola <sup>[24]</sup> who reported slight changes in test

water temperature and pH on exposure of *O. niloticus* juveniles to glyphosate herbicide. There was a significant relationship ( $p < 0.05$ ) between the rates of dissolved oxygen with atrazine concentration, as the mean values decreased with increase in concentration of the toxicant in the test solutions. The significant negative correlation between DO values and toxicant concentration as dissolved oxygen decreased considerably with increasing concentrations of atrazine is in consonance with reports of Ayoola and Ajani <sup>[25]</sup> and Ada *et al.* <sup>[6, 16]</sup> in exposed fish studied under laboratory conditions. This may be due to stress caused by atrazine which resulted in agitation and abnormal behaviours of the test fish, thereby, leading to reduced DO level of the test solutions. TDS and EC increased with increasing concentrations of atrazine indicating organic matter content and electrolyte concentrations; these are in agreement with the findings of Akaahan *et al.* <sup>[25]</sup> on the effects of zinc on water quality when *C. gariepinus* juveniles were examined and Seiyaboh *et al.* <sup>[21]</sup> on the toxicity of paraquat dichloride on water quality on exposure of adult *C. gariepinus*.

## 5. Conclusions

An indication of subtle but rapid deterioration of life due to the effects atrazine exerted on fish was evident in the blood parameters because small sublethal concentrations of toxicants in water bodies is equally as harmful as the release of its acute concentrations. Sublethal concentrations of atrazine herbicide on juveniles of *C. gariepinus* caused alterations in haematological parameters; Hb, RBC, WBC, PCV, MCH, MCV and MCHC of the fish causing a range of defects and health conditions like anaemia evident in increased white blood cells of the test fish. It is however imperative to note that the life of an organism is in the blood, so the enormous impact of atrazine on the blood characteristics posed an adverse damage to the treated fish. Atrazine application to enhance crop yield; although produces an immediate satisfactory result, but it consequently carries an accompanying side effect, moreover, when we act locally, the impact may be felt globally so let us be environmentally conscious and seek to achieve environmental sustainability for future generations.

## 6. Recommendations

Improved equipment and techniques of crop management should be developed and employed so as to reduce negative impacts of atrazine on the environment. Environmental law enforcement agencies such as NESREA (National Environmental Standards and Regulation Enforcement Agency) should ensure that companies that either manufacture pesticides in Nigeria or import from other countries for sale opt for less persistent or toxic chemicals to help in weed control. In some countries, sale of atrazine is done with restrictions (because it has been banned from usage); this should as well be employed in Nigeria when studies have been carried out on water bodies to ascertain the presence and levels of atrazine.

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