6.1 INTRODUCTION AND REVIEW OF LITERATURE

In recent years, haematological variables were extensively used when clinical diagnosis of fish physiology was applied to determine the effects of external stressors and disease conditions in fish (Hodson et al., 1978; Dhillion and Gupta, 1983; Benerjee and Kamar, 1988). This, according to Gill and Pant (1981), is as a result of their relationship with energetic (metabolite levels), respiration mechanics (haematological levels) and defence mechanisms (leucocyte levels). Haematological parameters provide an integrated measure of the health status of fishes. The introduction of toxicants into an environment, where fishes are found, stuns them and/or acts as a stressor of the fish and organisms found in such environment (Olatayo, 2008). The introduction of a toxicant to an aquatic system might decrease the dissolved oxygen concentration, which will impair respiration thus leading to asphyxiation (Warren, 1977). Stickney (1977) also reported that insufficient amount of dissolved oxygen is one of the contributory factors of mortality in some fish species. The darkening of the fish skin, respiratory distress and erratic swimming can be observed when fishes are exposed to acute concentrations of toxicants (De Silva and Ranasingle, 1989; Ayuba and Ofojekwu, 2002).

The tremendous increase in the use of heavy metals over the past few decades has inevitably resulted in an increased flux of metallic substances in the aquatic environment (Yang and Rose, 2003). The metals are of special concern because of their diversified effect and the range of concentration stimulated toxic ill effect to the aquatic life forms. Industrial wastes constitute the major source of metal pollution in natural water (Livingstone, 2001). A large part of these elements exert their toxic effect by generating reactive oxygen species (ROS), causing oxidative stress. Stress reaction involves various physiological changes including alteration in blood composition and immune mechanisms (Svoboda 2001; Witeska, 2003). Most of the heavy metal ions are toxic or carcinogenic in nature and pose a threat to human health and the environment (Damien et al., 2004; Farombi et al., 2007). The contamination of fresh water with a wide range of pollutants...
has become a matter of great concern over the last few decades, not only because of the threat
to public water supplies, but also with the damage caused to the aquatic life. The river systems
may be excessively contaminated with heavy metals released from domestic, industrial, mining
and agricultural effluents (Vander Oost et al., 2003). It has also been linked as one major factor
of disease outbreaks, low productivity and mortality in aquaculture. Stress is a general and non-
specific response to any factors disturbing homeostasis.

Other toxic endpoints include decreased growth, mobility and reproductive effects (Allen,
1995). Stress in fish may be induced by various abiotic environmental factors like changes in
water temperature, pH, dissolved oxygen and effects of pollution. Changes in environmental
quality can therefore be a major determinant of year-class strength and eventually the long-term
dynamics of many fish populations (Rose et al., 1993). Bioassay technique has been the
cornerstone of programmes on environmental health and chemical safety (Oshode et al.,
2008). Haematological variables remain veritable tools in determining the sub-lethal concentration
of pollutants such as heavy metals in fish (Witeska, 2003). The most common haematological
variables measured during stress included red and white blood cells count, haemoglobin content,
and haematocrit value and red blood cell indices. Fish haematological parameters are often
determined as an index of their health status (Oshode et al., 2008).

The count of red blood cells is quite a stable index and the fish body tries to maintain this
count within the limits of certain physiological standards using various physiological mechanisms
of compensation. Studies have shown that when the water quality is affected by toxicants, any
physiological changes will be reflected in the values of one or more of the haematological parameters
(Van Vuren, 1986). Blood cell responses are important indicators of changes in the internal and/
or external environment of animals. In fish, exposure to chemical pollutants can induce either
increase or decrease in haematological levels. Their changes depend on fish species, age, the
cycle of the sexual maturity of spawners and diseases (Golovina, 1996; Luskova, 1997). Like in
warm blooded animals, changes in the blood parameters of fish, which occur because of injuries
of the latter organs or tissues, can be used to determine and confirm the dysfunction or injuries of
the latter (organs or tissue). However in the fish, these parameters are more related to the response
of the whole organism, i.e. to the effect on fish survival, reproduction and growth. It should be
noted that although the mechanisms of fish physiology and biochemical reaction to xenobiotics
has not been investigated enough, it is obvious that species differences of these mechanisms exist.
Fish live in very intimate contact with their environment, and are therefore very susceptible to
physical and chemical changes which may be reflected in their blood components (Wilson
The adaptability of the organism, to frequent changes in environmental conditions is studied from the release of RBCs into the blood from those stored in spleen (Toft, 1955).

In fish, exposure to chemical pollutants can induce either increase or decrease in haematological levels. Blood tissue truly reflects physical and chemical changes occurring in an organism. Therefore, detailed information can be obtained on general metabolism and physiological status of fish in different groups of age and habitat. Early diagnosis is also possible when evaluating haematological data, particularly blood parameters (Folmar, 1993; Golovina, 1996; Luskova, 1997). Furthermore, it should be noted that, haematological indices are of different sensitivity to various environmental factors and chemicals (Lebedeva et al., 1998; Vosylienë, 1999a; 1999b). The use of haematological techniques in fish culture is growing in importance for ecotoxicological research, environmental monitoring and fish health conditions as reported by Mulcahy (1969).

Sampath et al. (1993) noted that studies in fish blood lies in the possibility that the blood will reveal conditions within the body of the fish long before there is any outward manifestation of symptoms of disease or effects of unfavourable environmental factors. Several studies have been done on the effects of toxicants on the haematology of *Clarias gariepinus* (Annune and Ahuma, 1998; Musa and Omoregie, 1999; Onusiriuka and Ufodike, 2000).

Haematological changes shown by fishes exposed to copper include; increase in ammonia (NH$_3$) levels, antibody and haematocrit values, haemoglobin level and glucose concentration. Sometimes, the changes are permanent while in other times they are temporary (Olurankinse, 2002). Changes in the haemoglobin concentration of the fish, due to the ingestion of copper may obstruct the uptake of oxygen, thus leading to asphyxiation and eventual death of the fish. A fall in RBC count, haemoglobin percentage and packed cell volume percentage, in the fish, *Channa punctatus* upon treatment with both copper and chromium was noticed along with acute anaemia (Singh, 1995). The metal entering into fish system is slowly eliminated (Newman and Mitz, 1988; James and Sampath, 1996; James et al., 1991). McKim et al. (1970) observed changes in the haematological parameters of the blood of the brook trout (*Salvelinus fontalis*) after a short term and long term exposure to copper. The measurement of specific and biochemical changes in *S. fontalis* exposed for short periods to sub lethal environmental stressors, which have provided a sensitive method for predicting the effects of chronic exposure on survival, reproduction and growth. Haematological alterations have therefore allowed for a relatively rapid evaluation of the chronic toxicities of a compound. Acute exposure of *Colisa fasciatus*, *Oreochromis mossambicus* to sublethal concentrations of lead, copper and zinc has been shown to produce haemolytic
anaemia due to lysis of erythrocytes with concomitant decrease in Hb%, PCV% value and the number of erythrocytes (Soiveo and Nikinmaa, 1981; Sampath et al., 1998). The metals responsible for toxicity are those whose toxicity and or mobility are enhanced by the variation of pH, hardness, alkalinity and decomposable organic carbon that typically accompany these events.

Zinc accumulates in the gills of fish and this indicates a depressive effect on tissue respiration leading to death by hypoxia (Crespso et al., 1979). Zinc pollution also induces changes in ventilatory and heart physiology (Hughes and Tort, 1975). Sub lethal levels of zinc have been known to adversely affect hatchability, survival and haematological parameters of fish (Cardeihac et al., 1981). Annune et al. (1994b) reported that zinc could cause subacute effects that change fish behaviours. Such observed behaviours include lack of balance since most fins are motionless in the affected fish, agitated swimming, air gulping, periods of quiescence and death. Apart from zinc, other toxicants have been known to adversely affect fish haematology. Gobacher and Skaya (1977) observed some chronic effects of organophosphate insecticide on fish haematology.

Haematological changes apart from those due to heavy metals have also been reported. Bouck and Ball (1966) reported that alterations in fish blood were observed due to the influence of capture. Bouck and Ball (1966) also investigated and reported the influence of capture methods on rainbow trout (Salmo gairdnerii) as well as a perturbation of blood parameters. Distinct differences in behaviour were noted in the captured fish which led to extensive mortality of some groups. It is, however known that the toxicity of heavy metals including zinc to fish is sometimes reduced in hard water. Jones (1983) demonstrated that Gasterosteus aculeatus survived longer in water containing zinc and calcium than when no calcium was present. Lloyd (1960) demonstrated that zinc was toxic to rainbow trout (S. gairdnerii) in water of hardness 12 g CaCO₃ L⁻¹ compared with water of 320 mg CaCO₃ L⁻¹. Eisler and Gardner (1973) reported that concentrations of cadmium (10 mg Cd²⁺) not easily toxic to Fundulus heteroclitus for 24 h (in sea water) exerted a negative effect on the survival of fish exposed to a test solution containing sublethal concentrations of copper and zinc.

The haematological characteristics of African mudfish, Clarias buthupogon have been reported (Kori-Siakpere and Egor, 1999). They have reported the chronic sublethal haematological effects of copper in fresh water teleost, Clarias isheriensis and some alterations in haematological parameters in Clarias isheriensis exposed to sublethal concentrations of water borne lead (Kori-Siakpere 1991,1995). Annune and Ahume (1998) observed sublethal haematological changes in mudfish, Clarias gariepinus when exposed to copper and lead. Apart from the studies on the
blood of *Clarias*, Olubah (1998) studied the effect of sublethal concentrations of copper II ions on the serum transaminase activity in *Clarias albopunctatus* and the effect of mercury and zinc on the plasma alanine aminotransferase activity in freshwater catfish, *C. albopunctatus* (Olubah and Amalu, 1998). Several studies have been done on fish haematology, mostly on marine temperate teleosts (Mishra and Srivastava, 1980; Waiwood, 1980; Koyama et al., 1982). However, not much work has been carried out on the effect of copper and zinc on the haematology of the fresh water fish, *Puntius parrah*.

Since the histological changes observed for both copper and zinc were more or less similar, the combined effects of both the metals on haematological parameters of the test fish under sublethal condition was investigated and presented in this chapter. Hence, the present study was undertaken to evaluate the haematological changes resulting from the exposure of the freshwater fish, *Puntius parrah* to sublethal concentrations of both the heavy metals copper and zinc.

### 6.2 MATERIALS AND METHODS

*Puntius parrah* used in this study was collected locally and was acclimatised to laboratory conditions. The collection, handling, acclimation of animals along with preparation of test solutions and other experimental conditions have already been described in Chapter 2. To study the haematological changes the fish were exposed to 1/10th concentration of 96hour LC$_{50}$ of both copper and zinc for 7, 14 and 28 days. After each exposure period blood from live fishes were collected and used for haematological studies. The fishes were caught and blood was collected in small vials by puncturing caudal peduncle. Blood was treated with EDTA to prevent coagulation. The different haematological analysis was carried out employing standard techniques (Hesser, 1960) unless specified.

#### 6.2.1 Total Erythrocyte Count

RBC counting was done with Neubauer Chamber as described as described by Davidson and Henry (1969).

**Procedure**

The blood was taken in a vial containing 1% EDTA as anticoagulant. Blood was drawn upto the 0.5 mark in the RBC pipette and immediately the diluting fluid (Hayem’s fluid) was drawn upto 101 mark (dilution 1:200). Pipette was shaken thoroughly and diluted blood was charged into counting chamber after discarding two drops. The solution was allowed to settle for few
seconds and the number of RBC was counted in five small squares of the RBC columns under high power microscope and the number of RBC’s per cubic millimetre was calculated.

\[
\text{Total number of RBC’s} = \frac{\text{Number of cells} \times \text{dilution factor} \times \text{depth factor}}{\text{Area counted}}
\]

### 6.2.2 White blood Corpuscle Count

WBC’s were counted according to the method described by Donald Hunter and Bomford (1968).

**Procedure**

Blood was collected in vials containing 1% EDTA as anticoagulant. The blood was drawn upto 0.5 mark of WBC pipette and immediately diluting fluid was drawn upto 11 mark above the bulb. Solution was mixed thoroughly and allowed to stand for 2 minutes. Solution was expelled and a drop of fluid was allowed to flow under the cover slip. It was allowed to stand for 2 minutes and the WBC’s were counted in the four corner square millimetres. The number of WBC’s per cubic millimetre was calculated accordingly.

\[
\text{Total number of WBC’s} = \frac{\text{Number of cells} \times \text{dilution factor} \times \text{depth factor}}{\text{Area counted}}
\]

### 6.2.3 Determination of Haemoglobin

The haemoglobin count was estimated by acid – Haematin method using Sahli’s haemocytometer.

**Principle**

The hydrochloric acid ruptures the red cells and haemoglobin is released into the solution (haemolysis). The acid acts on haemoglobin and converts it into acid haematin, which is brown in colour.

**Procedure**

Pure blood was drawn into Sahli’s pipette upto the 0.02 mark. The blood was expelled into a haemometer tube containing 0.1N HCl, upto the mark. After removing the pipette, the content was thoroughly mixed using a stirrer. The mixture was diluted with distilled water by adding few drops at a time with thorough mixing, until the colour of the solution matches with the glass plate.
of the comparator. The level of the fluid was noted at the lower meniscus. The amount of haemoglobin was directly read in gm%.

6.2.4 Determination of Haematocrit (Ht) or Packed Cell Volume (PCV)

Wintrobe’s apparatus was used in this method and microhaematocrit method was followed due to the limited amount of blood available.

Principle

RBC (1.090-sp-gr) being heavier than plasma (1.030-sp-gr) get packed towards the bottom of the tube by centrifugal force.

Procedure

The Wintrobe’s tube was filled with blood mixed with anticoagulant upto 10 mark. Blood was centrifuged for 30 mts at 3000 rotations per minute. The blood was separated into 3 layers - a tall bottom layer of red cells, a thin middle layer of WBC and top layer of clear plasma. After this the length of the packed RBC column was noted. Haematocrit was calculated as:

\[ Ht = \frac{L_1}{L_2} \times 100 \]

Where

\[ L_1 = \text{Height of RBC column in mm.} \]
\[ L_2 = \text{the total length of column (RBC + WBC + plasma) in mm (10)} \]

Ht is expressed as %.

6.2.5 Determination of Erythrocyte Indices

From the values of haemoglobin content, haematocrit and total erythrocyte count, erythrocyte indices were calculated using the respective formula (Dacie and Lewis, 1975).

A. Mean Corpuscular Volume (MCV)

It represents the average of individual erythrocyte in cubic microns (µ³/μL) and computed by the formula,

\[ MCV = \frac{Ht\%}{\text{RBC in million/mm}^3} \times 10 \]
B. Mean Corpuscular Haemoglobin (MCH)

MCH represents the average weight of haemoglobin in pictograms (pg) and calculated by the formula,

\[ \text{MCH} = \frac{\text{Hb} \% / \text{RBC} \text{ in million/mm}^3}{10} \]

C. Mean Corpuscular Haemoglobin Concentration (MCHC)

MCHC is the average haemoglobin concentration per 100ml of packed erythrocytes in percent and computed by,

\[ \text{MCHC} = \frac{\text{Hb} \% / \text{Ht} \%}{100} \]

Statistical Analysis

All replicates were used for the calculation of mean values. Differences in haematological parameters between exposure times has been subjected to statistical analysis using students t-test and ANOVA by SPSS, version 16.0 software to manifest the variations in comparison with control. The variations were reported in their significant levels. The haematological parameters were expressed as mean ± standard deviation.

6.3 RESULTS

The circulatory system of fish is in close association with the external environment and with every tissue. It is sensitive to foreign stimuli and reflects the homeostasis of the animal. Thus haematological studies help to check the systemic responses during stress conditions. The effect of heavy metals copper and zinc on various haematological parameters of fish *Puntius parrah* was studied and results are included here.

The variation in haematological parameters of *Puntius parrah* exposed to sublethal concentration of copper and zinc is presented on Table 6.1. At 1/10th of 96hour LC_{50} of copper and zinc for 7, 14 and 28 days showed significant increase in WBC (P<0.001) whereas total RBC showed slight decrease initially and then subsequent increase. The total RBC decreased from the control value 3.276x10^6/µl to 2.162x10^6/µl after 7 days of exposure and then slightly increased to 3.062x10^6/µl after 28 days of exposure (Fig. 6.1). However all the values were significant at P<0.001 when compared to the control value. Total WBC count showed significant increase after 28 days of exposure to the heavy metals. The count increased from the control value 47.58x10^3/µl after 7 days of exposure and then to 193.38x10^3/µl after 28 days of exposure...
Haemoglobin content of *Puntius parrah* showed significant decrease (P<0.001) from the control value of 15.38g/dL to 8.46g/dL to 9.54g/dL respectively (Fig. 6.3). Packed cell volume or haematocrit of experimental fish decreased after 7, 14 and 28 days of exposure. The reduction in haematocrit value was from control value 34.52% to 19.54%, 24.6% and 31.5% respectively after 7, 14 and 28 days (Fig. 6.4). PCV value was also significant at P<0.001 when compared to the control PCV.

Mean corpuscular volume or MCV of experimental fishes decreased from 105.6fL in control fishes to 90.4fL, 93fL and 102fL after 7, 14 and 28 days of exposure. Mean corpuscular haemoglobin or MCH of *P. Parrah* decreased from 47pg to 39pg after 7 days of exposure. It then slightly increased after 14 days to 43.66pg and again decreased to 31.14pg after 28 days of exposure to the heavy metals (Fig 6.5). All these mean corpuscular values on the treated fish were significant (P<0.001) when compared to the control value. Mean corpuscular haemoglobin concentration or MCHC of experimental fish also decreased from the control value 44.54g/dl to 43.28g/dl at first and then increased to 47.16g/dl after 14 days which were not significant. The MCHC value again significantly decreased to 30.26g/dl after 28 days of exposure (Fig. 6.6).

Table 6.2 shows the correlation coefficient of the 't’ value of haematological parameters of the control and treated samples of *Puntius parrah* exposed to sublethal concentration of copper and zinc.

**6.4 DISCUSSION**

Blood is a patho-physiological reflector of the whole body and therefore, blood parameters are important in diagnosing the structural and functional status of the animal exposed to toxicants. There was a significant decrease ((p<0.001) in erythrocyte count of *Puntius parrah* after exposure to sublethal concentration of copper and zinc. The exposure of *O. mossambicus* to sublethal levels of copper and zinc resulted in significant decrease in the RBC count, Hb content, oxygen carrying capacity of blood and Ht value, leading to anaemia (Sampath *et al.*, 1993). The anaemia may be due to the inhibition of erythropoiesis, haemosynthesis and increase in the rate of erythrocyte destruction in the haemopoietic organs. Goel and Kalpana (1985) reported that the RBC count, Hb content and Ht values significantly decreased resulting in macrocytic anaemia in *Heteropneustes fossilis* exposed to zinc. Kori Siakpere and Ubogu (2008) observed significant decrease in haematological indices MCHC, MCH and MCV in *Heteroclarias* sp. after exposed to zinc at sublethal concentration. Similar result was observed in the haematological indices of *Clarias gariepinus* exposed to zinc, lead and nickel (Emmanuel and Avoaja, 2005;
### Table 6.1 Variation in haematological parameters of *P. parrah* exposed to sublethal concentration of copper and zinc

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control</th>
<th>7 Days</th>
<th>14 Days</th>
<th>28 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (10^6/µl)</td>
<td>3.276±0.023</td>
<td>2.162±0.027*</td>
<td>2.654±0.024*</td>
<td>3.062±0.025*</td>
</tr>
<tr>
<td>WBC (10^3/µl)</td>
<td>47.58±0.31</td>
<td>52.62±0.28*</td>
<td>53.54±0.24*</td>
<td>193.38±0.39*</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>15.38±0.396</td>
<td>8.46±0.207*</td>
<td>11.6±0.223*</td>
<td>9.54±0.207*</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>34.52±0.258</td>
<td>19.54±0.230*</td>
<td>24.6±0.158*</td>
<td>31.5±0.291*</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>105.6±1.342</td>
<td>90.4±1.949*</td>
<td>93±1.141*</td>
<td>102±1.871</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>47±1.414</td>
<td>39±0.707*</td>
<td>43.66±0.773*</td>
<td>31.14±0.898*</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>44.54±1.205</td>
<td>43.28±1.532</td>
<td>47.16±0.965</td>
<td>30.26±0.487*</td>
</tr>
</tbody>
</table>

Each value corresponds to mean±SD

*Significant level at p<0.001

[Fig. 6.1 Variation in RBC of *P. parrah* exposed to sublethal concentration of copper and zinc](#)

[Fig. 6.2 Variation in WBC of *P. parrah* exposed to sublethal concentration of copper and zinc](#)
Chapter -6

Changes in Haematological ...

Fig. 6.3 Variation in Hb of *P. parrah* exposed to sublethal concentration of copper and zinc

Fig. 6.4 Variation in Haematocrit of *P. parrah* exposed to sublethal concentration of copper and zinc

Fig. 6.5 Variation in MCV of *P. parrah* exposed to sublethal concentration of copper and zinc

Fig. 6.6 Variation in MCHC of *P. parrah* exposed to sublethal concentration of copper and zinc
Table 6.2 Correlation in t value of haematological parameters of *Puntius parrah* exposed to sublethal concentration of copper and zinc

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Time of Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7Days</td>
</tr>
<tr>
<td>WBC</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>-54.348</td>
</tr>
<tr>
<td>Correlation</td>
<td>.758</td>
</tr>
<tr>
<td>RBC</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>454.789</td>
</tr>
<tr>
<td>Correlation</td>
<td>.999</td>
</tr>
<tr>
<td>HB</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>25.718</td>
</tr>
<tr>
<td>Correlation</td>
<td>-.986</td>
</tr>
<tr>
<td>HCT</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>85.916</td>
</tr>
<tr>
<td>Correlation</td>
<td>-.269</td>
</tr>
<tr>
<td>MCV</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>19.000</td>
</tr>
<tr>
<td>Correlation</td>
<td>.459</td>
</tr>
<tr>
<td>MCH</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>8.944</td>
</tr>
<tr>
<td>Correlation</td>
<td>-.750</td>
</tr>
<tr>
<td>MCHC</td>
<td></td>
</tr>
<tr>
<td>t value</td>
<td>1.043</td>
</tr>
<tr>
<td>Correlation</td>
<td>-.948</td>
</tr>
</tbody>
</table>
Ololade and Oginni, 2010) support the results of the present study. Obuotor et al. (2011) reported the changes in haematological parameters of Clarias gariepinus exposed to copper. Dharam Singh et al. (2008) observed significant variations in haematological parameters of freshwater fish, Channa punctatus exposed to copper. Acute exposure of Colisa fasciatus to sublethal concentrations of Pb produced haemolytic anaemia due to the lysis of erythrocytes with concomitant decrease in Hb content, Ht value and the number of erythrocytes (Srivastava and Shashikala, 1979). Observed depression in haematocrit and haemoglobin values coupled with decreased and deformed erythrocytes are obvious signs of anemia (Maheswaran et al., 2008). The significant reduction of RBC and Hb content were reported in fishes exposed to different heavy metals (Goel et al., 1985; Goel and Sharma, 1987).

Natarajan (1981) found a reduction of RBC, Hb content and Ht values resulting in hypochronic anaemia which was attributed to deficiency of iron and decreased utilization for Hb synthesis. The metal stress in the present study caused the macrocytic anaemic condition in P. parrah possibly by destroying mature RBCs, resulting in reduced RBC count and disrupting the iron synthesizing mechanisms. Oxygen carrying capacity of blood also declined in metal-exposed P. parrah due to the reduction of RBC count and Hb content. James and Sampath (1995) found that the oxygen carrying capacity of blood of Heteropneustes fossilis declined due to the reduction of RBC count and Hb content which reflected on tissue respiration. The red blood cell count of C. gariepinus was reported to have increased significantly by Annune et al. (1994a) when the fish was subjected to zinc treatment. They attributed the red blood cell elevation to blood cell reserve combined with cell shrinkage as a result of osmotic alterations of blood by the action of the metal (Tort and Torres, 1988). Annune et al. (1994b) also observed a non-significant decrease in red cells for O. niloticus. The nonsignificant decrease in erythrocyte count and erythrocyte sedimentation rate of Heterocllarias sp. may be attributed to the swelling of red blood cells. Flos et al. (1987) reported that the swelling of the red blood cells (erythrocytes) may be due to an increase in protein and carbon dioxide in the blood. Sampling procedure could also be as a result of hypoxia or stress that causes these changes.

High white blood cell counts indicate damage due to infection of body tissues, severe physical stress, and as well leukemia. Similar findings were also documented significantly higher in fish exposed to increased copper concentration (Nath and Banerjee, 1995; Mazon et al., 2002). Mishra and Srivastava (1980) also reported an increase in leucocytes count when they exposed fishes to heavy metals. Some of the most common causes of heavy metal toxicity are inflammatory
lesions associated with tissue damage, anaemia and neoplasia. Further, an increase in fibrinogen or serum globulins or a decrease in serum albumin, may also cause an increase in the erythrocyte sedimentation rate. Increase in erythrocyte sedimentation rate and mean corpuscular volume values and total leucocyte count suggested that, the anaemia was of macrocytic type (Sampath et al., 1998; Sinha et al., 2000). The white blood cells in fish respond to various stressors including infections and chemical irritants (Christensen et al., 1978). Thus increasing or decreasing numbers of white blood cells are a normal reaction to a chemical such as zinc and cadmium (Kori Siakpere et al., 2006), demonstrating the effect of the immune system under toxic conditions. An increase in WBC was observed in the present investigation that was statistically significant. This is in agreement with the findings of Sampath et al. (1993) when they exposed the Nile tilapia O. niloticus to a toxic environment. They attributed this to stimulation of the immune mechanism of the fish to eliminate the effects of the pollutants. The toxic effects of heavy metal on fish are multidirectional and manifested by numerous changes in the physiological and chemical processes of their body systems (Dimitrova et al., 1994). Sublethal toxicity of lead to fish produces haematological and neurological effects (Hodson et al., 1984). An increase in the metal concentration and exposure period resulted in the increased WBC, which was more pronounced in copper and zinc exposures. This increase might be due to the increase in the population of lymphocytes, neutrophils and basophils. The neutrophil increase in fish exposed to chosen metals may be due to tissue damage. Mahajan and Dheer (1979) reported that neutrophils showed greatest sensitivity to changes in the environment and were the most important of the leucocytes. Chronic exposure of fishes to sublethal levels of metals caused damage to various tissues (Gardner and Yevich, 1970; Overstreet, 1988). There are reports of phagocytosis of bacteria and cell debris by fish neutrophils (Suzuki, 1984; Parish et al., 1986). McLeay and Brown (1974) suggested that an elevation of neutrophil count was due to tissue damage. Similar elevation of neutrophil was also observed in fish exposed to pesticide (Sampath et al, 1993; James and Sampath, 1996; Bijoy Nandan and Nimila, 2012). An increase in the leucocyte count was mostly observed during the exposure period of stress reaction when fish tried to restore the disturbed homeostasis.

In the values obtained in the haematological indices, no significant change was recorded in the mean corpuscular volume (MCV) and mean corpuscular haemoglobin content (MCHC), but there was significant change (p<0.001) in the mean corpuscular haemoglobin (MCH). However, slight fluctuations were recorded in the MCV and MCHC when compared with the control. Spleen contractions after stress have been detected in fish (Abrahamsson and Nilsson, 1975).
Cells released from the spleen, which is an erythropoietic organ would have lowered the MCV values. A similar observation was made for Cyprinus carpio after cadmium exposure (Koyama and Ozaki, 1984). The significant change in the MCH may be due to the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis (Hodson et al., 1978).

Literature shows that, changes in haematological indices of fish caused by heavy metals and their mixtures are different. Vinodhini and Narayanan (2009) suggested that the presence of toxic heavy metals in aquatic environment has strong influence on the hematological parameters in the fresh water fish common carp Cyprinus carpio. In the light of the present study, the mean value of PCV was 34.5 in the control group, which decreased to 19.5, 24.6 and 31.5 respectively after 7, 14 and 28 days of exposure. A decrease in the erythrocyte count or in the percent of haematocrit indicates the worsening of the state of the organism and developing anaemia. Haemoglobin concentrations reflect the supply of an organism with oxygen and the organism itself tries to maintain them as much stable as possible. This study shows that mean haemoglobin in the control was 15.38. A decrease in the concentration of haemoglobin in blood is usually caused by the effect of toxic metals on gills, as well as decrease in oxygen, which also suggests anaemia or confirms toxic impact of copper and zinc in Puntius parrah. Haematological indices (RBC count, concentration of haemoglobin and haematocrit) have been reported to indicate secondary responses of an organism to irritants (Rogers et al., 2003). Therefore the changes observed in the haematological parameters can be used as an indicator of copper and zinc related stress in fish on exposed to elevated level of these metals.