Haematological Effect of Iron and Lead on *Clarias gariepinus* Juveniles

Omovwohwovie E. E.
Department of Fisheries Technology,
Federal Polytechnic Ekowe Bayelsa State

Paterson Adogbeji Ekokotu & Arnold Ebuka Irabor
Department of Fisheries,
Delta State University,
Asaba Campus.

Garba A. Abubaka
Department of Fisheries and Aquaculture,
Bayero University Kano
Nigeria

Egwono S. Osaide
Department of Biological Science Education,
Delta State College of Education Warri
Nigeria

Abstract
Laboratory study was undertaken to evaluate some physical and haematological changes resulting from the exposure of freshwater fish Clarias gariepinus to sub lethal concentrations ($0.1mg^{-1}$ and $0.4mg^{-1}$ of ion (fe) chloride, and ($0.1mg^{-1}$ and $0.4mg^{-1}$ of lead (Pb) chloride) in the water for a period of 15 days. Five(5) groups of twenty fishes each were subjected to serial dilutions of the stock solution of iron (fe) 0(control), $0.1mg^{-1}$ and $0.4mg^{-1}$ and lead (Pb) 0(control), $0.1mg^{-1}$ and $0.4mg^{-1}$ in a large plastic bowl of 60 litres capacity for 15days at the end , blood sample were taken from the control and experimental fish. Blood was assayed for selected haematological parameters (haematocrit, haemaglobin, red blood cells counts, white blood cell counts, differential white blood cell counts, erythrocyte sedimentation rate, and total plasma protein and plasma glucose concentration). The derived haematological indices of mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) were calculated. $0.1mg^{-1}$ and $0.4mg^{-1}$ of lead (Pb) when compare to control. There is no significant difference on differential white blood cell count in iron (fe) concentration except Neutrophill and lymphocytes and there is a decrease in red and white blood cells on different concentrations of lead (Pb) $0.1mg^{-1}$, $0.4mg^{-1}$ and iron(fe) $0.1mg^{-1}$, $0.4mg^{-1}$ treatment when compare to their control. In conclusion, the changes observed indicate the haematological parameters can be used as an indicator of iron and lead related stress in fish on exposed to elevated iron and lead levels.

Introduction

Iron and lead are essential elements; they are one of the most common heavy metals pollutants. The sources of iron and lead and other heavy metals in the aquatic environment maybe from geological rock weathering or from human activities such as industrial and domestic wastes water from discharge animals where it forms constituent functions in maintain cytoplasmic integrity (Wheatherly *et al.*, 1980). Intense activities in both the

industrial and agricultural sectors heave lead to increase the levels of heavy metals in natural waters (Nimmo *et al.*, 1998, Jordae *et al.*, 2002) heavy metals are serious pollutants of the aquatic environment because of their persistence and ability to bio-accumulate in aquatic organisms (Veena and Radhkrishnan 2007). The contamination of the water bodies with heavy metals has become a source of concern not only because of their threat but also due to the public health implication of such contamination (Farombi *et al.*, 2007).

Some research findings has shown that heavy metals in aquatic environment could accumulate in biota especially fish, the most common aquatic organism at higher tropic level (Olaifa and Wariaglhi 2013), other factors for bioaccumulation has been reported to depend upon the rate of excretion (Huany 2003) species differences as well as feeding habitat and tropic status of the fish. Most heavy metal have no beneficial function to the body and can be highly toxic, it enters the body through inhalation, ingestion and skin and can accumulate in the body tissue faster than the body's detoxification pathways and deposition (Ekpo *et al.*, 2008). In fish exposure to chemical pollutants induces either increase or decrease in the haematological and some biochemical parameter (Hilmy *et al.*, 1995), Juskova 1997, Annune and Ahuma 1998). Thus the changes in haematological parameters are good indicators of changes in the water quality.

Metals are non-bio degradable and form part of environmental pollutants in which elevated levels, form threats to human health through food chain (Goodwin, *et al*, 2003, Rant *et al.*, 2009) metals like lead which are toxic are known to present greater hazard when they are both persistent and bioaccumulative (De forest *et al.*, 2009).

Finally the use of haematological techniques is gaining importance for toxicological research, environmental monitoring and assessment of fish health conditions (Shah and Alfindag 2004). Blood parameters are considered patho-physiological indications of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants (Adhikari and Sarkar, 2004, Maheswaran *et al.* 2008).

This study is aimed at observing the mortality and survival rate as well as haematological effect on *Clarias gariepinus* exposed to iron (fe) and lead (Pb).

Materials and Method

Collection of Fish Sample for Study

A total of 100 catfish of (4) four weeks old, about the same size and weight were purchased from a notable fish farm in Asaba Delta State, and was transported in a 40 litres plastic water holding receptacle to the Fishery laboratory in Delta State University Asaba Campus. Only healthy and active fishes were reared for proper acclimation in open water receptacle kept within the project laboratory of the Department of fishery. They were fed with 1.5mm Coppens fish feed.

Table 1: Showing Analysis of Fish Feed

Compound/Nutrient	% composition
Crude protein	45
Crude fat	12
Ash	5.5
Preservatives	E280
Antioxidant	E324, EE 321
Vitamin A	1.5,000Iu/kg
Vitamin D3	2,000Iu/kg

Vitamin E	200mg/kg
Vitamin C	150mg/kg

Experimental Procedures

100 catfish of (4) four weeks old *Clarias gariepinus* with mean weight of 4.56g and mean length of 8.69cm were kept to acclimate in bowls containing clean tap water for 3 weeks (21 days) . The fishes were fed with 1.5mm Coppens fish feed daily at 2 percent body weight and the water was also changed daily. The bowls were covered with mosquito size nets to prevent them from jumping out. A total of five (5) bowls were used for the experiment. Two(2) bowls labeled 1^A – 2^A were used different concentration of 0.1mg and $0.4mg^{-1}$ of lead (Pb) treatment and another two(2)bowls labeled 3^A-4^A were used for iron(fe) of $0.1mg^{-1}$ and $0.4mg^{-1}$, the remaining one bowl was used as their control.

Preparation and Introduction of Chemicals

Hydrated salt of iron (fe) and lead (Pb) were used as toxicant. A concentration of $0.1mg^{-1}$ and $0.4mg^{-1}$ of iron (fe) and $0.1mg^{-1}$ and $0.4mg^{-1}$ of lead (Pb) were weighed using top rolling balance. Each concentration were dissolved in a litre of distilled water in a volumetric flask and distributed into the experimental bowls. Feeding of fish were stopped a day before the introduction of the toxicant after the acclimation of the fish for 3 weeks (21days) and the water were changed daily to maintain the toxicant for 15 days.

Haematological Procedures

After 15 days, the blood samples were taken from the experimental and control tank fish. The blood samples were taken by inserting 2ml syringe at the caudal vessel above the lateral line at the angle 45^oc and gently draw the blood via capillary action up to 1ml. immediately release the blood into the EDTA tube containing anti-coagulant to prevent blood clothing. The blood was analyzed for different haematological parameters.

Haemaglobin Method/HB Fluid

1. Measure 5ml of Draskin reagent or HB diluting fluid into a clean tube.
2. Using Hb pipette, suck the blood sample exactly to mark 20µl.
3. Release the blood into the tube containing Draskin solution (i.e Hb fluid).
4. Mix the blood thoroughly by gently shaking with the tube covered and allows it to stand for 10mins.
5. Take the readings on colorimeter at 540nm using Draskin reagent as blank.
6. Read the haemaglobin values from the standard tables in gram (g/dL).

Haematocrit Determination

Principles: Blood compartment is separated into three parts using capillary tube in a haematocrit centrifuge.

Methods:

1. Fills the blood into a micro haematocrit tube ($^3/_4$) and seal it with a sealant.
2. Centrifuge the centrife at 2000rpm for 5-10mins.
3. Read the values with haematocrit reader and record the result.

Plasma Protein Method

This is determine using a refractometer

A drop of plasma from capillary tube it release at the edge of the refractometer and then view to take your reading or values.

Red Blood Cells Method

Principles: A small amount of white blood cell is accurately diluted with a fluid, which is isonomic with blood method.

Method: manual haemacytometer method.

Procedures:

1. Using RBC pipette, take the blood into RBC pipette up to 0.5 mark and wipe the blood, clean the pipette with tissue paper.
2. Immediately draw the RBC diluting fluid up to mark 101(1:200 dilutions).
3. Rotate or twirled the pipette to mix the blood and the diluents.
4. Remove the pipette from diluting fluid and shake the pipette for few seconds.
5. Clean the counting chamber of haemacytometer and cover slip.
6. Place the cover slip in position over counting chamber by gently pressure.
7. Expel a drop of blood on the counting chamber by holding the pipette at angle 45^{o}c.
8. Allow the haemacytometer to stand for 2-3mins to settle down the red blood cell in counting chamber at an angle 45^{o}c.
9. The central square (millimeter) counting chamber is use for counting the erythrocytes.
10. The cells contained in 80 of the 400 small squares are counted.
11. The number of erythrocytes per cubic millimeter = cell counted ×10,000.

White Blood Cell Count

Principles: A small amount of blood is accurately diluted with 2% acetic acid, which destroys the non-nucleated erythrocyte and makes clearly visible the nuclei of the leukocytes.

Method: Haemacytometer.

Procedures:

1. Draw the blood into the white blood cell pipette up to 0.5 marks.
2. Wipe the outside of the pipette and adjust the level of blood carefully to the 0.5 mark.
3. Immediately plunge the pipette into the RBC fluid and draw to the 11 mark (1:20 dilution).
4. Remove the pipette from diluent and shake for few seconds.
5. Mix the content before counting the cells.
6. Clean the counting chamber of haemacytometer and cover slip.
7. Place the cover slip on the counting chamber with gentle pressure.
8. Expel the fluid in the pipette for 2mins to settle down the white blood cell.
9. Allow the fluid in the pipette for 2mins to settle down the white blood cell.
10. Count the white blood cell in 4 large squares in the corners of counting chambers (16 small squares).
11. Therefore total WBC = number of leucocytes per cubic mm = number of cells counted × 50.

Differential Leukocytes Count

a. Neutrophil
b. Eosinophil
c. Basophil
d. Monocyte
e. Lymphocyte

Method; Blood film (SMEAR) with wright's or Giemsa stain.

Materials/ Requirement for differential count

1. Clean glass slide.

2. Sterile needle, pipette.
3. Microscope.
4. Stain solution.
5. Oil immersion.

Procedure

Making the smear

1. Place a small drop of blood near an end of the slide.
2. Bring another slide in contact with the drop and allow distributing an angle of 30-40^{o}c.
3. Push to the left in a smooth and quick motion.
4. Dry the slide in air.
5. Stain the smear with Giemsa or wright's stain for 3-10mins.
6. Rinse the slide with distills water at room temperature.
7. Drain off the water and allow the slide to dry.
8. Place a drop of Canada balsam (oil immersion) on dried smear and mount the cover slip.
9. Examine under dry objective or oil immersion of × 100 objectives of the microscope.
10. Count about 100- 200 cells and take the average.

Plasma Glucose (Carbohydrates) determination in blood serum by biuret method

Principles

Glucose is determined after enzymatic oxidation in the presence of glucose oxidase. The hydrogen peroxide formed reacts, under catalysis of peroxidase, with phenol and of 4-aminophenazone to form a red-violet quinoneimine.

Calculating Red Blood Cell

1. MCV - Mean Corpuscular Volume

$$\frac{\text{Hct(Pcv)} \times 10}{\text{RBC}} = \text{fl(fintolitre)}$$

2. MCH - Mean Corpuscular Haemaglobin

$$\frac{\text{Hb} \times 10 \text{ Pg(Pictogram)}}{\text{RBC } (10^{6}\mu\text{L})}$$

3. MCHC - Mean Corpuscular Haemaglobin concentration

$$\frac{\text{Hb}}{\text{PCV}} \times 100 \text{ (g/dl)}$$

Data Analysis

The data collected from the haematology were analyzed using one-way Analysis of Variance (ANOVA), mean difference is separated using Duncan multiple range test. All analysis was carried out using SPSS VERSION 22

Results/ Discussion

Water Quality Parameter

The result of water quality parameters recorded during the experiment is presented in Table 2.

Table 2: Water Quality Parameter

Ph	6.55+0.36
Temperature	28.4 + 1.4^{o}c
Dissolved oxygen	6.36+ 1.07 mgL^{-l}
Alkalinity	33.8+1.72mg^{-l}
Turbidity	0.306 +0.08mg^{L}
Hardness	125.56 + 11.75mg^{L}

Source: Delta State University Research Lab. Asaba

Water quality in fish ponds is affected by interaction of different chemical components recorded above.

pH

It indicates whether water is acidic or alkaline. Water is considered acidic when pH is below 7 and the alkaline when it is above 7. The recommended pH range for aquaculture is 6.5 to 9.0 where 7 is the neutral.

Alkalinity

It is the quantity of base that is present in the water, such as carbonates, bicarbonates, hydroxides etc. A desirable range of total alkalinity for fish culture is between 75 and 200mg/L but not less than 20mg/L.

Hardness

It is a measure of the quantity of divalent ions such as calcium, magnesium etc. measured by chemical titration in mg/L. The degree of hardness is as follows: 0-75mg/L is soft, 75-150mg/L is moderately hard, 150-300mg/L is hard, and over 300mg/L is very hard alkalinity. Desirable levels for fish culture generally falls within the range of 20-300mg/L.

Temperature

The temperature of the water affects its activity, behaviour, feeding and growth etc. of the fish. Normal temperature for catfish is between 28-30^{o}C.

Turbidity

It is a measure of how particles suspended in water affect water clarity, slightly turbid water can be perfectly healthy while clean water contains unseen or unhealthy level of nutrient. A turbid water of 30-80cm is preferable for culturing fish.

Dissolved Oxygen

The amount of oxygen consumed by a fish is a function of its size, feeding rate, activity level and temperature .small fish consume more oxygen than to large fish because of their higher metabolic rate (Meade (1974).

Changes in Haematological Parameters

Result of haematological parameter of *Clarias gariepinus* subjected to different concentrations of iron (fe) are shown in table 3 below

Table 3: Analysis of Haematological Parameters.

Parameter	% control	0.1mg^{-1}	0.4mg^{-1}
Haematocnt (%)	39.00± 1.0^{b}	36.00±0.00 ^{b}	31.00±1.00^{a}
Haemaglobin (gd^{-1})	13.15±0.15^{c}	12.05±0.05^{b}	10.65±0.15^{a}
RBC (mm^{-3})	46.00±0.00^{c}	42.00±4.00^{ab}	35.00±50^{a}
WBC (mm^{3})	47.00. ±100.0^{c}	4050±50^{b}	3850±50^{a}
MCH (pg)	2.86±0.03^{a}	3.06±0.03^{a}	3.05±0.13^{a}
MCV (mg)	8.97. ±0.71^{a}	9.23±0.23^{a}	8.86±0.53^{a}
MCHC (%)	33.73±0.48^{a}	33.45±0.15^{a}	34.37±0.62^{a}
Plasma protein (gd -1)	7.40±0.40^{a}	6.9±0.70^{a}	6.30±0.30^{a}
Plasma glucose (mgd^{-1})	91.13±1.01^{b}	80.00±1.01^{a}	76.96±1.01^{a}

Note: The values are expressed as means ± S.E
Significance at 0.05 level and Non significance at α >0.05 level

The main haematological alteration from the exposure of *Clarias gariepinus* to various concentrations of iron in the water for 15 days, Haematocrit show mean value of 39.00±1.0% on the control, while 36.00±0% on 0.1mg and 31.00± 0% on 0.4mg^{-1}. Hb shows a significant decrease in all concentration. Plasma protein show decreasing value due to increase in concentration of iron (7.40±0.40) control, (6.9±0.7) 0.1mg^{-1}, 6.30±0.30 on 0.4mg^{-1}. This implies that plasma protein is not significant different as P0.304>0.05(95% confidence interval) 7.4±0.40 at 0% concentration, 6.90±0.40 at 0.1mg^{-1} and 6.30±30 at 0.4mg^{-1}. RBC shows no significant difference across the treatment at P1.00>0.05. The value of RBC is 46.00± 00±00 at 0% conc., 42.00±4.00 at 0.1mg^{-1} and 35.00± 50 at 0.4mg^{-1}. There is a significant difference in the value of WBC due to different concentration of iron at 4700±100 at 0%. 4050±50.00 at 0.1mg^{-1} and 3850± 50.00 at 0.4mg^{-1} as P 0.007<0.05. There is no significant different in the haematological indices of value of MCH after exposure to different concentration to iron as P0.279> 0.05. MCH is 2.86± 0.03 at 0%mg^{-1}, 3.06 ± 0.03a + 0.1mg^{-1} and 3.05± 0.13 at 0.4mg^{-1}, MCV P.0.885>0.05 and MCHC of P. 0.455>0.05 MCHC is 33.73±0.47 at 0%mg^{-1}, 33.45± 0.15mg^{-1} and 34.37±0.62 at 0.4mg^{-1}. There is a significant difference in mean value of glucose after exposure to different concentrations of iron as P0.03<0.05.

Changes in differential White Blood Cell Counts

Result of haematological parameter of *Clarias gariepinus* subjected to different concentrations of iron (fe) are shown in table 4 below.

Table 4: Analysis of Changes in differential White Blood Cell Counts

Parameter	% control	0.1mg^{-1}	0.4mg^{-1}
Lymphocytes	45.00±1.0^{a}	47.50±0.50^{ab}	40.00±0.00^{a}
Basophils	1.0±0.00^{a}	1.50±0.50^{a}	1.0±0.00^{a}
Neutrophils	51.00±1.0^{a}	50.00±0.00^{a}	58.00±2.0^{b}
Eosinophils	3.00±1.0^{a}	2.00±0.0.00^{a}	2.00±0^{a}
Monocytes	1.5±0.50^{a}	1.0±0.00^{a}	2.0±0.00^{a}

Note: The values are expressed as means ± S.E
Significance at 0.05 level and Non significance at α >0.05 level

There are no significant difference in white blood cell counts of *Eosinophylls*, *Monocytes*, *Basophyls* except *Neutrophills* and *lymphocyte* at (p<0.05) only on 0.4mg^{-1} and significant difference of p 0.008 <0.05 in lymphocyte.

Shows changes in Haematological Parameters

Result of haematological parameter of *Clarias gariepinus* subjected to different concentrations of lead (pb) are shown in table 5 below

Table 5: Analysis Changes in Haematological Parameters

Parameter	0% control	$0.1mg^{-1}$	$0.4mg^{-1}$
Hematocrit %	39.00 ± 1.0^{a}	30.00 ± 0.00^{b}	26.50 ± 0.50^{a}
Haemoglobin (gb^{-1})	13.15 ± 0.15^{a}	10.35 ± 0.50^{b}	8.90 ± 0.10^{a}
RBC (mm^3)	46.00 ± 0.00^{b}	42.00 ± 4.0^{a}	35.00 ± 1.00^{a}
WBC (mm^3)	4700.0 ± 100.0^{b}	4050.00 ± 50.00^{a}	3850.0 ± 50.00^{a}
MCH (Pg)	2.86 ± 0.03^{a}	3.06 ± 0.40^{a}	3.05 ± 0.13^{a}
MCV (mg)	8.47 ± 0.21^{a}	9.23 ± 0.23^{a}	8.85 ± 0.55^{a}
MCHC (%)	33.73 ± 0.48^{a}	33.45 ± 0.15^{a}	34.37 ± 0.62^{a}
Plasma protein (gd^{-1})	7.40 ± 0.40^{a}	6.10 ± 0.10^{a}	6.05 ± 0.45^{a}
Plasma glucose (mgd^{-1})	91.13 ± 0.03^{b}	80.00 ± 1.01^{a}	76.96 ± 1.01^{a}

Note: Mean = mean ± SER

Significance at 0.05 level and Non significance at $\alpha > 0.05$ level

Haematocrit show significant difference at P0.002<0.05, and there is decrease in value of PVC as lead contraction increase. There is a significant difference in mean due to variation in concentration of lead (Pb) at (P0.00<0.05) No significant difference in mean value of plasma P.0.118>0.05. Plasma protein is 7.40 ±0.40 at $0\%mg^{-1}$, 6.10±0.10 at $0.1mg^{-1}$ and 6.05 ±0.45 at $0.4mg^{-1}$. There is a significant difference in RBC and WBC in the mean values due to different concentration of lead, at RBC (P0.01>0.05) and WBC at (0.007<0.05). the haematologic indices of mean corpuscular volume (MCV) mean corpuscular haemoglobin concentration MCHC, and Mean corpuscular haemoglobin (MCH) show no significant difference after exposure to different concentration of lead (Pb) at MCH = P0.285>0.05, MCV = P=0.445>0.05, MCHC at P0.455>0.05 and there is slightly increase in $0.1mg^{-1}$ and slightly decrease in $0.4mg^{-1}$ when compared to control. Glucose shows a significant difference in the mean value after exposure to different concentration of lead as P 0.03<0.05.

Shows changes in differential white blood cells counts

Result of haematological parameter of *Clarias gariepinus* subjected to different concentrations of lead (pb) are shown in table 6 below

Table 6: Analysis of Changes in differential white blood cells counts

Parameter	% control	$0.1mg^{-1}$	$0.4mg^{-1}$
Lymphocytes	45.00 ± 1.0^{b}	47.50 ± 0.50^{b}	40.00 ± 0.00^{a}
Basophils	1.50 ± 0.50^{a}	1.00 ± 0.00^{a}	1.00 ± 0.00^{a}
Neutrophils	51.00 ± 1.08^{a}	50.00 ± 0.00^{a}	58.00 ± 2.0^{a}
Eosinophils	3.00 ± 1.00^{a}	$2.00\pm0.0.00^{a}$	2.50 ± 0.50^{a}
Monocytes	2.00 ± 0.00^{b}	1.00 ± 0.00^{a}	2.0 ± 0.00^{b}

Note: The values are expressed as means ± S.E

Significance at 0.05 level and Non significance at $\alpha > 0.05$ level

Neutrophils shows a significant difference in mean value across the different concentration P0.04<0.05, *Eosinophil* shows no significance difference in mean value after exposure to different level of lead concentration as P 0.372>0.05. *Monocyte* shows significant differences in mean as P 0.04<0.05. *Basophils* shows no significance difference in mean count after

exposure to different concentration of lead at P0.465>0.05. There is a significant difference in mean value of *lymphocytes* after exposure to different concentration of lead at P0.008<0.05.

Contamination of aquatic environment by heavy metals whether as a consequence of acute and chronic events constitutes additional source of stress for aquatic organisms. Sub lethal concentrations of toxicants in the aquatic environment will not necessarily result in outright mortality of survival of aquatic organisms. Omoregie *et al.,* (1990) reported that toxicants and pollutants have significant effects which can result in several physiological dysfunctions in fish. Dysfunction in fish induces changes in blood parameters possible as a result of blood water content.

Iron and lead are known to be an essential element of plants and animals. However at high concentrations, it exerts adverse effects by accruing structural damage which effects, the growth, development and survival of the fish (Tuurala and Solvio, 1982). The results obtained from this study indicated that *Clarias gariepinus* juveniles were affected by $0.1mg^{-1}$, $0.4mg^{-1}$ of iron (fe) and $0.1mg^{-1}$ and $0.4mg^{-1}$ of lead (Pb) solutions. Although there was no mortality during this study but it reduces their feed intake and thus affecting their growth when compared to the controls. The observed significant (P<0.05) reduction in feed intake with the concentrations of ion (fe) ($0.1MG_{-1}$), ($0.4mg^{-1}$) and lead (Pb) ($0.1mg^{-1}$) and $0.4mg^{-1}$ and duration of the exposure demonstrates both time level of uptake dependent response.

The behavioural condition of the fishes in both the control and treatment medium were note every 24 hours, the fishes showed a marked change in their behaviour such as reduced feed intake and slow moment (swimming) when exposed to different concentration at iron (fe) ($0.1mg^{-1}$ and $0.4mg^{-1}$) and lead Pb ($0.1mg^{-1}$ and $0.4mg^{-1}$) the fishes showed rapid swimming than the situation in the control more especially the higher concentrations of the solutions. The sub lethal exposure of *Clarias gariepinus* to sub lethal concentrations of lead and iron cause a significant decrease in haemoglobin and haematocrit of a fish. A similar reduction has been reported by (Annune *et al.*, 1994b). The decrease in haematocrit following iron and lead exposure in *Clarias gariepinus* may be an indication of haemodillution. (Tort and Torres 1998) reported decrease in haematocrit following 24hours exposure of dogfish, (*Scyhorhinus canicula*) to cadmium contamination. They attributed this decrease to haemodillution. There was no significant change in erythrocyte count and erythrocyte sedimentation rate in both the iron and lead concentration on *C. gariepinus* expose on them. The red blood cell count of *C. gariepinus* was reported to have increase significantly by (Annune *et al*., 1994a) when the fish was subjected to zinc treatment. They attributed the red blood cell evaluation to blood cell reserve combine with cell shrinkage as a result of osmotic alteration of blood by the action of metal (Tort and tores 1988). The decreasing value of plasma proteins in this study on exposure of iron (fe) treatment were attributed to renal excretion or impaired protein synthesis or due to liver disorder (Kori-Siakpere, 1995) on the other hand, the observe decrease of plasma protein could also result and possible into nitrogen and other elementary molecular.

A reduction of plasma glucose observed in this study on both lead and iron treatment may be due to changes in carbohydrate metabolism occur in fish exposed to various sub lethal concentration of pollutants, blood glucose has been employed as on indicator to environmental stress (Silbergeld 1974). Decrease glucose absorption has been reported in *Pontius conchnius* exposed to mercury nitrate (Gill and Pant, 1981) and *Isheriensis*, C. (Sydenham) exposed to water borne lead (Kori-Siakpere, 1995). The decreasing number of white blood cells from the lower to higher concentration of iron and lead are normal reaction in the present study and cadmium in a previous study by (Kori-Siakpere *et al.*, 2006). The

decrease number of white blood cells *(Ieucopaenia*) may be the result of bio-concentration of the test metal in the kidney and liver (Agrawal and Srivastara 1980) decrease number of white blood cells may also be related to an increased level of corticosteroid hormones whose secretion is a non-specific response to any environmental stress (Iwama *et al.*, 1976, Ellis 1981). The significant difference in *neutrophils* and *lymphocytes* of fish exposed to different concentration of iron treatment and significant difference in *neutrophil*, monocytes and lymphocytes in different concentration of lead treatment are in agreement with findings of (Sharma and Gupta 1984) when juveniles of mudfish, *Clarias batrachus* were exposed to carbon tetrachloride. (Musa and Omorgie (1999) also reported a decrease in *neutrophils* of *C. gariepinus* (Burchell) exposed to malachite green. This was attributed to tissue damage.

Conclusion/Recommendation

Heavy metals like lead and iron are those that are common pollutants in our water, that cause a wide range of stress to the aquatic organisms and environment when exceed their normal environmental condition that lead to pollution. There is a clear inter-relationship between the concentration of pollutant in the water and its effects on the blood of the fish. *Clarias gariepinus* exposed to even $0.1mg^{1}$ iron and lead concentration proved to be the highly toxic causing deleterious effects in their various parameters.

The under listed recommendation are steps which can be taken as a means of adjusting the effect of heavy metal on fish and their habitat. Discharge of effluent into the water bodies must be treated. A strict law controlling treatment standard of disposal of industrial and petroleum effluents should be enforced and by this it will reduce the rate of abnormal discharge of sewage and industrial refuse.

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