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# Toxicity of Butachlor on Juveniles of *Clarias gariepinus* (Burchell, 1822)

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## ABSTRACT

*Clarias gariepinus* is an important valued fish widely culture and occur naturally in freshwater. Herbicides produce cumulative deleterious effects thereby reducing the survival and growth of fish. The acute and sub-lethal toxicity of the Butachlor on Catfish were investigated. The fish were exposed to 0 mg/L, 10 mg/L, 15 mg/L, 20 mg/L, 25 mg/L and 30 mg/L acute concentrations of butachlor for 8weeks. Herbicides effects were measured using behavioural, heamatological, histopathological and growth parameters. The study revealed toxicity in a dose-dependent manner. Erratic swimming, gulping of air, restlessness, loss of balance, excessive secretion of mucus and finally death were observed in the exposed fish. Beats of the tail and operculum increased at 12 and 24 hours with increase in concentration, except at the 48th hour. The lethal concentration ($LC_{50}$) value of butachlor was 14.08 mg/L for 96 hours of exposure. Mean Red Blood Cells (RBC), Haemoglobin content (Hg), Packed Cell Volume (PCV), mean corpuscular volume (MCV) and neutrophils decreased as the concentration of toxicant increased while White blood cell (WBC), mean corpuscular haemoglobin concentration (MCHC) and lymphocyte increased with increase in the toxicant concentration. Histopathological changes of gills include high proliferation of nuclei, total obliteration of the general morphology, loss of epithelial and mucous cells, and presence of hemorrhage. In the liver, there was compressed nuclear architecture, evidence of enucleated cytoplasm reflecting necrosis and distortion of periportal vein. There was significant decrease ($p<0.05$) in growth rates and nutrient utilization. Its concluded that butachlor was moderately toxic to *Clarias gariepinus*.

**Keywords:** *Clariid fish,* Toxicity, Butachlor, Juveniles and Herbicides

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# INTRODUCTION

*Clarias gariepinus* belongs to the claridae family, which is extremely hardy and can withstand adverse environmental conditions and habitats instabilities. This species of fish does not easily succumb to diseases and is capable of feeding on all kinds of biowastes. *Clarias gariepinus* feeds on living, as well as dead animal matters, and is also capable of efficiently assimilating a wide variety of both animal and plant proteins. It is widely cultivated and found in water bodies in Nigeria hence used as biological indicators in ecotoxicological studies [1].

*Clarias gariepinus* has an average adult length of 1 - 1.5m and reach a maximum length of 1.7m and can weigh up to 60kg. Usually have slender bodies, flat bony heads, and broad terminal mouth with four pairs of barbels, having a well composed and modified gill arches with only the pectoral fin having spines [2].

Aquatic bioassays are necessary in water pollution control to determine whether a potential toxicant is dangerous to aquatic life and find a relationship between the toxicant concentration and its effects on the animals, therefore effective toxicology is the possible sure method of checking aquatic life preservation [3].

Herbicides are widely used for control of water plants which slow the movement of water mostly during the summer seasons. These agents used against pests, undesirable herbs and agricultural diseases were found to have adverse effects on the environment. As herbicides kill weeds, so they can kill human beings. Many of these chemicals are mutagenic and linked to the development of cancers as well as developmental defects [4].

Butachlor, (2-chloro-N-[2, 6-diethylphenyl] acetamide) is one of the most widely used chloroacetanilide herbicide for the control of annual grasses in rice fields and many broadleaf weeds. It can also be used in seed beds and seed transplant fields as well as in some crop fields such as wheat, barley, cotton, vegetables and peanuts. As rice fields are mainly located alongside rivers, this herbicide is often released into rivers and can affect its inhabitants. Butachlor is one of widely used herbicides in the northern region of Iran and its concentration in the Shahid Beheshti sturgeon fish hatchery has already been reported to be 0.67 ppb [5].

Butachlor is stable in the soil of farmlands and water systems. The remnants of the pesticide can enter ground waters used for human consumption and thus can be potentially hazardous for human health. The pesticide can degrade rapidly but under conditions of low temperature, low moisture, high alkalinity and lack of suitable microbial degraders, it may remain biologically active and persist in soils for a long time. Increase in sunlight enhanced photo degradation of butachlor in water and that the half-life of the herbicide in non-filtered river water was shorter than filtered samples. Butachlor has also been reported to be carcinogenic and can adversely disrupt the reproductive process and affect the thyroid and sex steroid hormones in Zebra fish [6].

Herbicides at certain high concentrations are known to reduce the survival, growth and reproduction of fish, and may also produce many visible effects on fish, usually concentrate in the tissues of the fish thereby producing cumulative deleterious effects by reducing the population of the fish or retarding their growth [7]. The research was aimed at determining toxicity of butachlor on the juvenile of *Clarias gariepinus.*

### Sample Collection

Juveniles of *Clarias gariepinus* of mixed sexes and fairly uniform size were obtained from Narayi fishpond in Kaduna, at latitude $10.48^0$N and longitude $7.45^0$E. The fish was transported at $30^0$C in a plastic container to the Zoological Laboratory, Department of Biological Sciences, Kaduna State University.

### Preparation of Bioassay Test Solution

The stock solution contains 500g/L of Butachlor and the definitive concentration for the bioassay were 10, 15, 20, 25 and 30ml respectively using dilution formular (1ml of the stock solution was diluted into 9ml of distilled water). 20 litres of dechlorinated tap water was poured into each plastic tanks while 10, 15, 20, 25 and 30mg/L of water were removed and replaced with butachlor from the stock solution in each of the respective plastic tanks to topped it to 20 litres mark. This was done same for sub-lethal bioassay [8].

### Acute toxicity test

Juveniles of fairly equal weight, total length and standard length were selected randomly, weighed and distributed into the plastic aquaria containing the treatments. The bioassay test was carried out in 15 plastic tanks each of size 44 x 29.5 x 24cm into which approximate quantity of butachlor were taken and to give a final volume of 20liters, with 3 plastic tanks which served as the control. The mixture was allowed to stand for 5 minutes to be evenly distributed via diffusion before the introduction of the fish. The solutions were stirred for homogenous mixing before each aquarium were randomly stocked in triplicates with 10 juveniles of fish while the test solution and control were renewed daily [9].

### Behavioural Studies of *Clarias gariepinus* Exposed to Butachlor Treatment

Following exposure of fish to the treatments, observations were made on the behavioural and morphological responses of the fish at 12, 24, 48, 72 and 96 hours. Control fish were also monitored along with the toxicant concentrations to provide a reference for assessing any behavioural or morphological changes. The behavioural and morphological characteristics that were monitored are erratic swimming, loss of equilibrium, general activity, increased excitability, mortality, vertical suspension, mucous secretion, startle response, deformities and haemorrhage. Each test tank was observed for 10 to 15 minutes which allowed sufficient time for an accurate evaluation of each fish [10].

### Opercular ventilation/tail fin movement rates

The opercular ventilation count and tail fin movement rates were counted using stop watch at 12, 24, 48, 72 and 96 hours per minutes [11].

### Mortality rate of $LC_{50}$

Juveniles of *Clarias gariepinus* were considered dead when there was no sign of opercular movement or no response to gentle prodding. The time and number of dead fish in each group were recorded. The dead fish were removed immediately to avoid fouling [12].

### Sub-lethal Test

Sub-lethal concentration of 1/10, 1/15 and 1/20 of 96 hours $LC_{50}$ was used to determine sub-lethal concentration; 1.4mg/l, 0.7mg/l, 0.5mg/l and 0mg/l served as the control.

At the time of exposure, fresh solutions were added in every 12 hours to maintain the concentration level before the wastes were siphoned out using rubber tube. The fish were fed on 35% crude protein feed at 5% body weight. Two fish per each replicate were sacrificed biweekly to isolate gill and liver which were stained using haematoxylin and Eosin for histopathological studies and the blood for haematological studies [13].

## Haematological Test

### Blood sampling

The blood was collected from the caudal artery at 2cm away from the caudal peduncle using a micro capillary and transferred into a sampling EDTA bottle treated with anticoagulant [14].

### Total Leucocytes Count

Leucocytes were counted by using Shaw's solution A (neutral red (25mg), sodium chloride (0.9g), distilled water (100mls) and B (crystal violet (12mg), sodium citrate (3.8g), distilled water (100mls)). The blood was drawn up to the 0.5 mark on the stem of a white cell pipette. Solution A was drawn to shake the bulb of the pipette half way and then filled to 101 mark with solution B. A few drops were dispensed in to the haemocytometer. The cells in the four large squares of the chamber were counted with 4mm objective and X40 eyepiece microscope. The number of cells were multiplied by 500 to obtain the total number of leucocytes per cubic millimeter (mm3) of blood [15].

### Total erythrocyte count

Hendricks solution was used for the erythrocyte count. Blood was drawn just beyond 0.5 mark of the haemoglobin pipette wiped with cotton wool and adjusted the volume to exactly 0.5 mark. The pipette was filled to 101 mark with the diluting fluid and shaken for 30 minutes to ensure thorough mixing. The diluted suspension of cells was thereafter drawn in to the Neubauer's chamber haemocytometer. The haemocytometer was placed under the microscope and the cells within the boundaries of five small squares of the haemocytometer were counted with 4mm objectives and x40 eyepiece of the microscope. The number of cells were multiplied by x10 and this gave the total number of cells per cubic millimeter (mm3) of blood [15].

### Packed Cell Volume

Micro-westegreen method was used to determine the packed cell volume (PCV). Blood samples were collected in heparinised capillary tubes. The end of the tubules was sealed with plasticine and the tubules spun for three minutes in a microhaematocrit centrifuge (Gallenkamp model) at a

speed of 12,500rpm. The haematocrit was measured with a micro-capillary reader to obtain the value of the packed cell volume (PVC) [15].

**Haematological Studies of *Clarias Gariepinus* Exposed to Sub-Lethal Concentrations of Butachlor**

Haematological indices were determined by an automated haemoglobin analyser (Cobus u 411) model. Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin(MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated by using:

$$MCV\ (m^3) = \frac{\text{Hematocrit (\%) x 10}}{\text{RBCC (cells/mm3)}} \quad [16]$$

Where,

RBCC= Red Blood Cell Count

MCV=Mean Corpuscular Volume

$$MCH\ (g/cell) = \frac{\text{Hemaglobin(g100ml) x10}}{\text{RBCC(cells/mm3)}} \quad [16]$$

Where,

RBCC= Red Blood Cell Count

MCH=Mean Corpuscular Haemoglobin

$$MCHC\ (G/100ml) = \frac{\text{Hb(g/100ml) x 100}}{\text{Hematocrit}} \quad [17]$$

**Histopathological Assessment of the Gills and Liver of juveniles of *Clarias gariepinus***

The gills and liver were obtained by severing the opercular and the liver by opening the stomach and fixed in formal-saline. The tissues were washed in running tap water for at least 2 hours to remove traces of formalin. This was followed by dehydration using successive percentages of alcohol (30, 50, 70, 90 and 100%). They were then infiltrated in chloroform and blocked in paraffin wax 58-60$^0$C melting point. Samples were embedded in fresh molten wax using L-shaped embedding molds. Sections of 8µm thickness were cut and stained in haematoxylin and eosin (H&E). Permanent slides were prepared with these sections and microphotographs taken with a magnification of X400. This were examined and compared with those for control. This method was adopted by [18].

**Growth Parameters**

*Clarias gariepinus* were acclimatized in tanks for two weeks, during which they were fed with commercial feeds. The fish were fed at 3% body weight because feeding was observed to be reduced. The average weight of the fish at commencement of the experiment was taken, and same at the end of the experiment [19].

Weight length of fish was measured using an electrical weighing balance and meter rule respectively at two weeks interval.

**Weight gain** (WG) = Fw-Iw [20]

Where,

WG = Weight gain

Fw = Final weight

Iw = Initial Weight

**Percentage Live Weight Gain (LWG %)**

Percentage live weight gain were computed as the difference between the initial and final fish weight, divide by the initial weight expressed as percentage.

$$LGW = \frac{Wt - Wo}{Wo} \times 100 \quad [21]$$

Where:

Wo = initial fish weight (g)

Wt = final fish weight (g)

**Specific Growth Rate (SGR)** was calculated as decribed by Hepher (1988)

$$SGR = \frac{LogWt - LogWo}{t - to} \quad [22]$$

Where:

Wt = final weight

Wo = initial weight

t - to = time interval between initial and final weight (days) or period of study in days.

**Feed Conversion Ratio (FCR)** was calculated as the dry weight of feed offered divided by the weight gain of fish

$$FCR = \frac{\text{Weight of feed offered (g)}}{\text{Net weight of fish (g)}}$$

**Protein Efficiency Ratio (PER)** was composed as a ration of fish weight gain to the crude protein consumed

$$PER = \frac{\text{Fish wet weight gain (g)}}{\text{Crude protein fed (g)}} \quad [22]$$

**Data Analysis**

Data generated from this experiment was subjected to Analysis of Variance (ANOVA) using SAS to determine the significant differences between means. Duncan's Multiple Range Test (DMRT) was used to separate the significantly different means.

# RESULTS

## Acute Toxicity Test

**Behavior**

The behavioural reactions on juveniles of *Clarias gariepinus* immediately introduced into the tanks containing the different treatments (10, 15, 20, 25 and 30mg/l) of butachlor herbicide in the order of their appearance were, agitated swimming, loss of equilibrium, opercular ventilation count, tailfin movement and period of quiescence. Fishes came to the surface of water much more frequently and occasionally tried to jump out of the water. As the activities decreased, the fishes became sluggish and lethargic. As the time of exposure increased fish stood in vertical position with their heads above the water surface. The fish showed abnormal swimming movements including loss of buoyancy, loss of orientation and spasm before death occurred. These reactions to the toxicants were more pronounced in tanks with higher concentrations of butachlor.

The fishes in the control tanks were swimming freely at the middle of the test tanks. The result showed that the symptoms were dose dependent for each tank containing the toxicant. The fish certified dead had swollen abdomen and their mouth wide opened, and there was also copious accumulation of mucus observed in the gill's filaments and body surface.

## Respiratory Rate

**Opercular ventilation beat**

Juveniles of *Clarias gariepinus* exposed to butachlor showed increased opercular ventilation and tail fin beat with increase in the concentration of the toxicant for 10, 15, 20, 25 and 30mg/L respectively. The result showed that the opercular count of the exposed fish at 12 and 24 hours were higher than that of the control fish (figure 1).

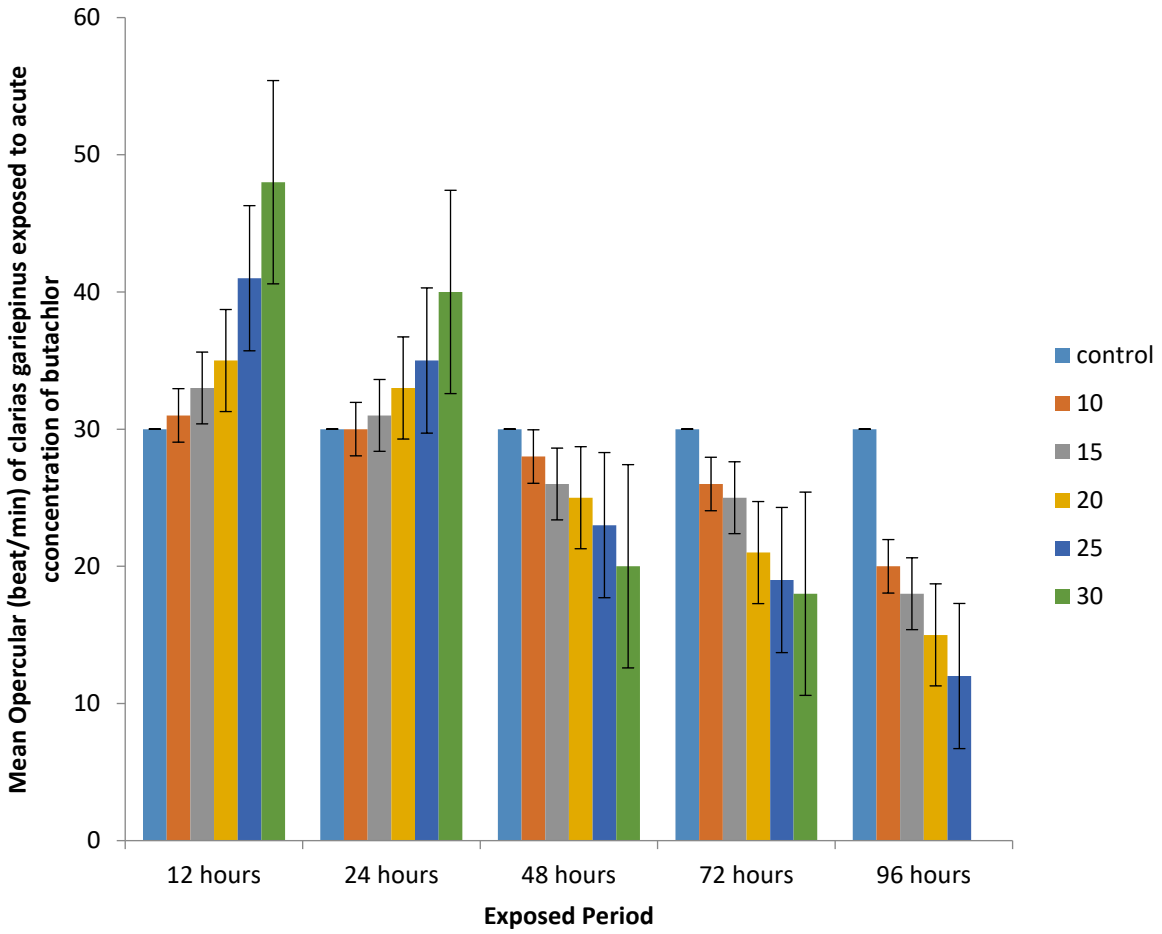


**Figure 1: Opercular ventilation rate of *Clarias gariepinus* exposed to acute concentrations of Butachlor**

## Tail fin beat

*Clarias gariepinus* exposed to butachlor showed increased tail fin beat with increase in the concentration of the toxicant of 10, 15, 20, 25 and 30mg/L. The 30mg/L concentrations had the highest mean values 52 and 48 respectively, with the control tank having 28 and 28 respectively. As the duration of exposure progresses, it led to more decrease in tail fin beat of the fish. There was significant difference between the tail fin beat of the treated fish (Figure 2).

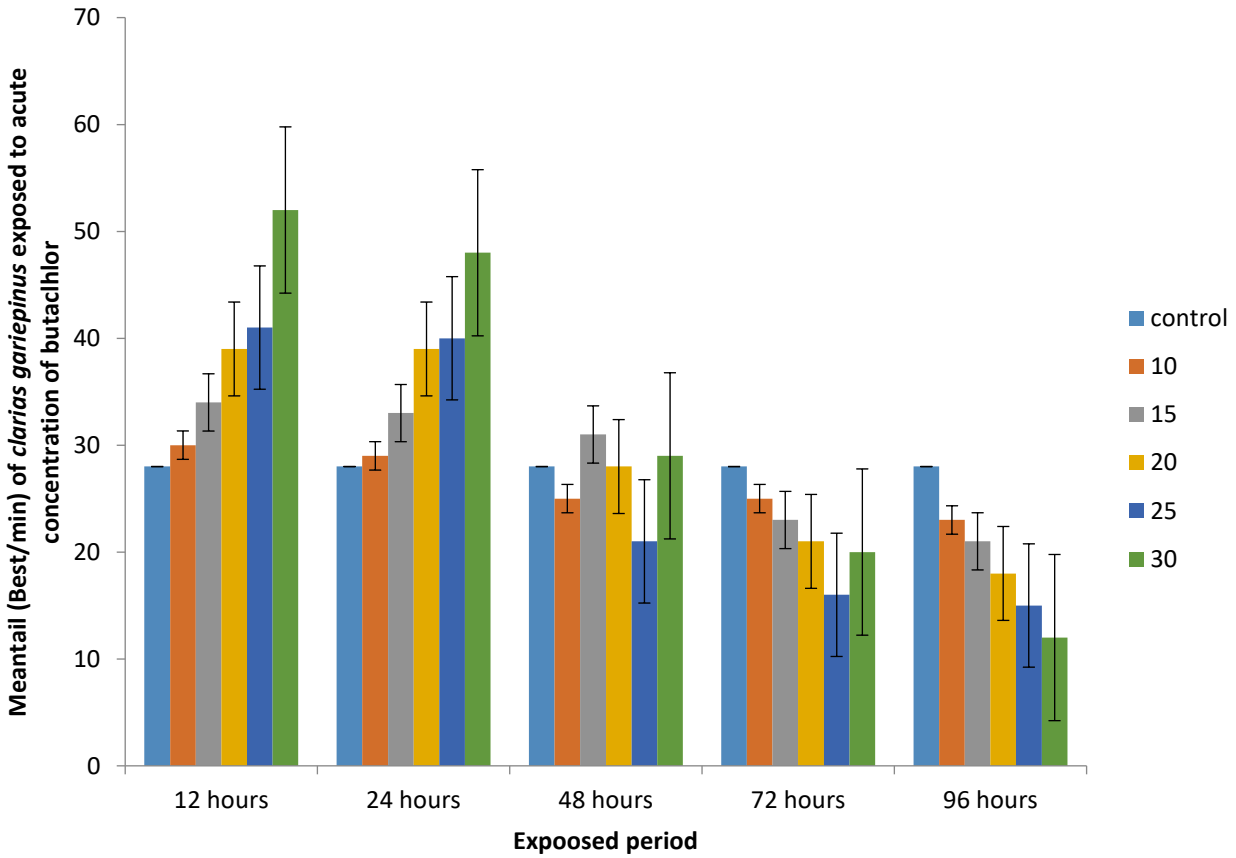


**Figure 2: Tail fin movement rate of *Clarias gariepinus* exposed to acute concentrations of Butachlor**

## Mortality Rates and Log Concentration in *Clarias gariepinus* exposed to Acute Concentration of Butachlor

The mortality rates and log of the concentration in *Clarias gariepinus* exposed to acute concentration of butachlor indicates that the concentrations; 10.0, 15.0, 20.0, 25.0 and 30.0mg/L had mortality rates of 6, 12, 14,15 and 17, there was no mortality observed in the control tank. 30.0mg/L had the highest mortality rate. The lowest mortality rate was observed in 10.0mg/L concentration.

**Table 1 Mortality Rates and Log of Concentration in *Clarias gariepinus* Exposed to Acute Concentration of Butachlor**

| Concentration mg/l | Mortality rates | % Mortality | Probit kill |
|---|---|---|---|
| 0 | 0 | 0 | 3.0 |
| 10 | 6 | 30 | 4.4 |
| 15 | 12 | 60 | 5.3 |
| 20 | 14 | 70 | 5.4 |
| 25 | 15 | 75 | 5.5 |
| 30 | 17 | 85 | 6.0 |

Values representing mortality rates and values of log conc. at different concentration.

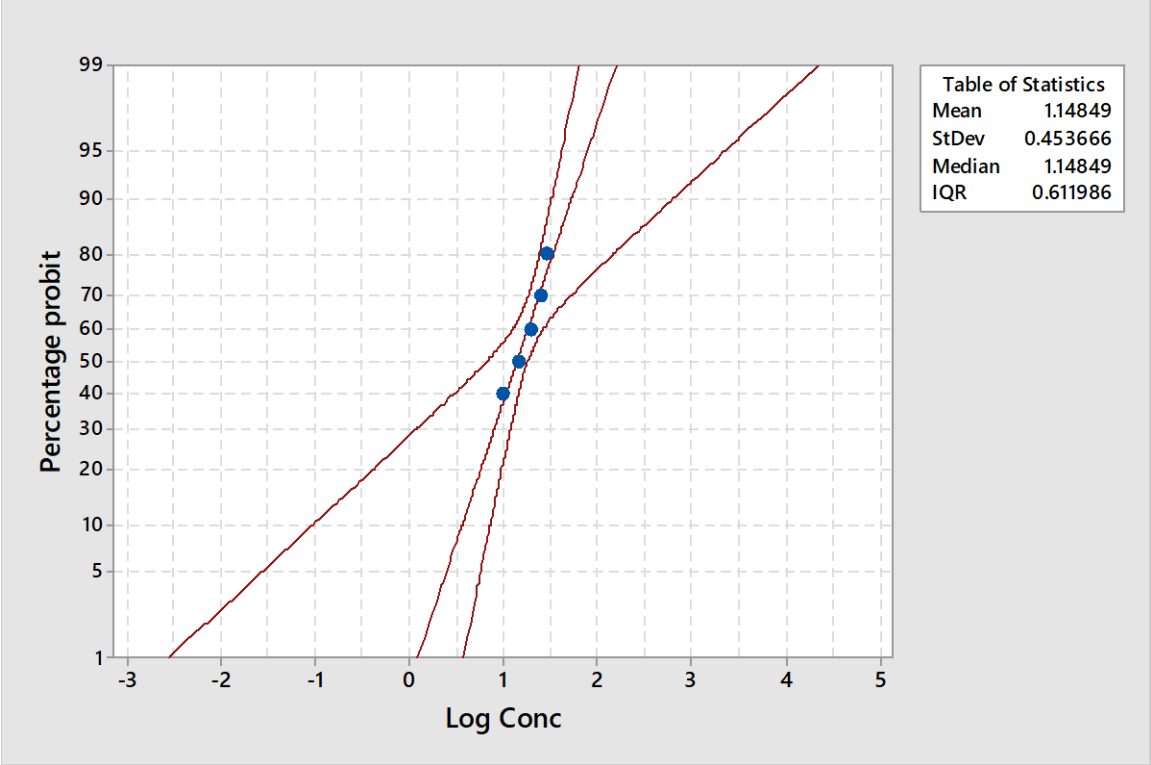


**Figure 3: 96-hours median lethal concentration ($LC_{50}$) of *Clarias gariepinus* juveniles exposed to butachlor**

## Haematological parameters of *Clarias gariepinus* exposed to sub-lethal concentration of Butachlor.

### Red Blood Cell (RBC)

The result of RBC from weeks 2 to 8, which was exposed to 0.47 mg/L, 0.71 mg/L and 1.45 mg/L showed a decrease in value compared to the control. The control has the highest value. The RBC

values of the control group were fairly constant. There was decrease in RBC values decreased from week 2 to 8 with increase in concentrations of toxicants (Table 2).

**Packed cell volume (PCV)**

The values of 2 and 8 weeks shows significant difference ($P<0.05$) in PCV. As the values of the toxicant increases there was decrease in PCV as observed in 0.47 mg/L, 0.71 mg/L and 1.45mg/L concentrations (Table 2).

**White blood cell (WBC)**

The result in Table 2 shows that WBC in all concentrations from weeks 2 to 8 increased with increase in 0.47 mg/L, 0.71 mg/L and 1.45 mg/L concentrations

**Haemoglobin (Hb)**

From weeks 2 to 8, as the concentration of the toxicants incr eases there was decrease in the Hb of the exposed fish. While the values of the control group were constant. With increase in the exposure time, Hb decreases showing a significant difference ($p<0.05$) as shown in Table 2.

**Mean corpuscular haemoglobin (MCH)**

Corpuscular haemoglobin showed an increase with increasing concentrations of the toxicant 0.47 mg/L, 0.71 mg/L and 1.45 mg/L concentrations (Table 2).

**Mean corpuscular volume (MCV)**

The result of weeks 2 to weeks 8 shows significant difference ($P<0.05$) in MCV. As the concentrations of the toxicants increased, there was decrease in value of MCV compared to the control tanks as shown in Table 2.

**Mean corpuscular haemoglobin concentration (MCHC)**

Table 2 shows significant difference ($P<0.05$) in MCHC values. At weeks 2 to 8 the MCHC values increased with increase in the concentrations of the toxicants, with control having the lowest values.

**Table 2 Sub-lethal Concentration of Butachlor on Some Heamatological parameters of *Clarias gariepinus***

| Parameters | Conc. (mg/L) | Weeks After Exposure | | | |
|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 |
| **RBC(x10$^6$mm$^3$)** | 0 | 5.28±0.32a | 5.27±0.06a | 5.27±0.06a | 5.26±0.05a |
| | 0.47 | 4.82±0.14b | 3.69±0.05c | 3.69±0.05c | 5.34±0.08a |
| | 0.71 | 3.44±0.08c | 4.46±0.04b | 4.46±0.04b | 4.77±0.33ab |
| | 1.45 | 3.22±0.04c | 4.50±0.04b | 4.50±0.04b | 4.44±0.04b |
| | P-value | 0.000 | 0.000 | 0.000 | 0.017 |
| **WBC(x500mm$^3$)** | 0 | 20.47±1.52a | 20.77±0.18b | 20.47±0.33a | 20.70±0.40b |
| | 0.47 | 21.83±1.62a | 21.50±0.63ab | 21.60±0.72a | 22.10±0.87ab |
| | 0.71 | 23.30±1.47a | 22.20±0.59ab | 22.10±0.92a | 22.60±0.72ab |
| | 1.45 | 23.50±2.97a | 23.30±0.97a | 22.53±1.31a | 23.23±0.67a |
| | P-value | 0.284 | 0.114 | 0.443 | 0.137 |
| **Hb(g/100ml)** | 0 | 13.77±1.06a | 13.87±0.61a | 13.87±0.80a | 13.97±0.71a |
| | 0.47 | 13.03±0.76a | 13.00±0.70a | 13.73±0.60a | 13.73±0.62a |
| | 0.71 | 12.73±1.11a | 12.73±0.64a | 13.63±0.52a | 13.60±0.44a |
| | 1.45 | 11.93±1.63a | 11.87±1.24a | 12.73±0.56a | 12.77±0.66a |
| | P-value | 0.358 | 0.455 | 0.596 | 0.566 |
| **PCV(%)** | 0 | 60.67±3.05a | 60.33±0.88a | 60.33±0.88a | 60.33±0.88a |
| | 0.47 | 33.33±2.52b | 30.33±0.88c | 42.20±0.47c | 49.23±0.67b |
| | 0.71 | 32.33±2.52b | 30.33±0.88c | 45.23±0.62b | 45.33±0.88c |
| | 1.45 | 30.33±1.53b | 46.33±0.88b | 30.13±0.52d | 30.20±0.53d |
| | P-value | 0.000 | 0.000 | 0.000 | 0.000 |
| **MCV(x10$^6$Pgcel)** | 0 | 115.67±1.20a | 115.33±0.88a | 115.60±0.65a | 115.10±1.18a |
| | 0.47 | 69.87±1.70c | 81.90±0.95c | 78.47±0.58c | 92.53±0.80c |
| | 0.71 | 95.07±1.42b | 68.67±1.20d | 101.50±0.55b | 102.33±0.88b |
| | 1.45 | 94.97±0.86b | 102.50±0.98b | 67.30±0.40d | 68.33±0.88d |
| | P-value | 0.000 | 0.000 | 0.000 | 0.000 |
| **MCH(x10$^6$Pgcel)** | 0 | 24.63±0.87d | 26.67±0.48b | 26.53±0.44b | 26.33±0.64b |
| | 0.47 | 26.87±0.38c | 28.83±0.90b | 25.13±0.52b | 25.57±0.56b |
| | 0.71 | 34.77±1.19b | 31.60±0.63a | 31.37±0.50a | 32.27±0.67a |
| | 1.45 | 43.67±1.00a | 31.60±0.67a | 31.37±0.56a | 32.27±0.71a |
| | P-value | 0.000 | 0.002 | 0.000 | 0.000 |
| **MCHC(g/100ml)** | 0 | 21.50±0.92d | 23.33±0.73c | 23.43±0.47d | 23.43±0.50d |
| | 0.47 | 34.83±1.05c | 44.40±1.23b | 32.17±0.61c | 28.17±0.50c |
| | 0.71 | 37.37±1.19b | 46.50±1.10ab | 40.67±0.76b | 40.43±0.38b |
| | 1.45 | 46.57±0.55a | 49.27±0.65a | 46.23±0.39a | 46.57±0.48a |
| | P-value | 0.000 | 0.000 | 0.000 | 0.000 |

Means with the same superscript along the columns are not significantly different (P>0.05)

## Leucocytes differential counts of *Clarias gariepinus* exposed to sub-lethal concentrations of butachlor.

### Lymphocytes

The results of 0.47mg/L, 0.71mg/L and 1.45mg/L concentrations of toxicants showed an increase in value compared to the control. While 1.45mg/L concentrations had the highest value. Lymphocytes values at weeks 2 to 8 increase with an increase in the concentrations of the toxicants (Table 3).

### Neutrophils

The control tanks had the highest values. The neutrophils values tend to decrease with increase in the concentration of the toxicant as shown in Table 3

**Table 3 The Effect of Sub-lethal Dose of Butachlor on Some Leucocytes Differential Count of *Clarias gariepinus***

| Parameters | Conc. (mg/L) | Weeks After Exposure | | | |
|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 8 |
| **Lymphocytes (%)** | 0 | 64.43±1.93a | 64.43±0.38a | 64.90±0.15a | 65.17±1.03a |
| | 0.47 | 66.57±2.46a | 65.47±0.60ab | 66.43±0.66a | 66.57±0.52a |
| | 0.71 | 67.33±1.18a | 67.03±0.96a | 66.33±0.49a | 66.63±1.00a |
| | 1.45 | 67.80±0.70a | 67.40±0.56a | 66.53±0.71a | 66.37±0.52a |
| | P-value | 0.156 | 0.040 | 0.196 | 0.563 |
| **Neutrophils (%)** | 0 | 33.53±0.85b | 33.17±0.73a | 32.57±0.80a | 32.47±0.66a |
| | 0.47 | 38.40±1.25a | 32.63±0.49ab | 31.40±0.36ab | 31.37±0.38a |
| | 0.71 | 31.43±1.14c | 31.40±0.46ab | 31.17±0.50ab | 31.17±0.87a |
| | 1.45 | 30.80±1.18c | 30.67±0.61b | 30.57±0.23b | 30.63±0.63a |
| | P-value | 0.000 | 0.058 | 0.122 | 0.322 |
| **Eosinophils (%)** | 0 | Nd | Nd | nd | Nd |
| | 0.47 | Nd | Nd | nd | nd |
| | 0.71 | Nd | Nd | nd | nd |
| | 1.45 | Nd | Nd | nd | nd |
| | P-value | Nd | Nd | nd | nd |
| **Monocytes (%)** | 0 | Nd | Nd | nd | nd |
| | 0.47 | Nd | nd | nd | nd |
| | 0.71 | Nd | nd | nd | nd |
| | 1.45 | Nd | nd | nd | nd |
| | P-value | Nd | nd | nd | nd |
| **Basophils (%)** | 0 | Nd | nd | nd | nd |
| | 0.47 | Nd | nd | nd | nd |
| | 0.71 | Nd | nd | nd | nd |
| | 1.45 | Nd | nd | nd | nd |
| | P-value | Nd | Nd | nd | nd |

Means with the same superscript along the columns are not significantly different (P>0.05).

nd = not detected

**Histopathology of *Clarias gariepinus* gills exposed to sub-lethal concentrations of butachlor**

The gills of the control fish and those of the treated fish exposed to different concentration of butachlor showed marked difference. The gills of the control fish had well supplied red blood cells, normal structure of filament with goblet cells and mucin and lamellae (plate I), with finger like projection (secondary lamellae) on each side and a well outlined nucleoplasmic architecture.

The gills of the treated fish were at various stages of distortion. Plate 4 with the highest concentration (1.45mg/l) of the toxicant, show high proliferation of nuclei which is an evidence of carcinoma, with total obliteration of general morphology.

There was also evidence of hemorrhage. In other tanks with lower concentrations there were mild differences in the gills when compared with the gills of the highest concentration. The gills gotten from tanks of 0.47mg/L in plate 2 had slight increase in red blood cells, increase in goblet cells and separation of the gill filaments at the surface border. The gills gotten from tanks of 0.71mg/L in plate 3 showed presence of hemorrhage, no definite goblet cells and no filaments showing evidence of total inflammation.

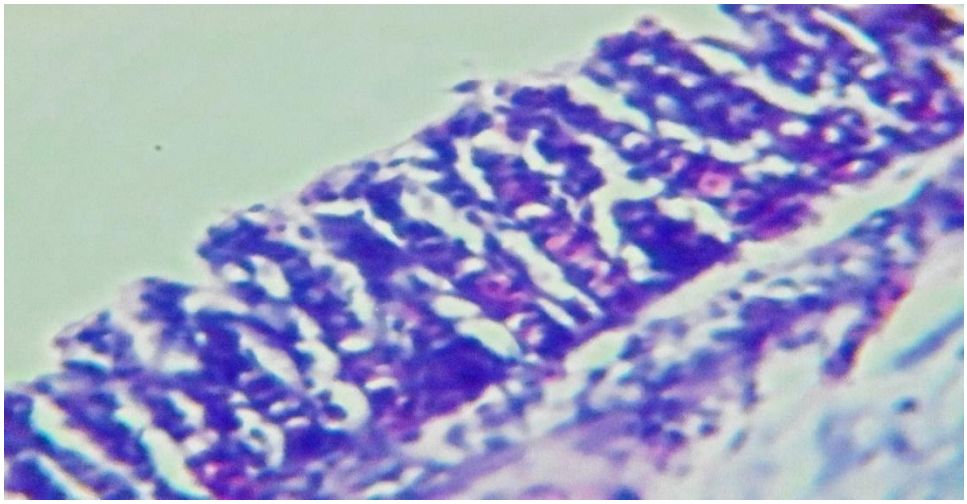

**Plate 1: Control 0.00mg/l. Photomicrograph of gill mg X40 well supplied with red blood cells B. compact filaments R raminified with goblet cells G and mucin M . Well outlined nucleoplasmic architecture.**

**Histopathology of *Clarias gariepinus* liver exposed to sub-lethal concentrations of butachlor**

The liver of the fish from tanks with the highest concentration (1.45mg/L) of the toxicant showed compressed nuclear architecture, there were evidence of enucleated cytoplasm reflecting necrosis and the periportal vein shows distorted outline with no visible blood cells. The liver from tanks with 0.71mg/L concentration also showed poor nuclear architecture with increase in the number of nucleus which was a sign of inflammatory reactions. 0.47mg/l concentration show the liver with good nuclear architecture, but thickened cell membrane and the periportal vein remain intact with visible blood cells.

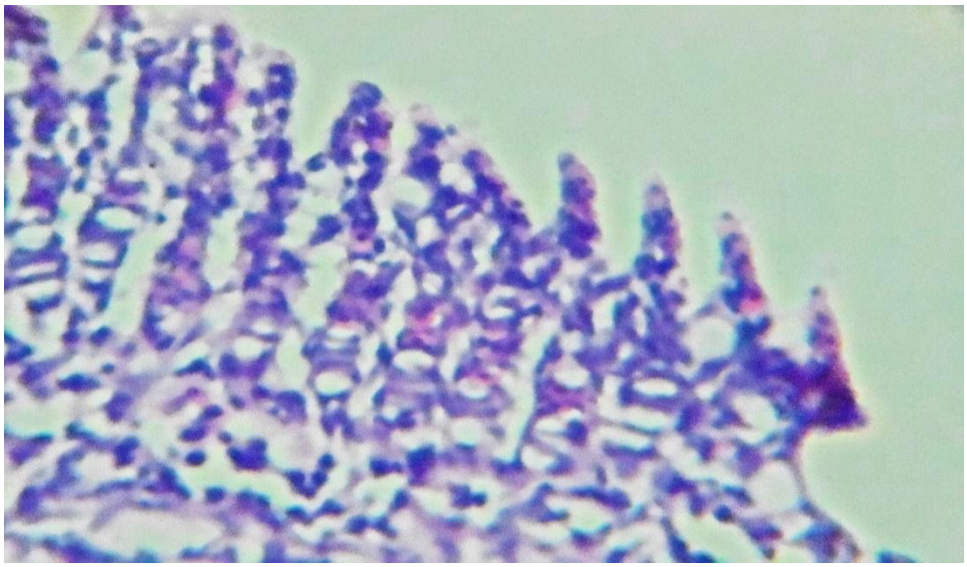

**Plate 2: 0.47mg/l. Photomicrograph of gill mg X40 with slight increase in red blood cells R. Well outlined nucleoplasmic architecture N, also increase in goblet cells, gill filaments G are relatively separated at the surface border indicating an earlier detachment**

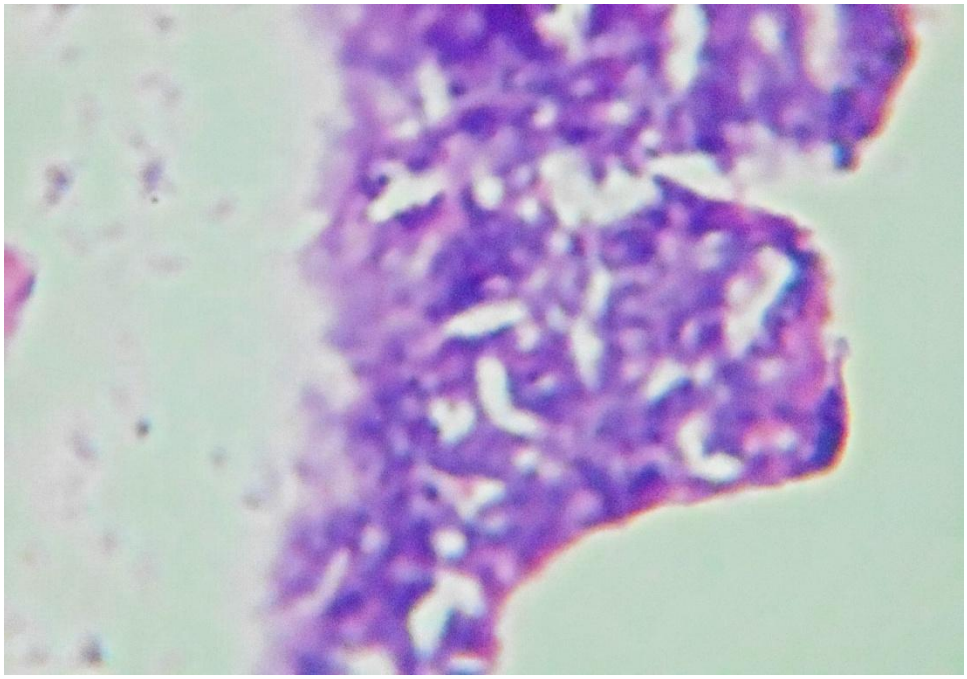

**Plate 3: 0.71mg/l Photomicrograph of gill mg X400 hemorrhage H no definite goblet cells and no filaments there is evidence of total inflammation I**

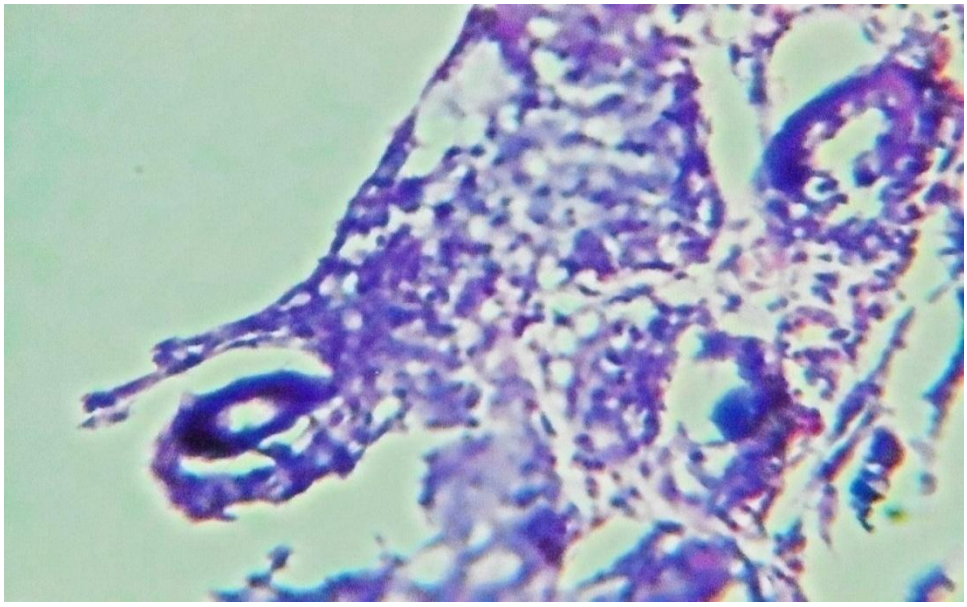

**Plate 4: 1.45mg/l. Photomicrograph of gill mg X400. High proliferation of nuclei N an evidence of carcinoma with total obliteration of general morphology. There is also evidence of hemorrhage H**

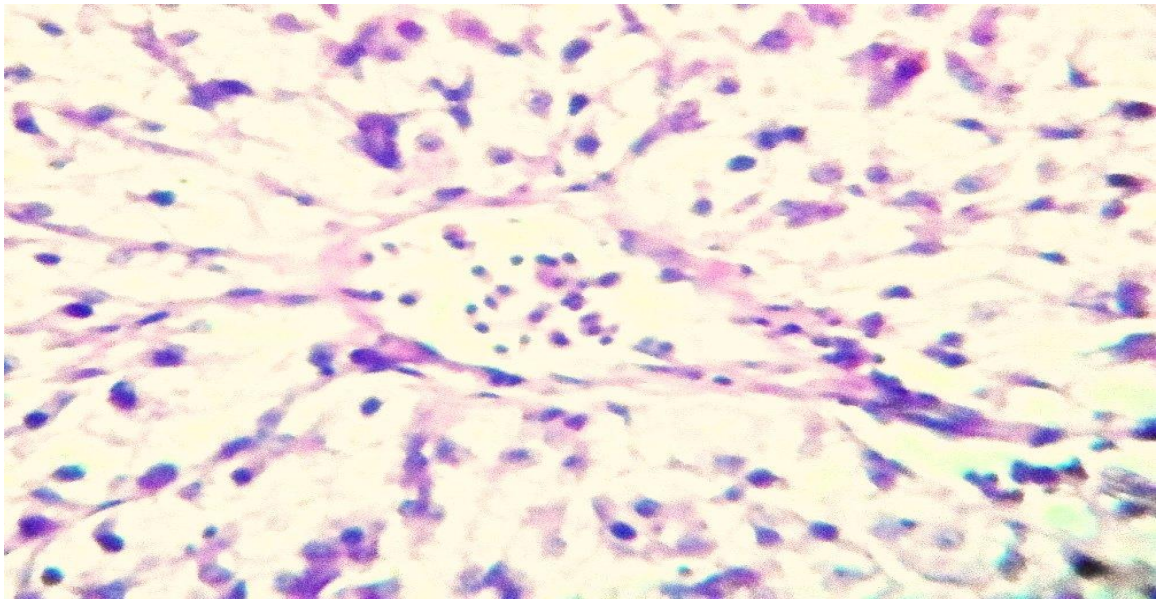

**Plate 5: Control 0.00mg/l Photomicrograph of normal liver mg X40 note the periportal vein PV with some blood cells. Well defined nucleus with chromatin materials. Well outlined nucleoplasmic architecture**

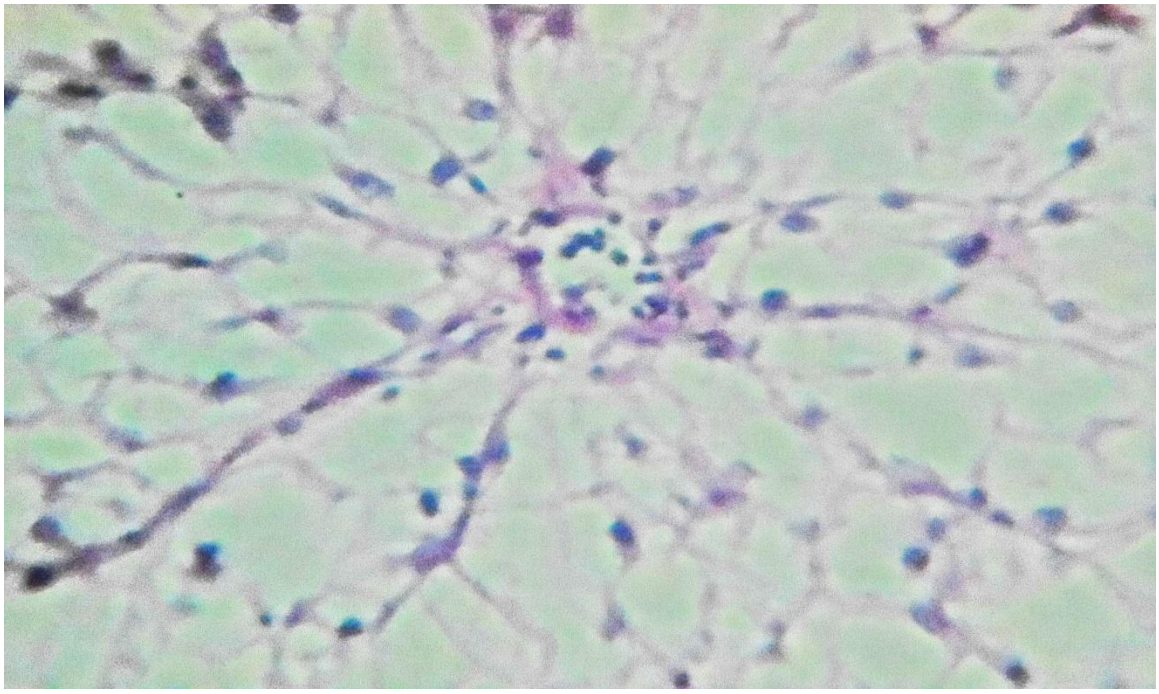

**Plate 6: 0.47mg/l concentration sublethal dose. Mg X 400. Photomicrograph shows the liver, good nuclear architecture( N) but with thickened cell membrane( CM) , periportal vein (PV) remain intact with visible blood cells.**

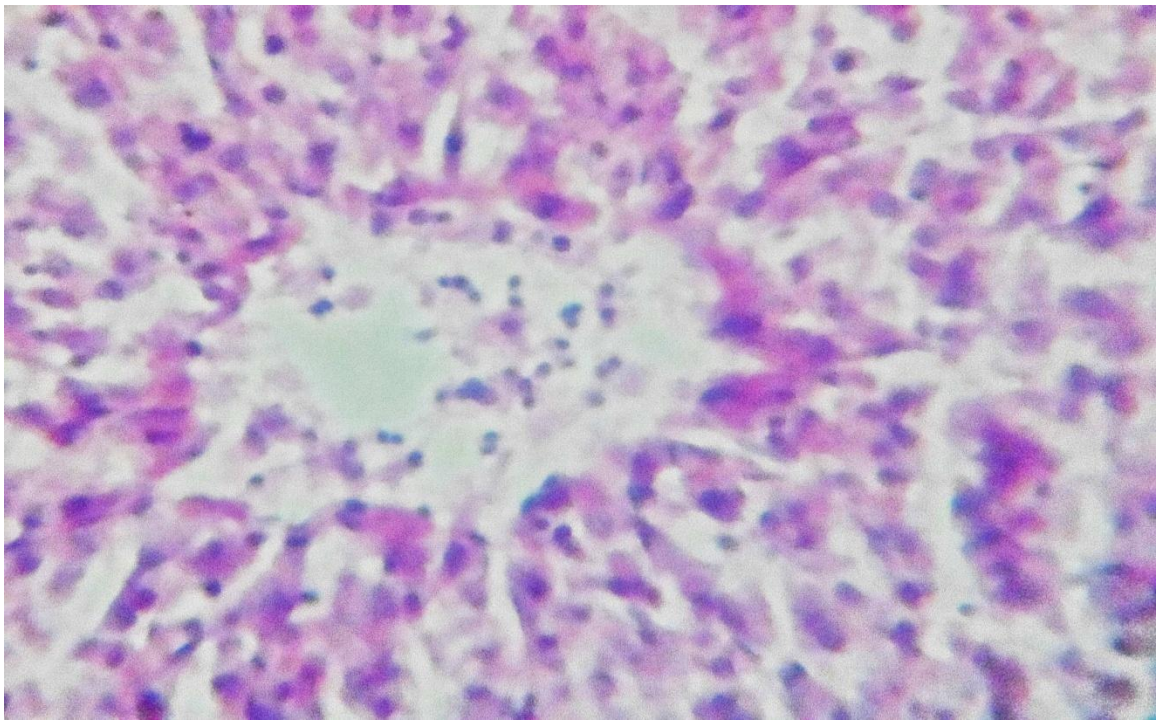

**Plate 7: 0.71mg/l concentration sublethal dose. Mg X 400. Photomicrograph showing the liver, poor nuclear architecture( N) With increase in the number of nucleus which is a sign of inflammatory reactions, periportal vein (PV) shows distorted outline with visible blood cells.**

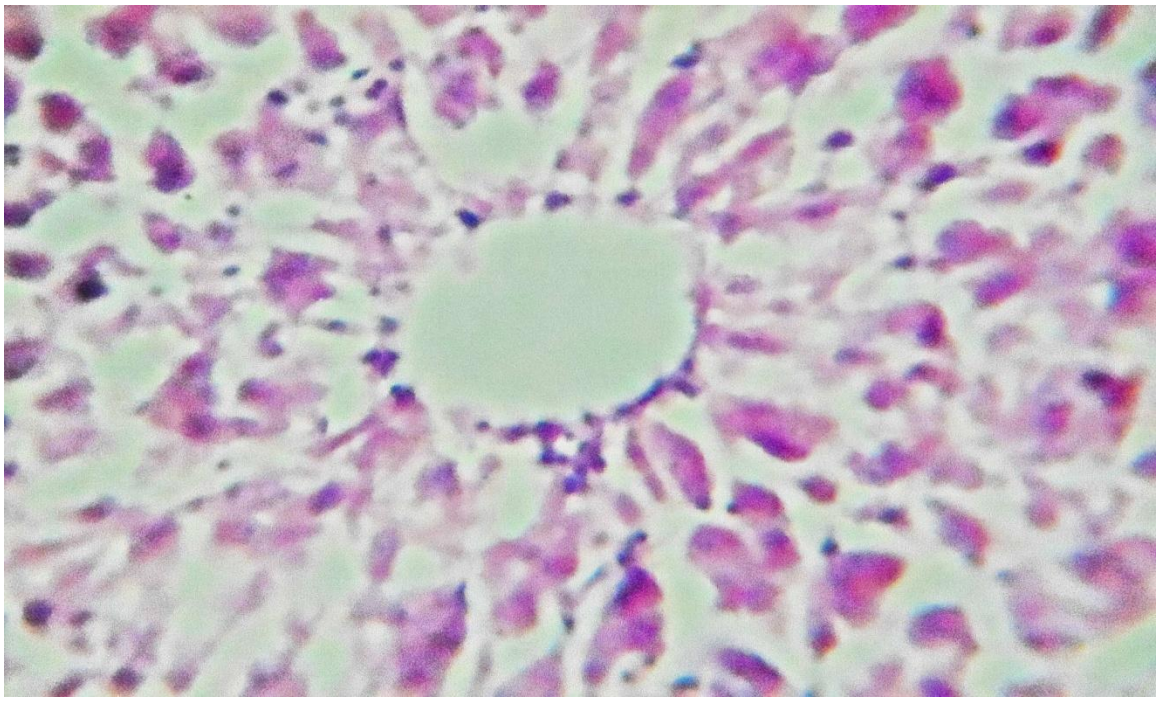

**Plate 8: 1.45mg/l concentration sublethal dose. Mg X 400. Photomicrograph showing the liver, compressed nuclear architecture( CN) which are often found outside the cytoplasm and very few, there is evidence of enucleated cytoplasm reflecting necrosis. periportal vein (PV) shows distorted outline with no visible blood cells.**

# GROWTH PERFORMANCE

## Effect of sub-lethal Concentration of butachlor on Growth of *Clarias gariepinus*.

The growth of *Clarias gariepinus* after exposure to sublethal concentrations of butachlor for 8 weeks. The results of weight gain and SGR clearly showed that the best growth performance was recorded in the control concentrations (70.36% and 0.058g). For the exposed fish, the best performance was recorded in concentration 0.47mg/L (26.27% and 0. 028g). The poorest growth performance was recorded in the highest concentration of 1.45mg/L. The growth performance was dose dependent, showing decrease in growth with increase in concentration (Table 4).

**Table 4 Growth of *Clarias gariepinus* exposed to sub-lethal concentrations of Butachlor for Eight weeks.**

| | Concentrations (mg/L) | | | |
|---|---|---|---|---|
| Parameters | 0 | 0.47 | 0.71 | 1.45 |
| Average initial (g) | 44.67 | 44.04 | 43.14 | 43.7 |
| Average final (g) | 76.1 | 55.61 | 47.53 | 46.01 |
| weight gain (g) | 31.43 | 11.57 | 4.39 | 2.31 |
| % weight gain (g) | 70.36% | 26.27% | 10.18% | 5.29% |
| SGR | 0.058 | 0.028 | 0.01 | 0.005 |

SGR = Specific Growth Rate

**Sublethal Effect of Butachlor Nutrient Utilization of *Clarias gariepinus* in 8 weeks.**

Table 5 showed a significant reduction in GFCE, FE, PER and NM in the fish exposed to the toxicant compared to that of the control fish. The result obtained also showed that FCR was higher in the fish exposed to the toxicant than the control. The best food utilization were obtained in the control tanks, while the poorest were obtained in the tanks containing different concentration of the toxicant.

**Table 5 Nutrient Utilization of *Clarias gariepinus* exposed to sub-lethal concentration of Butachlor for 8 weeks**

| | Concentrations (mg/L) | | | |
|---|---|---|---|---|
| Parameters | 0 | 0.47 | 0.71 | 1.45 |
| FCR (g) | 2.77 | 3.27 | 3.34 | 3.56 |
| GFCE (g) | 36.10 | 30.58 | 29.94 | 28.09 |
| FE (g) | 0.21 | 0.14 | 0.08 | 0.03 |
| PER (g) | 0.79 | 0.29 | 0.11 | 0.06 |
| NM (g) | 224.73 | 165.36 | 66.3 | 41.52 |

Keys: FCR= Feed Conversion Ratio, GFCE= Gross Feed Conversion Efficiency, FE= Feed Efficiency, PER= Protein Efficiency Ratio, NM= Nitrogen Metabolism

# DISCUSSIONS

The behavioral alteration observed may occur as a result of nervous impairment due to blockage of nervous transmission among the system and various effector sites. Similar observations were reported by [23] who reported that hyperactivity of fish on introduction to an unfavorable environment as the primary and principal sign of system failure due to pesticide poisoning which

affect the physiological and enzyme activities. Similar observations were reported by [24] and reported that when fish were exposed to acute concentrations of different toxicants, the fish were stressed progressively with time before eventually dying. The stressful ailment of respiratory impairment due to the toxic effect of butachlor on the gills was similar to the report of [25]. The accumulation of the mucus might have resulted from an increase in the activity of the mucus cells following exposure to the toxicant. Accumulation of mucus on the gills reduces respiratory activities in the fish and lack of ability of the gill surface to actively carry out gaseous exchange and this might be responsible for the observed mortalities [26].

The mortality observed indicated that the treatments could be poison when exposed to organisms beyond certain concentrations and time limit. The result of the present study showed that the mortality of fish was dose-dependent as reported by [27].

The increase in tail fin beat may be associated with sudden response of the fish to the shock of exposure to the toxicant [28]. This might be due to hyperventilation to cope at the initial period but become weak with increase in exposure, hence subsequent drop in opercular and tail fin beat and finally death due to suffocation. Similar findings were reported by [29].

The decrease in the value of HGb with increase in concentration is an indication of severe anaemia caused by the toxicant on the exposed fish. The anaemia condition in fish could be due to an inhibition in erythrocyte production and destruction of intestinal cells by the toxicant [30] . Erythropenia (deficiency in the number of red blood cells) was reflected by the reduced haemoglobin content and haematocrit value as well as erythrocyte sedimentation rate [31]. Haematocrit is used to determine the ratio of plasma to corpuscles in the blood as well as the oxygen carrying capacity in the blood. The decrease with increasing concentrations of RBC and MCV values could be attributed to the inhibition of the erythrocyte production. Similar trends RBC to various toxicants on exposed fishes [32].

The significant decrease in the packed cell volume (PCV) in this study could be attributed to gill damage and impaired osmoregulation causing anaemia and haemodilution [32].

The fluctuations in the MCH and MCHC in the present study clearly indicates that the concentration of haemoglobin in the red blood cells were much lower in the exposed fish than in the control fish, depicting aneamic condition [33]. Haemoglobin is very important in the tissue of vertebrates because it serves as an oxygen carrier. Therefore, tissue respiration and metabolism induce morbidity and mortality. White blood cells of the fish (WBC) increases with increase in the concentration of the toxicant, which is likely to be associated with an increase in antibody production which help in survival and recovery in the fish exposed to sub-lethal concentration of the toxicant [34].

Neutrophils decrease significantly with increase in the concentration of the toxicant. Butachlor exposure resulted in an enhancing effect on neutrophil values in the fsh. This may be due to the ability of butachlor to induce an immune response. Lymphocytes increase as the concentration of the toxicant increased. A reduction in lymphocyte values could be caused by the effects of butachlor as an (anti) androgenic endocrine disruptor, because androgens play a role in haematological homeostasis by mediating lymphocyte proliferation [35].[36]stated that lymphocytes consist majorly of white blood cell present in peripheral blood of *O. niloticus*, this

study also showed neutrophils and lymphocytes increased as the concentration of the toxicant increased compared with basophils, eosinophils and monocytes.

Butachlor is said to be a contact poison meaning that the gills, liver and kidneys are most affected by this chemical. The gill is one of the most important ant organs directly in contact with pollutant and any kind of damage to the gill tissue of the fish leads to disorder in the gas exchange process and also the decrease of ion regulation efficiency via this organ. Damage to gills upon exposure to herbicides can lead to respiratory distress [37].

Necrosis of some portions of the liver tissue that were observed may result from the excessive work required by the fish to get rid of the toxicant from its body during the process of detoxification, similar observations were reported by Hasan, Ferdous (38) also observed that the volume of the nuclei and nucleoli increased and led to necrosis of the liver cells due to disturbance in cellular and osmotic regulation power of cellular and biological membranes.

The reduction in the growth rate was due to both the concentration of the toxicant and exposure period increased. The suppressed reduction in growth was the effect of the toxicant in the water which would result to physiological disfunction in the fish, since water is essential for fish stability. Olivares-Rubio and Arce (39) reported that the presence of dominant, aggressive fish can increase the activity of others and cause a reduction in growth rate as well as an increase in their sensitivity to pollutants.

# CONCLUSIONS

The results of the present study revealed that butachlor was toxic to *Clarias gariepinus* with $LC_{50}$ value of 14.07mg/L. Sub-lethal exposal of butachlor to *Clarias gariepinus* showed a marked effect on the haematological parameter values, there was decrease in red blood cells, haemoglobin, packed cell volume, mean corpuscular volume, with values in the control higher than that of the exposed fish and erythrocyte count, with increase in white blood cell, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration and Lymphocytes values, with concentration 1.45mg/L having the highest values compared to that of the control. Histopathology observed in *Clarias gariepinus* include high proliferation of nuclei, total obliteration of the general morphology, loss of epithelial and mucous cells, presence of hemorrhage in the gill. While, compressed nuclear architecture, evidence of enucleated cytoplasm reflecting necrosis and distortion of periportal vein was observed in the liver. The herbicide has a negative effect on the growth of juvenile of *Clarias gariepinus*, at 0.47mg/L it was 11.57g while at 1.45mg/L it was 2.31g. The result showed that there was decrease with increase in concentration of the toxicant. The control has the highest weight gain of 31.43g and lowest value of 2.31g was recorded in concentration 1.45mg/L.

# REFERENCES

[1] Sogbanmu TO, Dauda AB. Native Nigerian Fish Species Used as Models in Ecotoxicological Studies: A Rapid Systematic Review. 2024.

[2] Asuquo P, Ifon H, editors. Growth and Haematological Status of Clarias gariepinus Fingerlings Fed Jatropha tanjorensis Leaf Powder Meal. Proceedings of the National Conference on Mariculture in India-Recent Advancement; 2021.

[3] Capela R, Garric J, Castro LFC, Santos MM. Embryo bioassays with aquatic animals for toxicity testing and hazard assessment of emerging pollutants: A review. Science of the Total Environment. 2020;705:135740.
[4] Ustuner T, Sakran A, Almhemed K. Effect of herbicides on living organisms in the ecosystem and available alternative control methods. Int J Sci Res Publ. 2020;10(8):633-41.
[5] Bamal D, Duhan A, Pal A, Beniwal RK, Kumawat P, Dhanda S, et al. Herbicide risks to non-target species and the environment: A review. Environmental Chemistry Letters. 2024;22(6):2977-3032.
[6] Soni R, Verma SK. Impact of herbicide pretilachlor on reproductive physiology of walking catfish, Clarias batrachus (Linnaeus). Fish physiology and biochemistry. 2020;46:2065-72.
[7] Lopes AR, Moraes JS, Martins CdMG. Effects of the herbicide glyphosate on fish from embryos to adults: a review addressing behavior patterns and mechanisms behind them. Aquatic Toxicology. 2022;251:106281.
[8] Bayero U. Toxicity of Atrazine (Herbicide) to Juveniles of the African Catfish, Clarias gariepinus (Bürchell, 1822): Ahmadu Bello University Zaria, Nigeria; 2017.
[9] Islam MA, Amin SN, Brown CL, Juraimi AS, Uddin MK, Arshad A. Determination of median lethal concentration (LC50) for endosulfan, heptachlor and dieldrin pesticides to african catfish, Clarias gariepinus and their impact on its behavioral patterns and histopathological responses. Toxics. 2021;9(12):340.
[10] Mahi TF, Chowdhury G, Hossain MA, Baishnab AK, Schneider P, Iqbal MM. Assessment of lead (Pb) toxicity in juvenile nile Tilapia, Oreochromis niloticus—growth, behaviour, erythrocytes abnormalities, and histological alterations in vital organs. Toxics. 2022;10(12):793.
[11] Hamisu Y, Auta J, Bawa BS. TOXICITY OF THE HERBICIDE 2, 4-D DIMETHYL AMINE SALT ON BEHAVIORAL, HISTOLOGICAL AND OXIDATIVE STRESS BIOMARKERS OF AFRICAN CATFISH CLARIAS GARIEPINUS (BURCHELL, 1822) FINGERLINGS. FUDMA JOURNAL OF SCIENCES. 2024;8(6):177-85.
[12] Ogueji E, Nwakpa J, Ekpenyong J, Olaolu M, Yaji A, Okey I, et al. Histological and hematological changes to Clarias gariepinus juveniles exposed to acute doses of Emamectin benzoate in a static bioassay. Ecotoxicology. 2023;32(5):569-82.
[13] Ukaegbu MC, Olatunde AO, Iheme PO, Uwa C. Sub-lethal effect of Round-up (a glyphosate-based herbicide) on juveniles of African cat fish (Clarias gariepinus). South Asian Journal of Experimental Biology. 2022;12(2).
[14] Amira KI. Comparative study on the growth, nutritional composition, pigment and hemato-biochemical index of Nile tilapia (Oreochromis niloticus) fry fed with selected microalgae: Chattogram Veterinary and Animal Sciences University Chattogram-4225, Bangladesh; 2021.
[15] SALIM A, YUSUF M, BICHI A. HAEMATOLOGICAL RESPONSES OF AFRICAN CATFISH (Clarias gariepinus, BURCHELL, 1822) JUVENILES EXPOSED TO ACUTE CONCENTRATIONS OF BUTACHLOR (HERBICIDE). FUDMA Journal of Agriculture and Agricultural Technology. 2021;7(2):110-5.
[16] Ruby P, Ahilan B, Antony C, Manikandavelu D, Selvaraj S, Moses T. Evaluation of effect of the different stocking densities on growth performance, survival, water quality

and body indices of pearlspot (Etroplus suratensis) fingerlings in biofloc technology. Indian Journal of Animal Research. 2022;56(8):1034-40.

[17] Onu E, Ugwu C, Onu M, Onyimonyi A. Hematological and carcass characteristics of finisher broilers fed varying levels of dietary pawpaw leaf meal. World Journal of Advanced Research and Reviews. 2024;21(1).

[18] Jena T, Chhatoi SK, Khandai SS, Mishra R, Mohanty A, Sharma KK, et al. Sublethal urea exposure in Nile tilapia: Morphological, behavioural, and histological alteration. Chemosphere. 2025;372:144086.

[19] Horstmann P, Maas RM, de Boer XV, de Jong TM, Staessen TW, Kokou F, et al. Effect of dietary protein source and ingredient grinding size on fish performance, faecal waste production and characteristics of yellowtail kingfish (Seriola lalandi) fed restrictively and to apparent satiation. Aquaculture. 2023;562:738875.

[20] Khattaby AERA, Samy B, Mohamed MA, Abd El-Nab AS, Ibrahim R. Effect of initial weights and/or feeding frequencies on growth performance and feed utilization on Litopenaeus vannamei. Egyptian Journal for Aquaculture. 2023.

[21] Aminu S, Ibrahim B. Growth Performance of Clarias gariepinus (Burchell, 1822) Fed Processed Moringa oleifera (LAM.) Seed Meal. International Journal of Science for Global Sustainability. 2022;8(2):7-.

[22] Saidu A, Babayi AG, Ibrahim B. Growth Performance of Clarias gariepinus (Burchell, 1822) Fed with Vigna subterranea at Different Inclusion Levels. Sahel Journal of Life Sciences FUDMA. 2025;3(1):126-31.

[23] Nualkaw C, Wattanakornsiri A, Nanuam J, Nanthanawat P, Prasatkaew W, Meeprom C, et al. Effects of paraquat-based herbicide on acetylcholinesterase (AChE), antioxidant status and histological alterations in freshwater fish: Nile tilapia (Oreochromis niloticus). Aquaculture, Aquarium, Conservation & Legislation. 2022;15(6):3197-211.

[24] Scott J, Grewe R, Minghetti M. Fish Embryo Acute Toxicity Testing and the RTgill-W1 Cell Line as In Vitro Models for Whole-Effluent Toxicity (WET) Testing: An In Vitro/In Vivo Comparison of Chemicals Relevant for WET Testing. Environmental Toxicology and Chemistry. 2022;41(11):2721-31.

[25] Reddy CPK, Manikandavelu D, Arisekar U, Ahilan B, Uma A, Jayakumar N, et al. Toxicological effect of endocrine disrupting insecticide (deltamethrin) on enzymatical, haematological and histopathological changes in the freshwater iridescent shark, Pangasius hypothalamus. Environmental Toxicology and Pharmacology. 2023;101:104201.

[26] Merkin GV, Girons A, Okubamichael MA, Pittman K. Mucosal epithelial homeostasis: reference intervals for skin, gill lamellae and filament for Atlantic salmon and other fish species. Journal of Fish Diseases. 2025;48(1):e14023.

[27] Ali I, Khan S, Shah K, Haroon, Kalimullah. Microscopic analysis of plant-mediated silver nanoparticle toxicity in rainbow trout fish (Oncorhynchus mykiss). Microscopy Research and Technique. 2021;84(10):2302-10.

[28] Salim A, Dauda A, Yusuf M. Behavioural responses of african catfish (Clarias gariepinus Burchell, 1822) juveniles Exposed to acute concentrations of butachlor (herbicide). FUDMA JOURNAL OF SCIENCES. 2021;5(1):472-6.

[29] Chris DI, Samuel EE, Sokiprim A. Haematological and behavioral response of African catfish (Clarias gariepinus)(Burchell, 1822) exposed to sub-lethal concentration of xylene. World Journal of Advanced Research and Reviews. 2022;14(1):554-65.

[30] El-Houseiny W, Anter RG, Arisha AH, Mansour AT, Safhi FA, Alwutayd KM, et al. Growth retardation, oxidative stress, immunosuppression, and inflammatory disturbances induced by herbicide exposure of catfish, Clarias gariepinus, and the alleviation effect of dietary wormwood, Artemisia cina. Fishes. 2023;8(6):297.

[31] Ahmed I, Zakiya A, Fazio F. Effects of aquatic heavy metal intoxication on the level of hematocrit and hemoglobin in fishes: a review. Frontiers in Environmental Science. 2022;10:919204.

[32] Al-Emran M, Hasan NA, Khan MP, Islam SM, Bashar A, Zulfahmi I, et al. Alterations in hematological parameters and the structure of peripheral erythrocytes in Nile tilapia (Oreochromis niloticus) exposed to profenofos. Environmental Science and Pollution Research. 2022:1-13.

[33] Arnaudov A, Arnaudova D. Erythrocytes and hemoglobin of fish: potential indicators of ecological biomonitoring. Animal Models and Experimental Research in Medicine: IntechOpen; 2022.

[34] Rohani MF. Pesticides toxicity in fish: Histopathological and hemato-biochemical aspects–A review. Emerging Contaminants. 2023;9(3):100234.

[35] Uzma S, Sharma A, Pankaj N, Kour H, Kishor K, Verma P. Effect of repeated oral administration of butachlor on haemato-biochemical parameters in Wistar rats. J Pharmacogn Phytochem. 2022;11:115-9.

[36] Kamila S, Shaw P, Islam S, Chattopadhyay A. Ecotoxicology of hexavalent chromium in fish: An updated review. Science of the Total Environment. 2023;890:164395.

[37] Ahmad W, Sultan A, Jan SMH, Ali A, Rahman IU, Usman H, et al. Environmental risk of atrazine (herbicide) to brain, gills and liver tissues of fish Ctenopharyngodon idella (Valenciennes, 1844). Journal of Geoscience and Environment Protection. 2021;9(2):1-16.

[38] Hasan J, Ferdous SR, Abi Rabiya SB, Hossain MF, Hasan AM, Shahjahan M. Histopathological responses and recovery in gills and liver of Nile tilapia (Oreochromis niloticus) exposed to diesel oil. Toxicology reports. 2022;9:1863-8.

[39] Olivares-Rubio HF, Arce E. Effects of chemical pollution on the behaviour of cichlid fish. Environmental Biology of Fishes. 2023;106(5):1149-76.