

## Haematological Changes in African Catfish (*Clarias Gariepinus*) Exposed to Mixture of Atrazine and Metolachlor in the Laboratory

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Received: 31.07.2017 / Accepted: 05.08.2017 / Published: 08.08.2017

### Abstract:

Seventy Two (72) male and female African catfish (*Clarias gariepinus*) juveniles of mean length ( $10.74 \pm 1.81$  g) were exposed to different concentrations of atrazine and metolachlor (0.00 mg/l-control, 0.01, 0.02, 0.03, 0.04 and 0.05mg/l) for 14 days to determine its effect on haematological parameters of the fish. The results obtained indicated significant ( $P<0.05$ ) reductions with increased concentrations of the chemical in haemoglobin (Hb), Red blood Cell (RBC), packed cell volume (PCV), lymphocytes, platelets and mean corpuscular volume (MCV). The white blood cell (WBC), neutrophils, monocytes, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) in fish exposed to the pesticides were significantly ( $P<0.05$ ) higher than that of the control. The data obtained from this work will contribute to the base line haematological parameters for use in monitoring health status of *Clarias gariepinus* in the wild and culture medium

**Keywords:** Haematology; Fish; Toxicity; Herbicide; Environment

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## Introduction

Herbicides are widely used all over the world to control the harmful effects of pests and weeds on agricultural productions and fish farm. However, despite the good results of using herbicides in agriculture, their use in the environment is usually accompanied by deleterious environmental and public health effects (Nwani *et al.*, 2010). The herbicide after being used, ultimately find their way into different aquatic ecosystems and have been found to be highly toxic to non-target organisms, especially aquatic life form and their environment (Nwani *et al.*, 2010). Herbicides pollution severely affects aquatic organism strophic levels including human beings. The effects of herbicides on fishes are of great concern (Bagheri and Nezami, 2000; Nwani *et al.*, 2010).

Atrazine is one of the most intensely used herbicides in the world. It is part of the 5-triazine chemical group. This herbicide inhibits photosynthesis by blockage of electron transport in the plant system (Roses *et al.*, 2013). It is included in the European Union (EU) priority substance list used for water chemical status definition due to its high mobility and persistence in the environment, causing adverse effects at low concentrations (European Commission, 2008). While Metolachlor is a chloroacetanilide that promotes the inhibition of cell division in seedling shoots and roots (Selck *et al.*, 2012).

The African catfish *Clarias gariepinus* is an ecologically and commercially important fish for the Nigerian aquaculture industry (Ita, 1980). These mud fish are frequently and widely cultured in ponds and they also occur freely in Nigerian's natural freshwater. Musa and Omoregie, (1999) reported that fish are intimately associated with the aqueous environment, such that physical and chemical changes in the environment are rapidly reflected as measurable physiological changes in fish.

The use of haematological technique in fish culture for toxicological research, environmental monitoring and fish health conditions have grown rapidly in recent times (Gabriel *et al.*, 2007a; Akinrotimi, 2008; Akinrotimi *et al.*, 2011a). Many works have been conducted on haematological changes of in the fish exposed to pesticides include but not limited to that of Das and Mukherjee (2000); Adebayo *et al.* (2005); Patnaik and Patra (2006); Gabriel *et al.* (2007b); Gabriel *et al.* (2011); Gabriel *et al.* (2012); Akinrotimi *et al.* (2011b); Akinrotimi and Amachree (2016). Haematology is an indicator of immunological status and can provide definitive diagnosis of fish during toxicant exposure (Akinrotimi *et al.*, 2007a; Campbell and Ellis, 2007; Nte *et al.*, 2011). Haematological indices are of different sensitivity to various environment factors and chemical (Akinrotimi *et al.*, 2013). Studies have shown that when the water quality is affected by toxicants, any physiological changes will be reflected in values of one or more haematological parameters of aquatic organisms (Akinrotimi *et al.*, 2007b; Gabriel *et al.*, 2007c). However, Sampath *et al.* (1993) and noted that there is a possibility that studies on fish blood might reveal conditions within the body of the fish long before there is any outward manifestation of disease.

Among all forms of chemicals, atrazine and metolachlor are considered to be the most hazardous with respect to environmental

pollution, since they are very persistent, non-biodegradable and capable of bio-magnification as they move up in the food chain. Exposure of fish to these compounds can result in mortality as well as sub-lethal impacts. In recent years, some herbicides are widely used on farms in Nigeria and are on the list of priority substances. Literature on the effects of metolachlor and atrazine on haematological parameters of *Clarias gariepinus* are scanty. Metalochlor and atrazine is relatively new and extensively applied in agriculture for weed eradication in Nigeria, so it is pertinent to study its hazardous effect on the aquatic system, it is assumed, that the residue might affect the fish from runoffs into the aquatic system. The study therefore investigated the effects of metalochlor and atrazine on some selected blood parameters of *Clarias gariepinus* under laboratory conditions.

## Material and Methods

### Experimental location and source of experimental fish

The experiment was carried out at the Toxicity laboratory in African Regional Aquaculture Centre (ARAC), Aluu, Rivers State, Nigeria. One Hundred and Eighty (180 juveniles) of *C. gariepinus* of (mean length  $8.74 \pm 2.64$  cm and mean weight  $56.68 \pm 1.81$  g) were harvested from earthen ponds in the center. They were transferred in six 50 liter plastic tanks to the laboratory for acclimation process.

### Acclimation and feeding of fish

The experimental fish were acclimated in four 150 L capacity circular plastic tanks containing 100 L 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 ARAC feed (35% CP).

### Procurement and preparation test solution

A commonly used pesticide and herbicide with brand name "Delta Force" (Atrazine and Metalochlor) was purchased off shelf, from "Analytical" chemical shop, Garrison, Port Harcourt, Rivers State. The solution of the chemical in water was prepared by serial dilution using the dilution formula of Grim Shaw (1978).

### Experimental design and procedure

The experimental design was a completely randomized design (CRD) with five treatments levels and a control with each level having three replicates Ten (10) *C. gariepinus* juveniles were introduced individually into 18, aquaria tanks of  $1.5 \text{ m} \times 1 \text{ m} \times 0.5 \text{ m}$  dimension, containing 0.00 (control), 0.10, 0.20, 0.30, 0.40 and 0.50 mg/L of Delta Force (metalochlor/atrazine). Each treatment and control had three replicates and lasted for 14 days. The solution for each concentration was renewed daily, with freshly prepared solution of Delta Force (metalochlor/atrazine). The tank were covered with netted materials and supported with heavy objects to prevent the fish from escaping.

### Evaluation of water quality parameters

The physico-chemical parameters were measured in the

experimental tanks on weekly basis. Ammonia, Alkalinity, Conductivity, Dissolved oxygen (DO), was determined using the methods described by APHA (1998). The water pH was measured using pH meter, and while Temperature was evaluated by using mercury in glass thermometer.

### Blood sampling and analysis

Blood sampling was conducted at the expiration of 14 days. Blood samples were collected from a total of 25 fish (five fish/treatment) with heparinized plastic syringe, fitted with 21 gauge hypodermic needle and preserved in disodium salt of ethylenediamine tetraacetic acid (EDTA) bottles for analysis. The Blaxhall and Daisley (1973), and Wedemeyer *et al.* (1983) haematological methods were adopted for this study. The cyano-haemoglobin method was used to determine haemoglobin (Hb) using diagnostic kits from Sigma diagnostics USA, and packed cell volume (PCV) was determined by the microhaematocrit method. Red blood cell (RBC), leucocrit (LCT) and thrombocyte count were determined with the improved Neubauer haemocytometer according to (Dacie and Lewis, 1991). White blood cells (WBC) was determined with the improved Neubauer counter, while differential counts such as neutrophils, lymphocytes and monocytes were determined on blood film stained with May-Grunwald-Giemsa stain (Mirale, 1982). The values of haematological indices were calculated Brown (1980).

### Mean cell volume (MCV)

Values of MCV were calculated using the formula:

$$MCV = \frac{PCV \times 10}{RBC}$$

### Mean cell haemoglobin (MCH)

Values of MCH were calculated using the formula:

$$MCH = \frac{Hb(g/dl) \times 10}{RBC}$$

### Mean cell haemoglobin concentration (MCHC)

Values of MCHC were calculated using the formula:

$$MCHC = \frac{Hb(g/dl) \times 100}{PCV(\%)}$$

## Statistical Analysis

Data obtained from the experiments were collated and subjected to ANOVA using Statistical Package for the social Sciences, (SPSS) version 10, differences among means were separated by Tukeys Comparative test at 0.05%.

## Results

The results of water quality variables in the experimental tanks during the exposure period are shown in **Table 1**. The result indicated an increase in the values of Alkalinity from 89.10 in the control to 156.34 mg/l at 0.05 mg/l of the chemical. Conductivity also increased from 121.50 to 271.21  $\mu$ S/cm. However, reductions were recorded in the values of dissolved oxygen and total dissolved solids, while the values for Temperature and pH were within the same range in all concentrations of exposure.

The result of hematological parameters in *C. gariepinus* juvenile males exposed to Atrazine/ metalochlor, for 14 days is presented in **Table 2**. The result indicated a consistent reduction relative to the control in the values of haemoglobin (Hb), red blood cell (RBC), packed cell volume (PCV), lymphocytes, platelets and mean corpuscular volume (MCV). While the values of WBC increased from 21.00 to 36.71, Neutrophils 40.00-59.12, monocytes 4.40-8.71, MCH 16.47 to 22.71 and MCHC 31.11 to 32.61. These was a decrease in the values of Hb 11.20 to 8.20, RBC 6.30 to 6.10 PCV 36.00 to 10.00%, Lymphocyte 59.66 to 36.12%, platelets 501.21 to 299.33% and MCV 52.94 TO 27.70 fl, with increasing concentration from control to 0.5 mg/L of the chemical. The result of hematological parameters, in female *C. gariepinus* juveniles exposed to Atrazine/metalochlor for 14 days is shown in **Table 3**. The results indicated that there was increase in the values of WBC from 24.00 to 40.81, Neutrophils 40.21 to 59.33%, and MCHC 34.00 to 74.00%. There was decrease in: Hb from 13.61 to 7.20, RBC 7.40 to 4.51, PCV 39.11 to 10.14, Monocytes 44.21-9.02%, Lymphocytes 59.21 to 35.62%, platelets 614 to 279.33% MCV 52.85 to 22.48 fl and MCH 18.39 to 15.96 pg. The summary of haematological parameters in *C. gariepinus* exposed to Atrazine and matelolachlor for 14 days is shown in **Table 4**. The values of Hb, RBC, PCV, Lymphocytes Platelets, and MCV reduced significantly ( $P < 0.05$ ) with increasing concentrations of

**Table 1:** Water Quality Variables (Mean  $\pm$  S.D) in the Experimental Tanks during the exposure period.

Variables	Concentrations of Atrazine/Metalochlor (mg/l)					
	0	0.01	0.02	0.03	0.04	0.05
Alkalinity (mg/l)	89.10 $\pm$ 10.16 <sup>a</sup>	98.20 $\pm$ 21.44 <sup>a</sup>	91.12 $\pm$ 21.15 <sup>ab</sup>	132.61 $\pm$ 11.77 <sup>b</sup>	144.62 $\pm$ 11.13 <sup>b</sup>	156.34 $\pm$ 22.52 <sup>b</sup>
Temperature ( $^{\circ}$ C)	29.62 $\pm$ 2.13 <sup>a</sup>	29.97 $\pm$ 0.58 <sup>a</sup>	29.95 $\pm$ 0.14 <sup>A</sup>	29.99 $\pm$ 0.69 <sup>a</sup>	29.80 $\pm$ 0.13 <sup>a</sup>	29.41 $\pm$ 0.94 <sup>a</sup>
pH	7.66 $\pm$ 0.87 <sup>a</sup>	7.67 $\pm$ 0.65 <sup>a</sup>	7.75 $\pm$ 0.43 <sup>a</sup>	7.64 $\pm$ 0.54 <sup>a</sup>	7.61 $\pm$ 0.68 <sup>a</sup>	7.60 $\pm$ 0.43 <sup>a</sup>
Conductivity (S/m)	121.50 $\pm$ 12.81 <sup>a</sup>	131.45 $\pm$ 18.81 <sup>a</sup>	176.45 $\pm$ 28.51 <sup>b</sup>	183.25 $\pm$ 14.11 <sup>b</sup>	210.15 $\pm$ 13.31 <sup>c</sup>	271.21 $\pm$ 18.32 <sup>c</sup>
DO (mg/l)	5.71 $\pm$ 0.28 <sup>c</sup>	4.06 $\pm$ 0.55 <sup>b</sup>	3.41 $\pm$ 0.87 <sup>b</sup>	3.06 $\pm$ 0.33 <sup>b</sup>	2.51 $\pm$ 0.33 <sup>a</sup>	2.04 $\pm$ 0.27 <sup>a</sup>
Total Dissolved Solid (mg/l)	0.01 $\pm$ 0.01 <sup>a</sup>	0.002 $\pm$ 0.01 <sup>a</sup>	0.03 $\pm$ 0.01 <sup>b</sup>	0.03 $\pm$ 0.01 <sup>b</sup>	0.04 $\pm$ 0.01 <sup>b</sup>	0.04 $\pm$ 0.01 <sup>b</sup>

Means within the same row with different super scripts differ significantly ( $P < 0.05$ ).

**Table 2:** Haematological Parameters in *C. gariepinus* juveniles Males exposed to Atrazine and Metalochlor for 14 days (Mean  $\pm$  S.D).

Parameters	0.01	0.02	0.03	0.04	0.05
Hb (gdl)	11.20 $\pm$ 0.91 <sup>d</sup>	11.20 $\pm$ 0.14 <sup>d</sup>	11.00 $\pm$ 0.11 <sup>c</sup>	11.00 $\pm$ 0.22 <sup>b</sup>	9.92 $\pm$ 0.27 <sup>b</sup>
RBC (Cells $\times$ 10 <sup>12</sup> )	6.80 $\pm$ 0.74 <sup>d</sup>	5.90 $\pm$ 0.28 <sup>c</sup>	5.70 $\pm$ 0.12 <sup>b</sup>	550 $\pm$ 0.27 <sup>b</sup>	4.10 $\pm$ 0.24 <sup>a</sup>
PCV (%)	36.00 $\pm$ 1.64 <sup>c</sup>	34.00 $\pm$ 1.871 <sup>c</sup>	32.00 $\pm$ 1.62 <sup>b</sup>	29.00 $\pm$ 1.11 <sup>b</sup>	18.00 $\pm$ 1.18 <sup>d</sup>
WBC (Cells $\times$ 10 <sup>9</sup> )	21.00 $\pm$ 1.92 <sup>a</sup>	21.00 $\pm$ 1.911 <sup>a</sup>	24.00 $\pm$ 1.81 <sup>a</sup>	28.14 $\pm$ 1.36 <sup>b</sup>	32.94 $\pm$ 1.36 <sup>c</sup>
Neutrophils (%)	40.00 $\pm$ 1.11 <sup>a</sup>	41.00 $\pm$ 1.71 <sup>a</sup>	46.00 $\pm$ 2.21 <sup>d</sup>	50.11 $\pm$ 1.18 <sup>b</sup>	52.61 $\pm$ 2.14 <sup>c</sup>
Monocytes (%)	40.00 $\pm$ 1.11 <sup>a</sup>	5.18 $\pm$ 0.14 <sup>a</sup>	5.41 $\pm$ 1.12 <sup>b</sup>	6.22 $\pm$ 10.27 <sup>b</sup>	7.18 $\pm$ 1.06 <sup>dc</sup>
Lymphocytes (%)	57.66 $\pm$ 6.41 <sup>d</sup>	55.00 $\pm$ 4.84 <sup>d</sup>	50. $\pm$ 12.97 <sup>c</sup>	45.12 $\pm$ 2.61 <sup>b</sup>	40.12 $\pm$ 3.14 <sup>b</sup>
Platelets (%)	501.21 $\pm$ 0.11 <sup>c</sup>	470.12 $\pm$ 0.12 <sup>b</sup>	450.11 $\pm$ 0.12 <sup>b</sup>	401.00 $\pm$ 0.14 <sup>b</sup>	381.14 $\pm$ 0.14 <sup>a</sup>
MCV (fl)	52.94 $\pm$ 3.86 <sup>a</sup>	57.62 $\pm$ 2.79 <sup>a</sup>	56.14 $\pm$ 3.63 <sup>b</sup>	52.98 $\pm$ 4.13 <sup>b</sup>	43.90 $\pm$ 3.28 <sup>b</sup>
MCH (pg)	16.47 $\pm$ 2.28 <sup>a</sup>	18.98 $\pm$ 1.86 <sup>a</sup>	19.29 $\pm$ 2.23 <sup>a</sup>	20.87 $\pm$ 1.79 <sup>a</sup>	24.19 $\pm$ 1.38 <sup>a</sup>
MCHC (%)	31.11 $\pm$ 1.84 <sup>d</sup>	32.94 $\pm$ 2.79 <sup>d</sup>	34.37 $\pm$ 3.25 <sup>c</sup>	37.93 $\pm$ 1.85 <sup>b</sup>	55.11 $\pm$ 1.78 <sup>b</sup>

**Table 3:** Haematological Parameters in *C. gariepinus* juveniles Females exposed to Atrazine and Metalochlor for 14 days (Mean  $\pm$  S.D).

Parameters	Concentrations of Atrazine and Metalochlor (mg/L)					
	0	0.1	0.2	0.3	0.4	0.5
Hb (gdl)	13.61 $\pm$ 0.111 <sup>d</sup>	11.21 $\pm$ 0.14 <sup>d</sup>	10.11 $\pm$ 0.11 <sup>c</sup>	9.11 $\pm$ 0.12 <sup>b</sup>	7.95 $\pm$ 0.17 <sup>b</sup>	7.20 $\pm$ 0.16 <sup>a</sup>
RBC (Cells $\times$ 10 <sup>12</sup> )	7.40 $\pm$ 0.64 <sup>d</sup>	6.11 $\pm$ 0.18 <sup>c</sup>	5.90 $\pm$ 0.12 <sup>b</sup>	5.61 $\pm$ 0.11 <sup>b</sup>	5.20 $\pm$ 0.14 <sup>a</sup>	4.51 $\pm$ 0.11 <sup>a</sup>
PCV (%)	39.11 $\pm$ 1.64 <sup>c</sup>	38.11 $\pm$ 1.871 <sup>c</sup>	30.21 $\pm$ 1.62 <sup>b</sup>	29.21 $\pm$ 1.11 <sup>b</sup>	13.22 $\pm$ 1.18 <sup>d</sup>	10.14 $\pm$ 1.21 <sup>a</sup>
WBC (Cells $\times$ 10 <sup>9</sup> )	24.00 $\pm$ 1.92 <sup>a</sup>	22.00 $\pm$ 1.811 <sup>a</sup>	27.12 $\pm$ 1.71 <sup>a</sup>	29.14 $\pm$ 1.26 <sup>b</sup>	34.94 $\pm$ 1.26 <sup>c</sup>	40.81 $\pm$ 2.02 <sup>c</sup>
Neutrophils (%)	40.21 $\pm$ 1.11 <sup>a</sup>	40.21 $\pm$ 1.71 <sup>a</sup>	65.00 $\pm$ 2.21 <sup>d</sup>	51.00 $\pm$ 1.18 <sup>b</sup>	54.60 $\pm$ 2.14 <sup>c</sup>	59.33 $\pm$ 7.61 <sup>d</sup>
Monocytes (%)	44.21 $\pm$ 0.12 <sup>a</sup>	5.2 $\pm$ 0.14 <sup>a</sup>	5.21 $\pm$ 1.12 <sup>b</sup>	7.9210.27 <sup>b</sup>	8.99 $\pm$ 1.06 <sup>dc</sup>	9.02 $\pm$ 1.21 <sup>c</sup>
Lymphocytes (%)	59.21 $\pm$ 6.41 <sup>d</sup>	54.21 $\pm$ 4.84 <sup>d</sup>	49.21 $\pm$ 3.97 <sup>c</sup>	46.21 $\pm$ 2.61 <sup>b</sup>	41.11 $\pm$ 3.14 <sup>b</sup>	35.62 $\pm$ 2.69 <sup>a</sup>
Platelets (%)	614 $\pm$ 0.11 <sup>c</sup>	510.00 $\pm$ 0.12 <sup>b</sup>	422.00 $\pm$ 0.12 <sup>b</sup>	341.00 $\pm$ 0.14 <sup>b</sup>	321.00 $\pm$ 0.14 <sup>a</sup>	279.33 $\pm$ 0.12 <sup>a</sup>
MCV (fl)	52.85 $\pm$ 3.86 <sup>a</sup>	62.37 $\pm$ 2.79 <sup>a</sup>	51.20 $\pm$ 3.63 <sup>b</sup>	52.06 $\pm$ 4.13 <sup>b</sup>	25.42 $\pm$ 3.28 <sup>b</sup>	22.48 $\pm$ 3.22 <sup>c</sup>
MCH (pg)	18.39 $\pm$ 2.28 <sup>a</sup>	18.34 $\pm$ 1.86 <sup>a</sup>	17.13 $\pm$ 2.23 <sup>a</sup>	16.23 $\pm$ 1.79 <sup>a</sup>	15.28 $\pm$ 1.38 <sup>a</sup>	15.96 $\pm$ 2.75 <sup>b</sup>
MCHC (%)	34.79 $\pm$ 1.84 <sup>d</sup>	29.41 $\pm$ 2.79 <sup>d</sup>	33.46 $\pm$ 3.25 <sup>c</sup>	31.18 $\pm$ 1.85 <sup>b</sup>	60.13 $\pm$ 1.78 <sup>b</sup>	71.00 $\pm$ 1.73 <sup>a</sup>

Means with the same superscript in the row are not significantly different (P>0.05)

**Table 4:** Pooled Data of Haematological Parameters in *C. gariepinus* juveniles exposed to Atrazine and Metalochlor for 14 days (Mean  $\pm$  S.D).

Parameters	Concentrations of Atrazine and Metalochlor (mg/L)					
	0	0.01	0.02	0.03	0.04	0.05
Hb (gdl)	12.40 $\pm$ 0.83 <sup>d</sup>	11.20 $\pm$ 0.04 <sup>d</sup>	10.55 $\pm$ 0.01 <sup>c</sup>	10.05 $\pm$ 0.12 <sup>b</sup>	8.93 $\pm$ 0.17 <sup>b</sup>	7.7 $\pm$ 0.16 <sup>a</sup>
PCV (%)	37.55 $\pm$ 1.64 <sup>c</sup>	36.05 $\pm$ 1.871 <sup>c</sup>	31.10 $\pm$ 1.62 <sup>b</sup>	29.10 $\pm$ 1.11 <sup>b</sup>	15.61 $\pm$ 1.18 <sup>d</sup>	10.07 $\pm$ 1.21 <sup>a</sup>
WBC (Cells $\times$ 10 <sup>9</sup> )	22.5 $\pm$ 1.92 <sup>a</sup>	21.5 $\pm$ 1.811 <sup>a</sup>	25.56 $\pm$ 1.71 <sup>a</sup>	28.64 $\pm$ 1.26 <sup>b</sup>	33.94 $\pm$ 1.26 <sup>c</sup>	38.76 $\pm$ 2.02 <sup>c</sup>
Neutrophils (%)	40.10 $\pm$ 1.11 <sup>a</sup>	40.60 $\pm$ 1.71 <sup>a</sup>	55.5 $\pm$ 2.21 <sup>d</sup>	50.55 $\pm$ 1.18 <sup>b</sup>	53.60 $\pm$ 2.14 <sup>c</sup>	77.22 $\pm$ 7.61 <sup>d</sup>
Monocytes (%)	24.300 $\pm$ 0.12 <sup>a</sup>	5.19 $\pm$ 0.14 <sup>a</sup>	7.31 $\pm$ 1.12 <sup>b</sup>	7.07.27 <sup>b</sup>	8.08 $\pm$ 1.06 <sup>dc</sup>	8.86 $\pm$ 1.21 <sup>c</sup>
Lymphocytes (%)	58.43 $\pm$ 6.41 <sup>d</sup>	54.60 $\pm$ 4.84 <sup>d</sup>	49.60 $\pm$ 3.97 <sup>c</sup>	45.66 $\pm$ 2.61 <sup>b</sup>	40.61 $\pm$ 3.14 <sup>b</sup>	35.87 $\pm$ 2.69 <sup>a</sup>
Platelets (%)	557.5 $\pm$ 0.11 <sup>c</sup>	490.06 $\pm$ 0.12 <sup>b</sup>	436.05 $\pm$ 0.12 <sup>b</sup>	371.00 $\pm$ 0.14 <sup>b</sup>	351.07 $\pm$ 0.14 <sup>a</sup>	289.33 $\pm$ 0.12 <sup>a</sup>
MCV (fl)	52.88 $\pm$ 3.86 <sup>a</sup>	59.58 $\pm$ 2.79 <sup>a</sup>	53.62 $\pm$ 3.63 <sup>b</sup>	51.04 $\pm$ 4.13 <sup>b</sup>	53.56 $\pm$ 3.28 <sup>b</sup>	24.80 $\pm$ 3.22 <sup>c</sup>
MCH (pg)	17.46 $\pm$ 2.28 <sup>a</sup>	18.51 $\pm$ 1.86 <sup>a</sup>	18.18 $\pm$ 2.23 <sup>a</sup>	18.36 $\pm$ 1.79 <sup>a</sup>	19.20 $\pm$ 1.38 <sup>a</sup>	18.96 $\pm$ 2.75 <sup>b</sup>
MCHC (%)	33.02 $\pm$ 1.84 <sup>d</sup>	31.06 $\pm$ 2.79 <sup>d</sup>	33.92 $\pm$ 3.25 <sup>c</sup>	34.53 $\pm$ 1.87 <sup>b</sup>	57.20 $\pm$ 1.78 <sup>b</sup>	76.46 $\pm$ 1.73 <sup>a</sup>

Means with the same superscript in the row are not significantly different (p>0.05).

the chemical. While significant increases (P<0.05) comparable to control were recorded in the values of WBC, Neutrophils, MCH, and MCHC in both male and female fish.

## Discussion

The temperature values recorded during the experiment maybe

considered to be good enough for the normal growth of *Clarias gariepinus* as it falls within the range reported by Hogendoorn (1983) at 27.5-32°C for optimal growth of fish. The chemical also did not significantly change the pH of the various treatments, such that control and treatment were all slightly alkaline. The values of dissolved oxygen recorded for all treatment were below recommended values of 5-6 ppm for the rearing of *Clarias*

*gariiepinus* (Janseen, 1985). This may be due to the fact that water was contaminated by the toxicants and therefore the amount of dissolved oxygen was relatively low. There was an increase in the values of alkalinity and conductivity. The rise in alkalinity and conductivity falls in line with the study at Eleiyele reservoir at Ibadan, Nigeria (Mombeshora *et al.*, 1981), where all the physical and chemical parameters were within acceptable limits for fishes, but were increased above tolerant limits as the parent river was used as a depository for wastes. The changes in the physical and chemical composition of natural aquatic environment from internal and external influences and their effects on fauna and flora are well documented (Binay *et al.*, 1994).

The result obtained in the exposure of *C.gariiepinus* to different concentrations of atrazine and metalochlor indicated that there was increase in WBC, neutrophils, monocytes, MCH, MCHC in both male and female *C. gariiepinus*. This falls in line with the works of Akinrotimi *et al.* (2011b) on acute haematological study of cichlid fish. *Sarotherodon melanotheron* exposed to toxicants. The rise in WBC, neutrophils and monocytes shows an immune response to the toxicants. The result is in agreement with work of Akinrotimi and Gabriel (2012a) on submission of remarkable richness of toxicants on the fish blood, where they found that more of these white blood cells and its components are recruited to combat the stressor in the blood stream of the fish. The result shows the values of RBC, Lymphocytes, platelets, haemoglobin and packed cell volume of *C. gariiepinus* were higher in the control group than the experimented group exposed to Atrazine/Metolachlor for 14 days, which was in agreement with the studies of Akinrotimi *et al.* (2011c) and Oriakpono *et al.* (2012). The derived variables of the haemoglobin indices such as MCV, and MCH excluding MCHC were observed to be lower than expected and the reason could be as result of the size, and age of fish used and duration of exposure (Gabriel *et al.*, 2011; Akinrotimi *et al.*, 2012a).

Literature shows that changes in haematological indices of fish caused by toxicants and their responses varied depending on the species, chemical and duration of exposure. They are also influenced by the type, quality and concentration of the chemicals in the water. These factors can cause reversible and irreversible alterations in the haematological parameters of fish (Sovio *et al.*, 2004; Cameron, 2010; Akinrotimi *et al.*, 2012b). It is well known that Atrazine/metolachlor promotes the reticence of cells, causes premature mortality of mature red blood cells and inhibition of haemoglobin formation. This lead to anaemia at high exposures and erythropoiesis at lower exposure of the chemical (Hodson *et al.*, 1984; Akinrotimi *et al.*, 2010a). In the light of the present study, the mean value of PCV decreased progressively in the experimented group compared to the control. The result agreed with the work of Akinrotimi *et al.* (2009) in haematological indices of *Tilapia guineensis* subjected to handling stress. The decrease in the PCV indicates the worsening of the condition of the organism and developing of anaemia.

Haemoglobin concentration reveals the status of an organism oxygen level and the organism itself tries to maintain them as much as possible in the face of any stressor. This study shows that the mean haemoglobin reduced considerably when compared to

the control values. Reduction in the values of haemoglobin in the blood of exposed fish, is usually caused by the effect of chemicals on the blood, as well as decrease in its oxygen carrying capacity, which also imply anaemia or validate the toxic effect of Atrazine/metolachlor on *Clarias gariiepinus* (Akinrotimi *et al.*, 2010b; Gabriel *et al.*, 2011).

A dose dependent reduction in RBC level was observed in *C. gariiepinus* treated with atrazine/metolachlor. Haematological indices like RBC count, concentration of haemoglobin and PCV have been reported to indicate secondary responses of an organism to pollutants (Rogers *et al.*, 2003; Akinrotimi *et al.*, 2012b; Akinrotimi *et al.*, 2012c). The MCV, MCH, and MCHC increased considerably in all treatment compared to the control. The increase in MCV, MCH and MCHC is in agreement with the work of Ariweriokuma *et al.* (2011) following a short term exposure of *C. gariiepinus* to cypermethrin in the laboratory. These alterations were attributed to direct or feedback response of structural impairment to RBC membranes resulting in haemolysis and impairment in haemoglobin production, stress-related release of RBCs from the spleen and hypoxia stimulated by exposure to atrazine and metolachlor (Shah, 2006; Nte and Akinrotimi, 2011; Akinrotimi and Gabriel, 2012b).

## Conclusion

The result obtained in this study revealed that atrazine/metolachlor is highly toxic to the *C. gariiepinus*. Toxicity of atrazine/metolachlor on *C. gariiepinus* increased with increasing concentration of the pesticides. In the aquatic environment, this can negatively affect the ecosystem. Although, the aquatic environment is not the target of such pesticide but the widespread use of them has led to some serious problem in aquatic biota. Hence discharge of this pesticide in aquatic environment should be restricted.

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