Some Haematological Changes in *Oreochromis niloticus* Juveniles Exposed to Butachlor

Ada, Fidelis Bekeh¹, Ndome, Chris B.² and Bayim, Peter-Robins B.³

1. Department of fisheries and Aquatic Sciences, Cross River University of Technology, Faculty of Agriculture and Forestry, Obubra Campus, Cross River State, Nigeria.
2. Department of Zoology and Environmental Biology, Faculty of Science, University of Calabar, Cross River State, Nigeria.
3. Department of Chemical Sciences, Faculty of Science, Cross River University of Technology, Calabar, Cross River State, Nigeria.

---

**ABSTRACT**

Tilapia is an important aquaculture species in Nigeria. As natural fisheries are dwindling, Aquaculture is where we place our hope for the continual supply of alternative animal protein. An experiment was carried out to observe the haematological changes which result from exposing the juveniles of *Oreochromis niloticus* to Butachlor. Observation revealed that haemoglobin, mean cell haemoglobin, erythrocyte sedimentation rate, red blood cell count and white blood cell count were statistically higher than control at 0.05 level of alpha. Mean cell volume and mean cell haemoglobin did not show significant reduction as compared to control while packed cell volume was significantly reduced in exposed groups.

**Key words:** haematology, *Oreochromis niloticus*, Butachlor, herbicide.

---

**INTRODUCTION**

Because of the ease with which tilapia can reproduce, their ability to adapt to different artificial culture systems (Osman and Caceci, 1991, Popma and Masser, 1999 and Gomez-Marquez et al., 2003), their omnivorous nature as well as their general hardiness in culture environment, they have been farmed in different culture systems and in many countries of the World. This is because they have been looked upon as aquaculture candidate that can narrow the gap between demand and supply of animal protein in developing countries.

In spite of their hardy nature, tilapias must obey the forces of nature, for all organisms have their maximum and minimum limits for responding and adapting to environments. The increasing introduction of wastes in form of pesticides and industrial wastes obviously have their hazardous effects on the aquatic species among which is tilapia that is of immense importance. The target organs which toxin exert their influence in fish include skin, gills, liver, kidney, gut and blood due to the roles they play in homeostasis and general physiology (Baker et al., 2001 and Adeyemo, 2005).

The chemical method of weed control, which is cheaper and involves less labour and time spent, has led to increases in food production from rice, cassava and yam within the Cross River State. These chemicals have removed the bottle neck in expansion of food production. Because of that, industrial task forces are presently campaigning for the use of herbicides since they undoubtedly improve food production and financial profit at large scale. Like in the tobacco companies where the dangers of smoking is well known, there is reasonable amount of pressure for the wider-spread utilization of herbicides (Chinalia et al., 2007).

Among the numerous studies carried out on the effects of agrotoxicants on fish are those of Oloruntuyi et al. (1992) who worked on the toxicity of Glyphosate and Gramoxone to *Clarias gariepinus*; Jiruungkoorskul et al. (2002) who worked on the toxicity of commercial formulation of Glyphosate to the Nile tilapia to observe the Histopathological changes in the liver, gills and kidneys; Abd El Gowad (1999) who studied the histopathology of the liver and gills of *Tilapia niloticus* exposed to lead acetate and zinc sulphate; Kovaniznych and Ubancikova (1998) that determined the effects of Acetachlor on the guppy and zebra fish; Visoottiviseth et al. (1999) who studied the histopathological effect of triphenyltinhydroxide on liver, kidney and gill on one month old Nile tilapia; Babatunde et al. (2001) determined the acute toxicity of Paraquat to fingerlings of tilapia in Southern Nigeria. Agbon et al., (2002) carried out a renewable static experiment on the toxicity of Diamxonon on rotifers, Cyclops, mosquito larvae and fish; Kori-Siakpere et al. (2007) investigated the effects of sublethal Paraquat on blood plasma and organic constituents of African catfish. Also, Ayotunde (2006) determined the toxicity of *Moringa oleifera* seeds on the juvenile and adults of Nile tilapia and African catfish and more recently, Ayotunde et al. (2010) exposed adult *Clarias gariepinus* to pawpaw seed extracts to observe their haematological changes.

---

*Corresponding Author: * Ada, Fidelis Bekeh, Department of fisheries and Aquatic Sciences, Cross River University of Technology, Faculty of Agriculture and Forestry, Obubra Campus, Cross River State, Nigeria.
The present study was initiated to investigate the influence of Butachlor, a herbicide on the haematology of Oreochromis niloticus which is a widely cultured species in Cross River State and many parts of Nigeria. According to Couch (1984), monitoring both form and functions of organ in discrete population of fish is a useful tool for long term low concentration exposure to pollutants and other stressors.

**MATERIALS AND METHODS**

**Specimen collection and acclimation**

Tilapia juveniles were obtained in the early hours (between 7.00 and 9.00) from the Faculty of Agriculture and Forestry fish farm, Obubra campus of CRUTECH, Cross River State. These were transported in plastic buckets to the laboratory of the Department of Fisheries and Aquatic Sciences, Faculty of Agriculture and Forestry, Cross River University of Technology, Obubra Campus for acclimation. The feeds of pellet size 1.8 to 2mm were obtained from COPPENS, www.coppens.eu. The fish were fed at 6% of their body weight. The daily ration was divided into two (3 % body weight) and fed at 10.00AM and 4.00PM (Gilbert, 1996; Ajani et al., 2007) The fish were acclimated in the laboratory for two weeks and fed with industrially made feed pellets containing zero percent concentration of pesticides. The fish were not fed for 48 hours prior to the commencement of the experiment and during the experiment (Omitoyin et al., 2006 and Mills, 1986). This is because oxygen consumption is reduced when fish are not feeding. Fish are more apt to withstand treatment in no food conditions (Beitlich, 1995) compared to when they are fed.

Plastic aquaria of 52 cm length, 38 cm width and 30 cm height were filled with stream water up to 20.3cm level giving a volume of 40 litres of water per tank. These were subjected to five different concentrations of Butachlor (Rizenee) and labeled T0, T1, T2, T3, T4 and T5 in the range finding experiment; as described by Beitlich (1995) and FAO (1977) manual of aquatic Science research (Martins et al., 2008). T0 had zero concentration of pesticide and served as control. Ten fish were selected randomly and stocked in each aquarium (APHA, 1981; Cengiz et al., 2001; Adeyemo, 2005; Ayoola, 2008). A static bioassay method was used in the experiment, which was replicated three times (Ayoola, 2008).

**Haematological analysis**

Blood was removed from the dorsal blood vessels lying below the vertebral column (Lewbert, 2001). 3 – 5 ml of blood was taken from one fish each from the various tanks for haematological analysis. Fish from the various treatments were analysed for the following haematological parameters: packed cell volume (PCV), erythrocyte sedimentation rate (ESR), Haemoglobin (HB), mean cell Haemoglobin (MCH), mean cell Haemoglobin concentration (MCHC), erythrocyte count, white blood cell count and mean corpuscle volume (MCV). The syringes were exposed to anti coagulant (tetra acetic acid). The blood was stored in labelled bottles (Akiwande et al., 2004) at 0°C in deep freezer waiting for the analysis.

The Haemoglobin was estimated using a haemoglobinometer. A Shali graduated tube was filled with N/100 HCl acid up to 20 ml mark, to which 0.02 ml of blood was added. Distilled water was added drop by drop with gentle shaking to mix. This continued until colour change to match a standard. The amount of mixture in the tube gives Haemoglobin concentration in percentages (Baker et al., 2001). Mean cell Haemoglobin (MCH) which is the average Haemoglobin present in one red blood cell was obtained by:

\[
\text{Hb} \times \frac{10}{\text{RBC}} \text{picogram}
\]

Mean cell Haemoglobin concentration which is the amount of Haemoglobin present in 100 ml of packed red cells was obtained by: 

\[
\text{Hb/PVC}
\]

To estimate Erythrocyte sedimentation rate (ESR), fresh blood was collected in microhaematocrit tubes by capillary action. The lower side of the tube was sealed with a synthetic sealant and placed on a rack for 40 minutes at an angle of 45°, and determined with the help of a haematocrit reader and the value obtained as length of settled blood cell column divided by total blood column expressed as percentage (Svoboda et al., 1991).

Packed Cell Volume (PCV) was estimated by centrifuging the sample anticoagulated blood at 12,000g for 10 minutes using centrifuge model Gallenkamp CFA 400 (Baker et al., 2001). The PCV was obtained by the formula, Height of packed cell column/ Height of whole blood column

The blood sample was diluted with formol citrate in the ratio 1:200 blood to diluents. 0.5 ml of blood sample was added to 100 ml of formol citrate. The 0.5 ml of sample was taken in a pipette and then added to a round bottom flask containing 100 ml of the formol citrate. Formol citrate was prepared by mixing 10 ml of formalin (40% formaldehyde) with 1litre of 3.13g/l of trisodium citrate solution. The fluid was filtered and stored in a clean glass container (Baker et al., 2001).

The diluted sample of blood was mixed and loaded into a haematocytometer and allowed to settle and be counted. The minimum area of the haematocytometer considered was five of the 0.04 mm² to give a total area of 0.2 mm² (Baker et al., 2001). Cells per mm² was obtained by:

\[
N \times 10^6 / A \times D \text{per litre} (Baker et al., 2001).
\]

The diluent used during white blood cell count was 2 % acetic acid tinged with Gentin Violet in the ratio of 1:20 blood sample to diluents by volume. This diluent makes the white blood cells visible by staining their nuclei as well as dissolving the red blood cells. The diluents was filtered using a filter paper and fondle to prevent the debris from giving counting error.

The blood sample was loaded into a haematocytometer and the white blood cells were counted as in the red blood cells and estimated using a formula as for the red
blood cells. Mean cell volume or mean corpuscular volume was derived by dividing the value of the packed cell volume by red blood cell count expressed in femtolitres as in the formula by Baker et al. (2001). This manual method was preferred because Baker et al. (2001) pointed out that cell size estimation as may be obtained by machines have been shown to be affected by the shape of the cells. Abnormal shapes produce abnormal pulses which, is 1.5 times greater than the normal, yet such electrical devices are difficult to come by. The haematological parameters were analysed using analysis of variance (ANOVA) at 0.05 % alpha level by SPSS, version 13.0. The post hoc comparison of means was carried out using Duncan’s multiple range tests (Frank and Althoen, 1995).

RESULTS

Haematological analysis showed that the haemoglobin of fish exposed to lower concentrations of Butachlor produced no significant different between control and treated animals. However, there was a significantly higher concentration of haemoglobin content of the group given the highest concentration of the herbicide (1.8mg/l) as shown in Figure 1. Mean cell haemoglobin did not show significant different at 0.5 alpha level (Figure2). However, the mean cell haemoglobin concentration showed differences between control and exposed groups. Cells exposed to high concentration were observed to have higher haemoglobin per cell. This results were displayed Figure1. There was a steady rise in the mean cell haemoglobin concentration, which is the average amount of haemoglobin in 100 red blood cells as the poison concentration increased as in figure 3. Packened cell volume had significant difference at 0.05 alpha level between control and treated animals (Figure 4). The packend cell volume was significantly different from that of concentration 0.6mg/l, 0.9mg/l and 1.5mg/l. However it was not different from those exposed to 0.3mg/l, 1.2mg/l and 1.8mg/l.

The erythrocyte sedimentation rate was observed to be influenced by concentration of Butachlor. However, the highest concentration of 1.8mg/l is not statistically different from the control. The rate of red blood cell sedimentation is shown on figure 5.

Red blood cell count showed differences between treatments at 0.5 alpha level. Though the number of cells did not reduce linearily with concentration, the red blood cell in control was higher than those exposed to 0.3mg/l and 1.5mg/l. At 1.8mg/l, the number of cells was higher than others as seen in figure 6.

White blood cell count increased with toxicant concentration. Figure 7 shows that the control group has significantly lower cells than the groups treated with 1.2mg/l and 1.8mg/l of Butachlor. The white blood cell count is similar in the control groups and those treated in the lower concentrations.

Observation showed absolute reduction in mean cell volume (Figure 8). It was not high enough to result in a statistically significant difference in mean cell volume between the groups exposed to Butachlor herbicide and the control tilapia juveniles. The cell volume was not seen to be correlated to Butachlor concentrations.

TABLE 1: Effect of Butachlor herbicide on the haematological parameters of juvenile Nile tilapia

<table>
<thead>
<tr>
<th>SN</th>
<th>concentration/parameters</th>
<th>PVC (%</th>
<th>Hb (g/mm)</th>
<th>ESR (%)</th>
<th>WBC 10^3/mm³</th>
<th>RBC 10^6/mm³</th>
<th>MCH (pg)</th>
<th>MCHC (g/dl)</th>
<th>MCV(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0mg/l</td>
<td>13.67</td>
<td>3.83b</td>
<td>10.00b</td>
<td>2.60b</td>
<td>1.30</td>
<td>2.70b</td>
<td>12.71</td>
<td>5.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.06</td>
<td>1.36</td>
<td>1.00</td>
<td>0.65</td>
<td></td>
<td>5.38</td>
<td>76.40</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.17 0.88</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.3mg/l</td>
<td>10.00a</td>
<td>3.43b</td>
<td>5.00a</td>
<td>1.83a</td>
<td>0.65</td>
<td>2.10a</td>
<td>16.22</td>
<td>4.81</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
<td>1.66</td>
<td>1.00</td>
<td>0.30</td>
<td></td>
<td>4.76</td>
<td>141.29</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.81 0.66</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.6mg/l</td>
<td>9.00b</td>
<td>3.03b</td>
<td>2.65</td>
<td>2.73b</td>
<td>0.67</td>
<td>2.53b</td>
<td>11.80</td>
<td>3.52</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.97</td>
<td>1.52</td>
<td></td>
<td>2.59</td>
<td></td>
<td>62.56</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.52 0.75</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.9mg/l</td>
<td>10.33b</td>
<td>9.36b</td>
<td>3.70a</td>
<td>5.33a</td>
<td>1.15</td>
<td>2.57b</td>
<td>15.54</td>
<td>4.97</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.06</td>
<td>0.37</td>
<td>1.15</td>
<td>0.72</td>
<td></td>
<td>6.38</td>
<td>150.24</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.97 3.82</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.2mg/l</td>
<td>9.00b</td>
<td>3.3b</td>
<td>0.85</td>
<td>8.67b</td>
<td>2.52</td>
<td>2.87b</td>
<td>13.44</td>
<td>3.67</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
<td>0.85</td>
<td></td>
<td>0.68</td>
<td></td>
<td>3.33</td>
<td>43.78</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3.67 1.24</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.5mg/l</td>
<td>9.00b</td>
<td>3.40b</td>
<td>0.85</td>
<td>8.00b</td>
<td>1.00</td>
<td>2.50b</td>
<td>18.62</td>
<td>4.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.33</td>
<td>0.53</td>
<td></td>
<td>0.27</td>
<td></td>
<td>7.60</td>
<td>131.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.93 1.23</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>1.8mg/l</td>
<td>10.00b</td>
<td>5.67b</td>
<td>1.10</td>
<td>11.67b</td>
<td>1.52</td>
<td>4.17b</td>
<td>16.25</td>
<td>2.83</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.00</td>
<td>0.35</td>
<td></td>
<td>0.97</td>
<td></td>
<td>4.96</td>
<td>137.61</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.83 0.62</td>
<td></td>
</tr>
</tbody>
</table>
Figure 1: Haemoglobin concentration of Oreochromis niloticus juvenile exposed to concentrations of Butachlor herbicide

Figure 2: mean cell haemoglobin of Oreochromis niloticus juveniles exposed to different concentrations of Butachlor herbicide

Figure 3: mean cell haemoglobin concentration of Oreochromis niloticus juveniles exposed to different concentrations of Butachlor herbicide

Figure 4: Erythrocyte sedimentation rate of Oreochromis niloticus juveniles exposed to different concentrations of Butachlor herbicide

Figure 5: packed cell volume of Oreochromis niloticus juveniles exposed to different concentrations of Butachlor herbicide

Figure 6: Red blood cell count of Oreochromis niloticus juveniles exposed to different concentrations of Butachlor herbicide
Haematological parameters are important in the health status of any organism (Baker et al., 2001 and Taylor et al., 1997). In fishes, they are used for clinical diagnosis of fish physiology, which is determined by the effect of the internal and external physical environment (Adeyemo, 2005). Pycnotic and granular micronuclei were observed by Ateeq et al. (2002), while Anisochromasia and anisocytosis of erythrocytes were also observed in Ateeq et al. (2002). The herbicide may therefore said to be genotoxic and may be responsible for the reduction in packed cell volume in the exposed fish in this study, due to cell death as shown in figure 5. The effects of Butachlor on the haematological parameters were not seen to be increasing linearly with toxin concentration in this experiment. Kori Siakpere et al. (2008) equally reported reduction with concentration of Zinc in haematological parameters such as haemoglobin value, haematocrit value as well as red blood cell count. These were obvious signs of anaemia caused by Zinc intoxicification. That means observation of some haematological parameters suggested that changes were not necessarily concentration dependent. These none linearity is often reported in the toxicity of Aluminium and other heavy metals in fish. As Brown and Sadler (1989) noted, Calcium and Aluminium are the most important chemical variables for determination of fish status in a water body. They reported that Aluminium dissolves least at pH 5.5 and continues to increase in solubility with increase or decrease in pH. Though Aluminium concentration may be more in water at pH 5.5, it does not dissolve much to be made available to the fish at this pH (5.5). When substances dissolve in water, they may produce different species of products in different environmental conditions (such as pH, temperature and even availability of other elements (Vangenechten, et al., 1989). Vangenechten, et al. (1989) showed that acidity pH interferes with Na⁺ and Cl⁻ transport in gills. Vijyan et al. (2001) reported a 20% increase in gill epithelial cells activities in Oreochromis transferred from fresh water to sea water in order to maintain ion balance. But the mortality increased with concentration. It could be argued that mortality did not primarily result from haematological alterations alone, but also by causes made to other tissues as well (Forambi et al., 2008). Squibb (2010) noted that effects of exposure to organochlorides are difficult to be defined because they are general nervous system alterations that can occur through many causes. Tilak et al. (2006) pointed that Butachlor toxifies by respiratory distress, accumulation of which affect the biochemical pathway at cellular neurological level and finally culminating in decrease/inhibition of the neurological enzyme, acetyl cholinesterase. Butachlor is said to be a contact poison. It therefore shows that the gills, liver, gut and kidney are most affected by this chemical.

However, the white blood cell were shown to be significantly different in number in samples exposed to 1.2mg/l and 1.8mg/l of Butachlor compared to the controlled group. Increase in white blood cell number is usually an adaptation to fight toxin (Baker et al., 2001). A similar observation was made by Daryani et al. (2007) in humans exposed to Butachlor where the chemical was observed to raise eosinophills population by 20% (Ateeq et al., 2002). Butachlor can destroy the cell structure when in high concentration (Tilak et al., 2007). Mahonia Ecological Consulting (2005) explained that contact herbicides destroy cell membrane and may cause desiccation, necrosis and eventual death of part or the whole organism. At lower concentration, it acts as protein synthesis inhibitor or amino acid inhibitor (Mahonia Ecological Consulting, 2005; and Ateeq et al., 2002). This effect could result in the death of active cells in the gills and other tissues which are among the first point of contact in a respiratory fluid (Jiraungkoorskul et al., 2003). The destruction of primary and secondary lamellae could distort the normal respiratory function of gaseous exchange (Olorin et al., 2006), and this is capable of stressing the fish due to oxygen deficiency in blood and this might have probably led to mortality. Erosion of the skin with complete degeneration at higher concentrations
disturbs normal physiological function of this organ. Daryani et al. (2007) demonstrated that Butachlor produced exfoliative dermatitis in man. The destruction of the skin tissue paves the way to free movement of substances in and out. Essential fluid could ooze out at will resulting in morbidity and mortality of the fish. Ateeq et al. (2002) pointed out that Butachlor is a spindle fibre inhibitor which may therefore produce liver cells with abnormal sets of chromosomes. Such abnormal structures lead to abnormal physiological functions that are likely to stress the fish to death.

Forambbi et al. (2008) observed Clarias gariepinus to be seriously affected when exposed to Butachlor in terms of excretory functions. The production of increased number of cells (hyperplasia) would probably enable the fish to carry out their excretory functions since they could be unduly stressed with resultant accumulation of the poison in the tissue as pointed out by Shallangwa and Auta (2008).

REFERENCES

Abd El-Gawad, A. M. 1999. Histopathological studies on the liver and gills of Tilapia nilotica (Oreochromis niloticus) exposed to different concentrations of Lead acetate & Zinc sulphate. Journal of Egypt’s society of zoology 30 (c):13-22


