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Full Length Research Paper

Hematological, organic and behavioral changes in Oreochromis niloticus (Linne 1757) adolescents presented to Paraquat herbicide

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The Nile Tilapia, *Oreochromis niloticus* was exposed to Paraquat for 24 h so as to monitor its effects on the fish's haematology, and general behaviours. The haematological parameters estimated include haemoglobin, mean cell haemoglobin, mean cell haemoglobin concentration, packed cell volume, mean cell volume, erythrocyte sedimentation rate and white and red blood cell counts. The hepatosomatic index was measured for different concentrations of Paraquat exposed. Results showed that the hepatosomatic index was decreasing with increasing concentration of Paraquat. Haemoglobin, mean cell haemoglobin, mean cell haemoglobin concentration and erythrocyte sedimentation rate were observed to be negatively related to concentration of Paraquat. Packed cell volume, white blood cell count (WBC), red blood cell count (RBC) showed positive relationship with concentration while mean cell volume was not significantly changing with change in concentration of Paraquat.

Key words: *Oreochromis niloticus*, Paraquat, herbicide, haematology, hepatosomatic, Index, biological responses.

INTRODUCTION

Tilapia is commonly available and easy to manipulate as both capture and culture fisheries due to its qualities. Tilapinnes are commonly acclaimed as having qualities that make them highly cultivable species. Cichlids are native to Africa, but they have migrated to other parts of the world such that, they are now present in all the continents of the world (Fitzsimmons, 2002; Peterson et al*.,* 2004). Cichlids were originally fresh water fishes but some can adapt to brackish water and others capable of adapting to full strength salt water (Gomez-Marquez et al., 2003; and Peterson et al., 2004). Tilapinnes are good candidates for Aquaculture especially in developing countries where there are high levels of animal protein deficiencies. The admirable characteristics of these fishes as cultivable species include resistance to diseases, the ease with which to reproduce in captivity, switching of diet and tolerance to poor water qualities such as low dissolved oxygen levels and over crowding.

Some works carried out on the effects of agrotoxicants on fish include those of Oloruntuyi et al. (1992), Jiraungkoorskul et al. (2002), Abd El Gowad (1999), Koviznych and Ubancikova (1998), Visoottiviseth et al. (1999) and Babatunde et al. (2001). Agbon et al. (2002) carried out a renewable static experiment on the toxicity of Diaxonon on rotifers, Cyclops, mosquito larvae and fish; Kori-Siakpere et al. (2007) investigated the effects of sub lethal Paraquat on blood plasma and organic constituents of African catfish. Ayotunde et al. (2010) exposed adult *Clarias gariepinus* to pawpaw seed extracts to observe their haematological changes.

The uses of these chemicals have influence on non target organisms and information on this is growing. The toxins can be carried from one place to another or one organism to another along a food chain (Shallangwa and

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Auta, 2008). Their role in degradation of the aquatic ecosystem cannot be ignored (Omitoyin et al., 2006). They could therefore be accumulated in the tissues of these non target organisms, thereby influencing their ability to adapt to the environment. Herbicides could hamper reproduction, food conversion efficiency, growth and mortality of inhabitants (Ateeq et al., 2002).

It is therefore pertinent to determine the tolerance limit of some of these agrochemicals to fish in general and the most widely cultured species in our area in particular. This is because these chemicals are increasingly used in weed control in the area. Any human activity, which seems to be a threat to this promising species that its production is likely to reduce the present lag (Daramola et al., 2007) of about nine million metric tonnes between supply and demand of fish and animal protein must be given watchful eyes. So investigation into the influence of some of the pesticides in the histopathology of aquatic organisms namely, *Oreochromis niloticus* as a widely cultured species in Cross River State, Nigeria, is carried out here. The choice of Paraquat in this experiment is because it is among the most frequently used chemical in Cross River State for the control of weeds on land, which find their way to aquatic environments (Ye et al., 2002). Paraquat treated *Tilapia niloticus* showed significant changes in hepatosomatic and gonadosomatic indexes as compared to control fish (Figueiredo-Fernandes et al., 2006). Historically paraquat causes some hepatic alterations of parenchyma cell such as vascularization, necrosis and increased microphage aggregation and eosinophilic granular cells. The intensity of toxicity of various pesticides was observed by Nishiuchi (1979) to be influenced by dissolved oxygen level, pH and the amount of pesticides involved. For Couch (1984) stressed that monitoring both form and functions of organs in discrete population of fish is a useful tool for long term low concentration exposure to pollutants and other stressors.

MATERIAL AND METHODS

Identification and taxonomy of species

O. niloticus, like other tilapias has deep laterally compressed body (Pinnoy, 2006). Tilapias were re-classified into *Tilapia, Sarotherodon and Oreochromis* based on their breeding habits. Members of the genus tilapia are substrate breeder and both parents guard the eggs and fry. Survival of fry is low because they are released too early. Soratherodons are those that orally rear eggs and fry. Survival is relatively higher than the tilapia. Care for the young is paternal though sometimes bi-parental. In the *Oreochromis*, the females solely brood the eggs for an extended period of time. They spend a lot of time doing this. So they remain smaller than males. Fry survival is high due to extended period of brood care. The maximum length (La) of this species could range from 340 to 630 mm depending on the locality. The male is larger than the female. The fish has 18 to 26 gill rakers.

Six aquaria of 59280 cm^3 capacity were filled with 40 L of water per tank. Ten fish were selected randomly and stocked in each aquarium (APHA, 1981; Cengiz et al., 2001; Adeyemo, 2005; Ayoola, 2008). These were subjected to six different concentrations of Paraquat labeled T0, T1, T2, T3, T4 and T5 in the range finding experiment; and T1, T2, T3, T4, T5 and T6 in the definitive test. T1 to T6 had graded concentrations of pesticides prepared as described by Beitlich et al. (1995); and FAO (1977) manual of Aquatic Science Research (Martins et al., 2008). T0 had zero concentration of pesticides and served as control. This experiment was replicated three times (Ayoola, 2008) for each concentration. A static bioassay was used. Cengiz et al. (2001), Svoboda et al. (2001), Ajani et al. (2007) and Kori – Siakpere and Ubogu (2008) used a semi static method, because according to them, some herbicides are known to be adsorbed to solid particles and are made unavailable to the column. Ayoola (2008) used a static renewal method in which all the liquid environment was changed with the same concentration of the chemical every 24hours. Tilapia fingerlings were obtained from the Faculty of Agriculture and Forestry fish farm, Obubra campus of CRUTECH, Cross River State. These were transported in plastic buckets between 7.00 and 11.00 h to avoid heat stress, to the Department of Fisheries and Aquatic Sciences laboratory for acclimation for 14 days and fed with industrially made feed pellets containing zero percent concentration of pesticides. These feeds were purchased from COPPENS, www.coppens.eu. The fish were fed at 6% of their body weight divided into two rations (Gilbert, 1996; Ajani et al., 2007). The fish were not fed for 48 hours prior to the commencement of the experi-ment (Omitoyin et al., 2006). This is because oxygen consumption is reduced when fish are not feeding. Fish are more likely to withstand treatment during food deprivation (Beitlich et al., 1995) compared to when they are fed.

The average weight and length of fish used in the experiments were 10.54±1.47 g and 6.72±0.85 cm respectively. Juvenile fish, according to Ayoola and Ajani (2008) are better used in toxicity experiment because they are more sensitive to toxins. They also form a sensitive stage in the life cycle of the organisms. From the start, the fish were observed every 30 min for abnormal behaviours. Interval of observation increased to every one hour after 12 h for the remaining period of the experiment. Death fish were immediately removed and preserves in 10% formaldehyde (Ayoola, 2008, Lewbert, 2001). The fish were weighed. The liver was removed and weighed using a digital balance (Scout-pro SPU402). The hepatosomatic index was obtained by dividing the weight of the liver by the total weight of the fish (Koumi et al., 2008) expressed as a percentage (Gomex-Marquez et al., 2003). Five fish were mea-sured in each tank and their averages taken. Blood was removed from the mid dorsal blood vessel lying below the vertebral column (Lewbert, 2001) using a heparinised syringe and needle. 3 to 5 ml of blood was taken from fish in each tank for haematological analysis. The blood was temporally kept in labeled heparinised bottles (Akinwande et al., 2004) at 0°C in deep freezer waiting for the analysis. Blood from the various treatments were analysed for the following haematological parameters: packed cell volume (PCV), erythrocyte sedimentation rate (ESR), heamoglobin (HB), mean cell haemoglobin (MCH), mean cell haemoglobin concen-tration (MCHC), erythrocyte count, white blood cell count and corpuscle volume (MCV). The haemoglobin was estimated using a *haemoglobinometer*. Mean cell haemoglobin (MCH) was obtained by Hb X 10/ RBC pictogram. Mean cell haemoglobin concentration was obtained by dividing Hb by PCV. The erythrocyte sedimentation rate was 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., 2001).

Packed Cell Volume (PCV) was determined by centrifuging the sample anti coagulated blood at 12,000 revolutions per minute (RPM) for 10 min using centrifuge model SHADON AS325. The blood sample was diluted with formol citrate in the ratio 1:200 blood to diluents for red blood cell count and 1:20 for white blood cell count. The diluted sample of blood was mixed and loaded into a

Table 1. Behavioural changes and biological responses in O*reochromis niloticus* juveniles exposed to different concentrations of Gramoxone herbicide (Range find

	12 h						16 h						20 h					
Concentration (mg/l)	0.0	5.5	11.0	16.5	22.0	27.5	0.0	5.5	11.0	16.5	22.0	27.5	0.0	5.5	11.0	16.5	22.0	27.5
Loss of reflex	N	N	N	N	Y	Y	N	N	N	N	Y	Y	N	N	N	N	Y	Y
Molting	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	Y
Discolouratrion	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Air gulping	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y	N	Y	Y	Y	Y	Y
Erratic swimming	N	N	N	Y	Y	Y	N	N	N	N	Y	Y	N	Y	Y	Y	Y	Y
Haemorrhage	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Loss of scale	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N

N = No change in behavior found
Y = Yes, change in behavior found

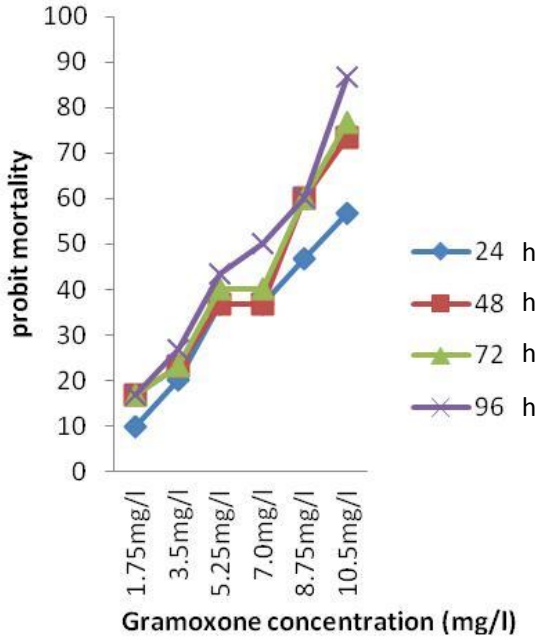

Figure 1. The LC50 24, 48, 72 and 96 h of *Oreochromis niloticus* juvenile exposed to different concentrations of Gramoxone (Definitive test) was 10.40, 7.65, 7.50 and 7.00 respectively.

haematocytometer and allowed to settle. The white blood cells blood cells were also counted in the haemato-cytometer. The minimum area of the haematocytometer considered was five of the 0.04 mm^2 to give a total area of 0.2 mm^2. 0.2 mm^2 was exceeded only when the cells counted in this area were not up to 500 as advised by Baker et al. (2001) to increase the confidence in the final result by decreasing inherent statistical counting error. The diluents used during white blood cell counting were 2% acetic acid tinged with Gentian Violet to increase their visibility by staining their nuclei as well as dissolving 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 (Baker et al., 2001).

RESULTS

Loss of reflex and molting were observed within 16 hour, at concentration of 5.7 mg/l. Discoloura-tion was delayed till the 72nd h and occurred at concentration of 7.6 mg/L. Air gulping was observed within 24 h except at 1.9 mg/L but this concentration caused the fish to gulp air after 24 h. Similar response was seen in erratic swimming where response was delayed in 3.8 mg/L to after 24 h. There was haemorrhage observed at

concentration o gulping and e within 24 h at c respectively. Th within the 48th 9.5 mg/L respe scales were ob tions of 9.5 and
1). The Lc_{50} 24,
7.50 and 7.00 tion which kille decreasing with The temperatur dissolved oxyge in the exposed are displayed i same superscrip those carrying statistically. Th *niloticus* juvenil with increment hepatosomatic i It reduced almo poison administr The differences

Table 2. The physicochemical properties of water exposed to different concentrations of Gramoxone during range finding and definitive tests.

Range finding test				Definitive test			
Dose (mg/l)	Dissolved oxygen	Acid pH	Temperature	Dose (mg/l)	Dissolved oxygen	Acid pH	Temperature
0.0	$7.13^{a}\pm0.83$	$7.23^{a}\pm0.35$	$26.91^{a}\pm0.29$	1.75	$7.02^{a}\pm0.53$	$7.31^{b}\pm0.40$	$25.57^{a}\pm0.61$
3.5	$6.78^{a}\pm0.75$	$7.09^{a}\pm0.27$	$27.00^{a}\pm0.22$	3.50	$6.82^{ab}\pm0.54$	$7.17^{b}\pm0.40$	$26.74^{a}\pm0.39$
7.0	$6.68^{a}\pm0.79$	$7.06^{a}\pm0.41$	$27.04^{a}\pm0.19$	5.25	$6.67^{ab}\pm0.63$	$7.40^{ab}\pm0.41$	$25.74^{a}\pm0.29$
10.5	$6.76^{a}\pm0.90$	$7.26^{a}\pm0.39$	$27.05^{a}\pm0.39$	7.00	$6.48^{abc}\pm0.65$	$7.23^{b}\pm0.50$	$26.75^{a}\pm0.58$
14.0	$6.36^{a}\pm0.1.00$	$7.24^{a}\pm0.36$	$27.17^{a}\pm0.28$	8.75	$6.19^{bc}\pm0.86$	$7.72^{a}\pm0.48$	$26.64^{a}\pm0.63$
17.5	$6.37^{a}\pm0.89$	$7.14^{a}\pm0.44$	$27.03^{a}\pm0.36$	10.50	$5.99^{c}\pm0.79$	$7.19^{b}\pm0.43$	$26.36^{a}\pm0.64$

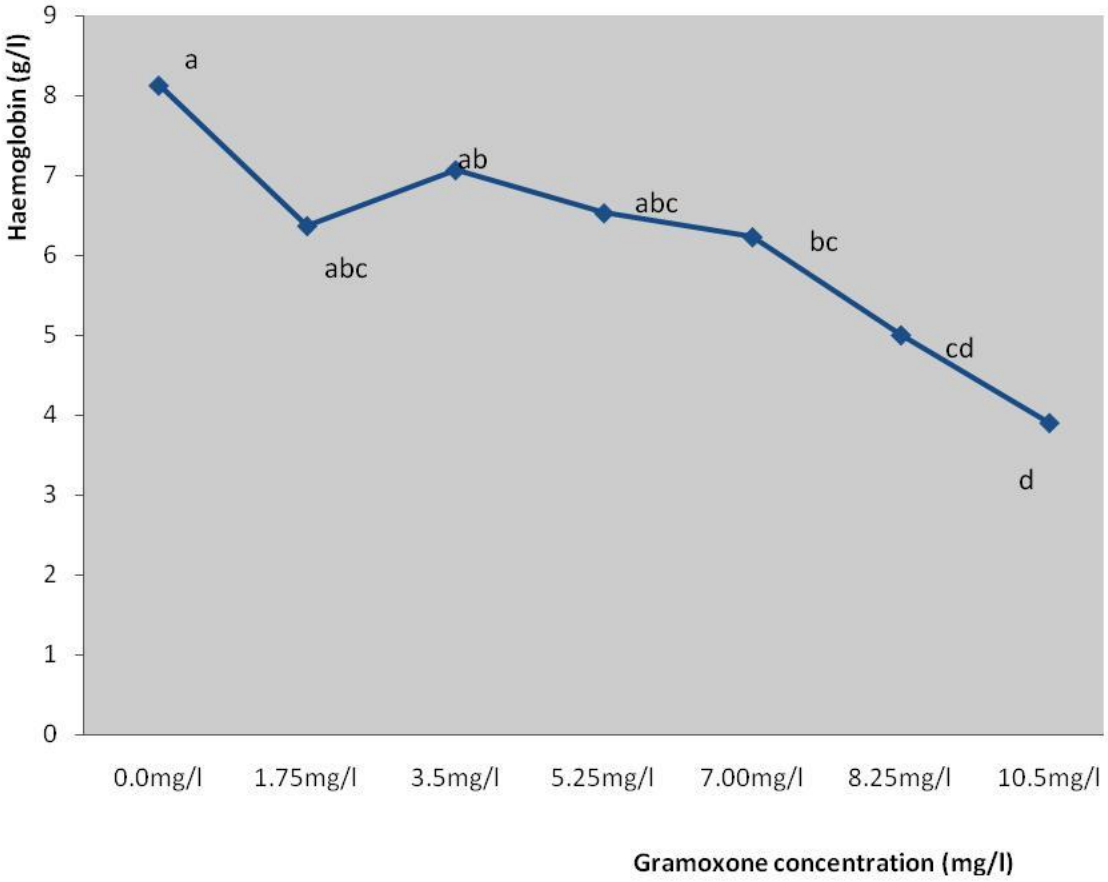

Figure 3. Haemoglobin of *Oreochromis niloticus* juveniles exposed to different concentrations of Gramoxone herbicide.

analysed using ANOVA. Haemoglobin (Hb), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) were observed to be decreasing with Paraquat concentration. These are shown in Figures 3, 4 and 5 respectively. Erythrocyte sedimentation rate and packed cell volume showed inverse relationship. While the erythrocyte sedimentation was decreasing with increase inconcentration of Gramoxone (Paraquat), the packed cell volume was increasing with increasing concentration of the poison as shown in Figures 6 and 7 respectively. Both red and white blood cell counts decreased with toxicant concentration (Figures 8 and 9) to a point and increase with further increase in poison concentration. There was no statistical significant change in the mean cell volume as shown in Figure 10.

DISCUSSION

The rate of poisoning is often measured by the gross observation in terms of death or physical or biological and behavioural changes which occur in organisms (Shallangwa and Auta, 2008; Ramesh et al., 2009). The mortality was observed to be concentration and time

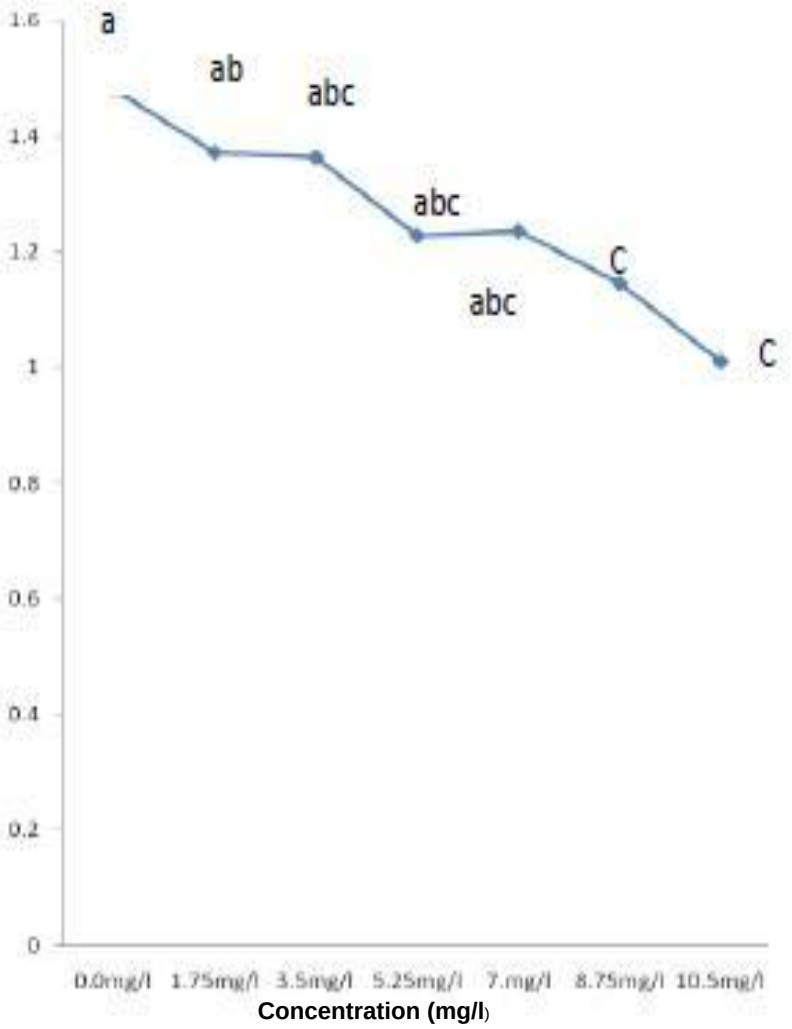

Figure 2. The hepatosomatic index of *Oreochromis niloticus* juveniles exposed to different concentrations of Gramoxone. Significantly different means carry different letters.

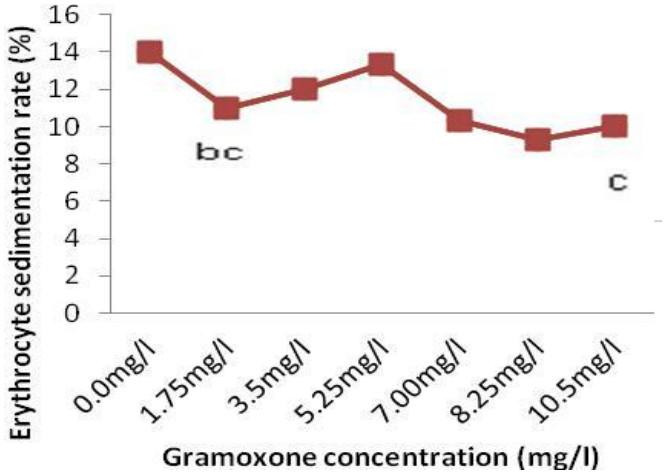

Figure 4. Mean cell haemoglobin of *Oreochromis niloticus* juveniles exposed to Gramoxone herbicide. Means carrying different letters are significantly different.

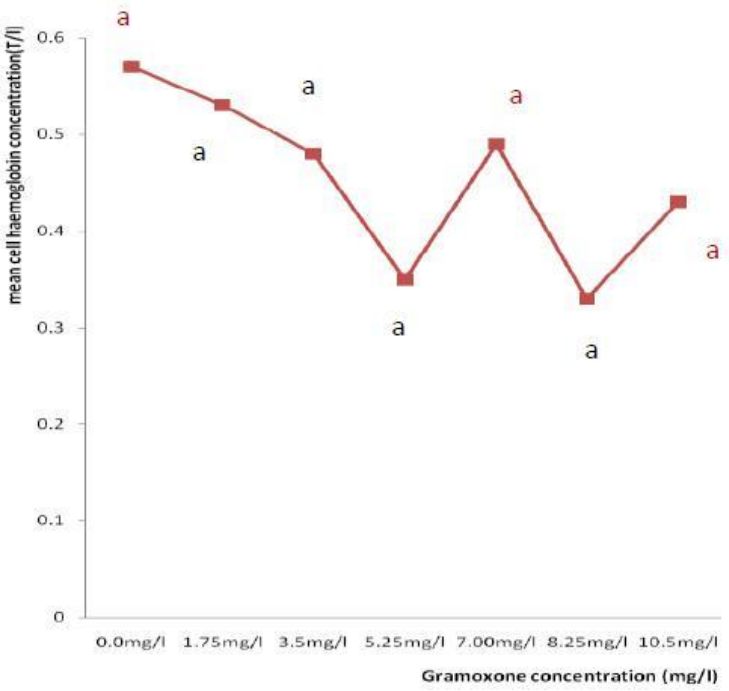

Figure 5. Mean cell haemoglobin concentration of *Oreochromis niloticus* juveniles exposed to different concentrations of Gramoxone herbicide, means carrying the same letters are sie not significantly different.

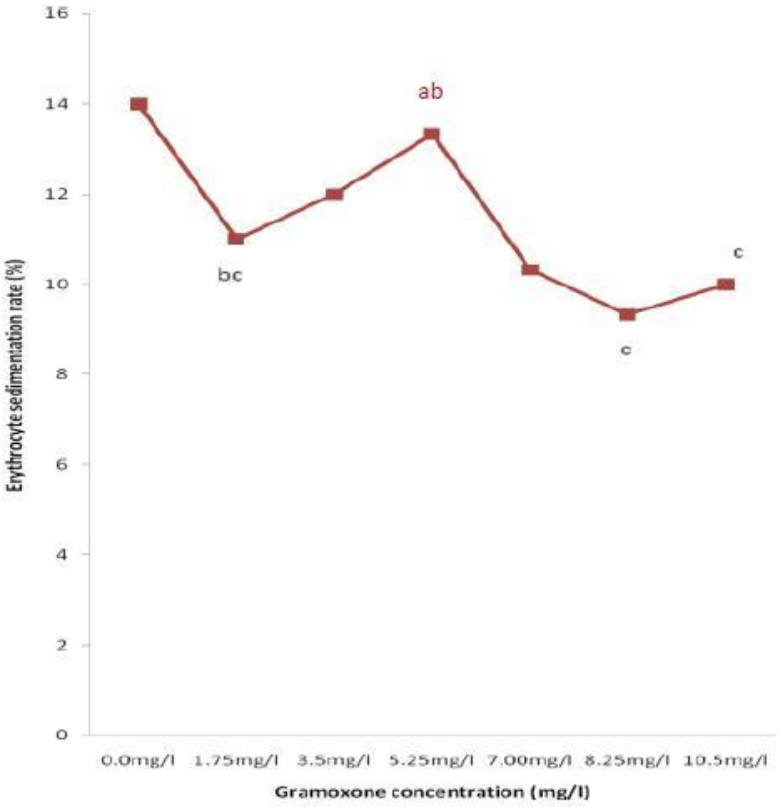

Figure 6. Erythrocyte sedimentation rate of *Oreochromis niloticus* juveniles exposed to different concentrations of Gramoxone herbicide. Means carrying different letters are significantly different.

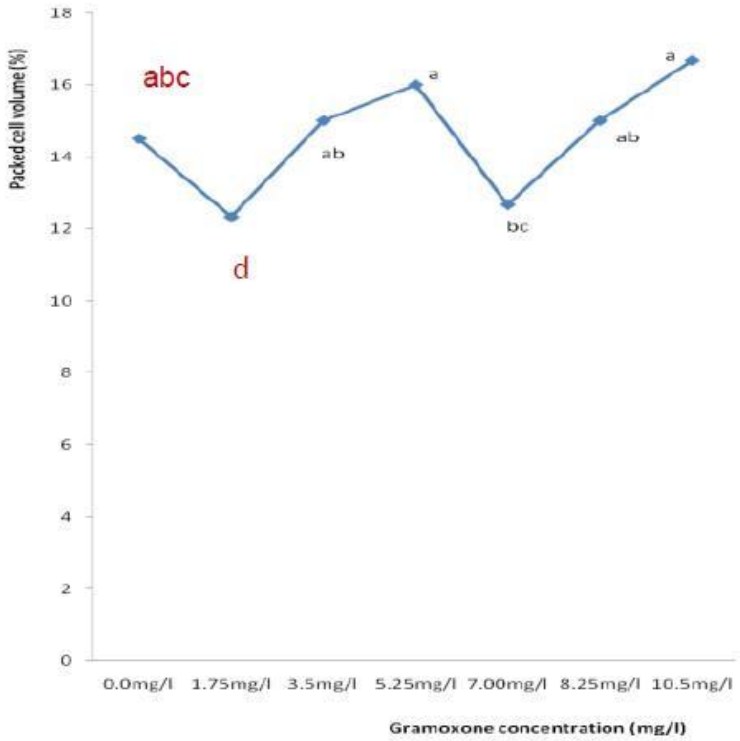

Figure 7. Packed cell volume of *Oreochromis niloticus* juveniles exposed to different concentrations of Gramoxone herbicide. Means which carry different letters are significantly different.

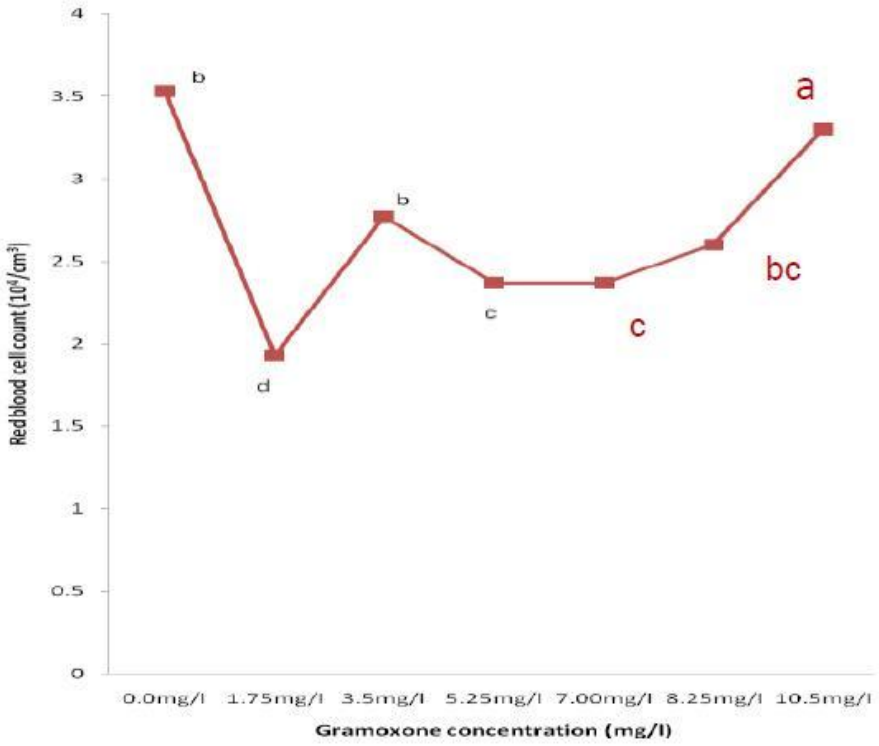

Figure 8. Red blood cell count of *Oreochromis niloticus* juveniles exposed to different concentrations of Gramoxone herbicide. Means which carry the same letters are not significantly different.

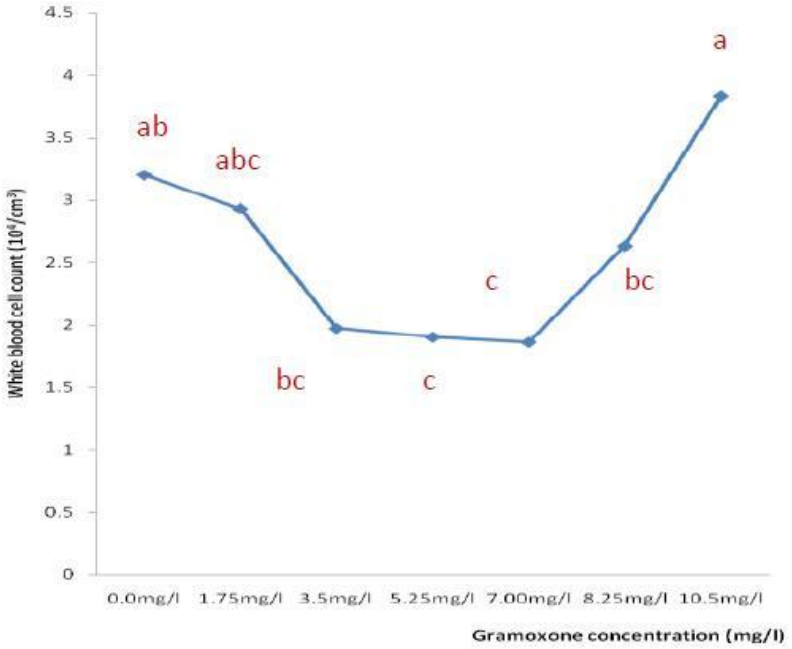

Figure 9. White blood cell count of *Oreochromis niloticus* juvenile exposed to different concentrations of Gramoxone herbicide. Means bearing different letters are significantly different.

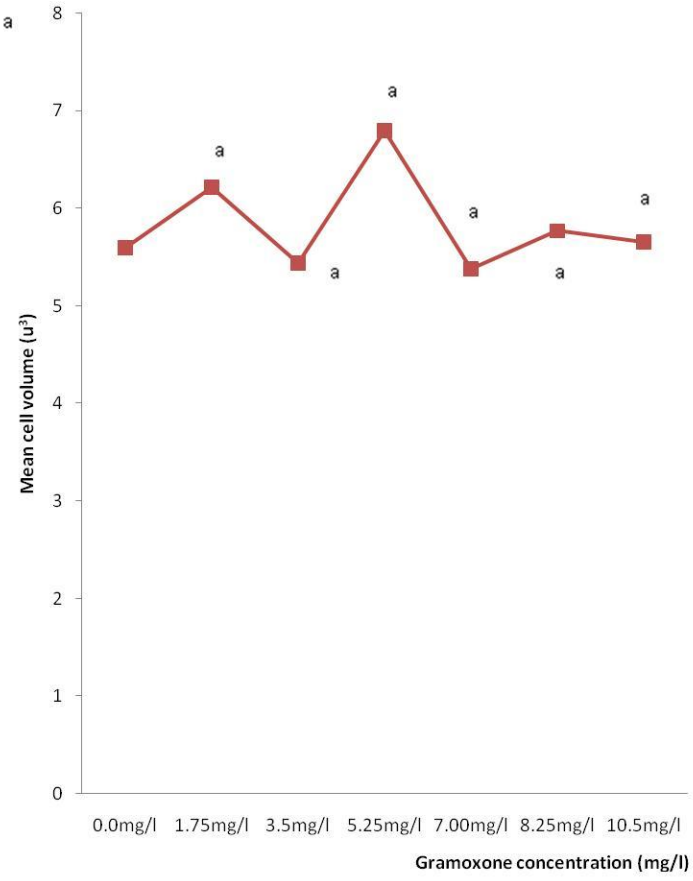

Figure 10. Mean cell volume of *Oreochromis niloticus* juvenile exposed to different concentrations of Gramoxone.

dependent in this experiment. These were similarly observed by Ateeq et al. (2005) and Olurin et al. (2006). It has also been stated that all substances could be poison when exposed to organisms beyond certain concentrations and time limit (Jouncey and Ross, 1982; Ross and Chills, 1996; Ogundele et al., 2004; Wikes University, 2010).

Gramoxone is a contact poison even in the plant kingdom (Kori-Siakpere et al., 2007). It could attack aquatic organisms especially those that flush water through their openings (mouth and anus) for the purpose of gaseous exchange. Paul and Simonin (2007) made similar observation in the soft-shell turtles in North America.

Gramoxone is marked as class II by WHO classification of toxic substances (Akobundu, 1987). It's LC_{50} for Nile tilapia in this experiment for 24, 48, 72, and 96 h of 10.40, 7.65, 7.50 and 7.00 mg/L shows that it is more toxic than organo-phosphorus insecticides as was reported by Santhakumar and Balaji (2000). Though these fish were not exposed to the same fish and yet different ages and environment. Santhakumar and Balaji (2000) found the LC_{50} 24, 48, 72, and 96 h of organophosphorus insecticide to be 22.65, 21.20, 19.75 and 19.00 ppm respectively in *Anabas testudineus*. Jiraungkoorskul et al. (2003) noted that the toxicity of toxin is species and environmental factor dependent. Gramoxone has appeared to be of less toxicity than an insecticide ethofenprox when compared with the report of Muniyan and Veeraraghaven (1999); who reported the LC_{50} 24, 48, 72 and 96 h of ethnofenprox to be 1.85, 1.79, 1.76, and 1.74 ppm respectively in a static experiment conducted using *Oreochromis mossambicus*.

In the presence of adverse environment, every organism intends to adjust itself for survival. The reduced oxygen in the water could be caused by increased uptake by fish (Shallangwa and Auta, 2008; Vijayan et al., 2001). Exposed fish may have got increased energy demand at their gills and other tissues. Vijayan et al. (2001) demonstrated that even slight change in hyalinity (salinity) was capable of creating energy deficiency in the tissues of *O. niloticus*. This may have resulted from the fact that there was increased glucose production in the liver, making the glucose (energy) highly demanding. The exposed fish would have to give off when its total ability to adapt or adjust is exhausted. This becomes more realistic when it is known that in nature stressed fish will more readily reject food.

The abnormal physiology, morbidity and mortality were time and concentration dependent the organism is exposed to. Mortality may arise due to the poison interfering with metabolism. Useful nutrients may not be able to be utilized. El-Gawad et al. (2007) said similarly that vitamin C deficiency could result to reduced growth, haemorrhage, reduced wound repair and anaemia.

Kori-Siakpere et al. (2007) observed reduced levels of plasma glucose, reduced triglyceride concentration, and increased concentration of cholesterol and lower levels of plasma protein in *Clarias gariepinus* exposed to Paraquat. Hypoglycaemia could have resulted from extensive physical effort which exhausted available blood glucose present (Kori-Siakpere, 1998). The stored glucose in form of glycogen could be rendered not useable due to the liver's inability to convert it to glucose and its subsequent elimination because the liver is degenerated. The fish metabolism using glucose is diverted to the necessary energy being supplied by oxidation of fatty acids, which may have caused the rise in cholesterol levels in exposed fish (Kori-Siakpere et al., 2007).

The temperature and acid pH were relatively the same or not statistically different between groups possibly because the herbicide may not have reacted as in exothermic or endothermic reactions. But oxygen concentration reduced with increase concentration of herbicide. It may be possible that the herbicide needs oxygen for its decomposition. The heighten activities of the fish due to poison can also remove oxygen from the water body (Shallangwa and Auta, 2008).

There was a significant decrease in both the white and red blood cell counts with increasing concentration of the toxicant to a point. They then increased with increase concentration. Increase in blood cell count in this experiment agrees with the reports of Ayotunde et al. (2010) and Ayoola (2006). This may have accounted for the significant rise in packed cell volume, which was higher in the exposed groups compared to the control group. This is because the packed cell volume is influenced by both the number (population) of cells and their size. In this experiment, the mean cell volume did not change significantly in the exposed groups compared with the control. It could therefore be assumed that the increase in packed cell volume was due to increase in cell population. The observation in this experiment that the mean cell haemoglobin was reducing with increasing concentration of poison may have triggered the multiplication of the blood cells to compensate for the low load of haemoglobin per cell. Because haemoglobin is an oxygen carrier, its availability in tissues of vertebrate is important. Its reduction invariably means anoxic internal environment that confers stress on the organism. Tissue respiration and metabolism induces morbidity and mortality. Increase in blood cell count is seen as adaptation of organism to fight cell poisons. Baker et al. (2001) explained that the population of blood cells exposed is usually made of younger and smaller cells because the old ones are killed. The surviving ones have to multiply rapidly to fight their destroyers. The increase in number of younger and smaller cells may have been responsible for the slight reduction but statistically similar cell volume observed between exposed and controlled groups.

Gramozone has been described as a free radical generator in the body of organisms (Asada and Barba, 2004). All amino acid residues are susceptible to attack

by –OH. The reactive oxygen species (ROS) may cause fragmentation, aggregation, amino acid modification and change in proteolytic susceptibility (Stadmann, 1992, 1993).

Environmental pollutants such as air pollutant, photo-chemical smog, industrial chemical, ionizing radiations as well as the metabolism of xenobiotics, as was pointed out by Ames (1990) contribute to cellular steady state con-centration of reactive oxygen species. Reactive species are formed as a response to a diverse stimulation by physiological reactions. All cell components such as lipids, nucleic acids, proteins and carbohydrates are sensitive to damage by reactive oxygen species (Aikens and Dix, 1991). The explanation of mechanism of histological damage made to the fish tissue could be drawn from Asada and Barba's (2004) that super oxide and hydrogen peroxide will form the destructive hydrogen radicals and initiate the oxidation of organic substrates.

Death in fish is the end product of the various effects caused in the various tissues and organs. When these tissues collectively stop functioning due to the toxin, the fish dies. For instance, the degeneration of gills causes a dysfunction of its gas exchange ability causing an anoxic internal environment (Ajani et al., 2007). The blood is a homeostatic organ in fish. Any attack made on it, if intense, do cause damage that will result to outright death of the organisms. Its reduction of the hepatoso-matic index is in line with destruction of the liver tissue. The liver being the target point as it is used as organ of poison modification or detoxification (Taylor et al., 1988). As the liver is overwhelmed, it is consequently degenerated so that this gives way to become open for the poison to attack other tissues more freely and intensively.

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