CHAPTER 5
EFFECT OF COPPER AND MERCURY ON THE HAEMOGLOBIN, HAEMATOCRIT AND MEAN CELL HAEMOGLOBIN VALUES

Fishes are susceptible to any changes that may occur in the environment. It is expected that these changes would be reflected in the physiology of fish, particularly in the values of haematological parameters and haematology has been used as an index of health status of a number of fish species (Blaxhall, 1972).

Blood takes part directly and indirectly in almost all the activities of fish and thus it can be a good indicator of stress conditions. The use of haematological parameters as indicators of sublethal stress can provide information on the physiological responses that the fish make to a changing environment. This is the result of the close association of the circulatory system with the external environment and with tissues. When values are obtained under abnormal conditions it should be possible to monitor the changes in the quality of water (Mawdesley-Thomas, 1971). Since haematological tests have been an important diagnostic tool in medicine for many years, it is speculated that they may be an equally valuable indicator of stress or disease condition of fish (Larsson, 1975).

Haematological changes have been detected in response to diseases, pollutants, surgical procedures, hypoxia etc. (Eisler and Edmunds, 1966; DeWilde and Houston, 1967; Gardner and Yevich, 1969; McKim et al., 1970; Soivio and Oikari, 1976; Duthie and Tort, 1985). Blood alterations or damage to the haemopoietic organs in these organisms may also be associated with pathological conditions related to water-borne pollutants (Reichenbach-Klinke, 1966; Gardner and Yevich, 1969; Saad et al., 1973).

One of the important functions of blood is the transportation of oxygen and carbon dioxide in the body. The immature red blood corpuscles (RBC) is uniquely concerned with the synthesis of Hb; it is otherwise comparable to many other cells in its metabolic activity. When its maturation is complete the RBC functions primarily in the transportation of haemoglobin (Hb). The haemoglobin present in the red blood cell enables the blood to carry
adequate amount of the gases to different tissues as the capacity of the Hb to carry these gases is very high. Hence an estimation of the Hb in the blood provide us information about the physiological status of the body. The increase or decrease of the Hb content and RBC, variation of the packed cell volume (PCV) or haematocrit (Hct), mean cell haemoglobin concentration (MCHC) etc. indirectly indicates the oxygen carrying capacity of the blood. Alterations of the haematological parameters can be due to factors like retention of metabolites, metabolic problems, oxidation of Hb, increased or decreased erythropoiesis, haemodilution or haemoconcentration.

RBC count is a long cumbersome procedure which nowadays is replaced by haematocrit determination which express PCV (Packed Cell Volume) as the percentage of the whole blood volume. Haematocrit provides a rapid approximation of the volume of circulating RBC and is used as a routine method for haematological diagnosis of fish health in field studies. This method has the advantage of speed and simplicity and is suitable for the capillary blood.

Heavy metals are one class of pollutants which have a disruptive influence on the structural organisation of the gill tissues because the gills are intimately associated with ionic regulation and it is predictable that heavy metals will influence aspects of osmotic and ionic regulation in fish which may influence the composition of blood.

McKim et al. (1970) compared data for Hb, Hct, RBC count etc of blood from male and female of fishes exposed to copper and found that no significant differences at 95% level. Bell (1968) also did not find any difference in the Hct values between male and female fishes.

In the present study also both the male and female fishes were used for the experiment. In this chapter the effects of the metals, copper and mercury on the Hb, Hct, and MCHC values of Oreochromis mossambicus are described.
MATERIAL AND METHODS

Specimens of *O. mossambicus* were collected and acclimatized in the laboratory for a month as described in Chapter 2. Later they were transferred to five large experimental tanks containing 200 l dechlorinated tap water. Calculated volumes of 1000 mg/l solutions of copper sulphate and mercuric chloride were separately added to different tanks to give concentrations of 100 µg/l and 200 µg/l for copper and 150 µg/l and 100 µg/l for mercury. The last tank without any toxicant, served as the control. The test medium was renewed every 24 h. The temperature in the tanks was maintained at 28 ± 1°C. Fishes were not fed during the experiment. Samples of blood were collected at 24, 72, 120 and 168 h. The fishes were caught and immobilised with a hard blow on the head. Immediately the caudal peduncle of the fish was cut and the blood was collected in tubes rinsed with heparin. From this, the Hb and Hct were determined.

**Estimation of Hb**

Haemoglobin was determined by the cyanmethaemoglobin method (Drabkin, 1946). To 5 ml of Drabkin's reagent, 0.02 ml of blood was added and mixed thoroughly. The potassium ferricyanide present in the reagent converts the Hb iron from ferrous to ferric state to form methaemoglobin. The methaemoglobin thus formed combines with potassium cyanide of the Drabkin's reagent to produce a stable pigment, cyanmethaemoglobin. This represents the sum of oxyhaemoglobin, carboxyhaemoglobin and methaemoglobin. The cyanmethaemoglobin formed is measured colorimetrically at 540nm.

**Haematocrit values**

Haematocrit values of the samples were determined, following the procedure described by Hesser (1960). Heparinized microhaematocrit capillary tubes were filled with blood and one end was sealed with plastic clay. Blood-filled capillary tubes were centrifuged at 12000 rpm for 5 minutes and haematocrit values were measured with a Spiracrit reader.

Mean corpuscular haemoglobin concentration (MCHC) of the different blood samples was calculated from the Hb and Hct values.
RESULTS

The results are presented in Tables 15, 16 and 17 and Figs. 15, 16 and 17.

Haemoglobin values

There was no significant variation in the Hb values in the control fishes between days even though the Hb values declined by the end of the experiment. The Hb values of the copper-dosed fishes were significantly lower than that of the control fish at 24 h. But mercury-dosed fishes did not show any significant difference in the Hb content. However at 72 h the copper-exposed fishes showed a different trend in Hb values. A highly significant increase in the Hb content of copper-treated fishes was seen at 72 h. The Hb content increased significantly in the copper and mercury-dosed fishes at 120 and 168 h.

Haematocrit values

In spite of a slight decrease in Hct values, the controls did not show any significant variation in Hct values. Haematocrit values did not alter significantly from that of controls in the copper and mercury-dosed fishes at 24 h. But at 72 h the copper-treated fishes and fishes exposed to the higher concentration (150 µg/l) of mercury showed a significant increase in the haematocrit values over the controls. And at 120 and 168 h the haematocrit values increased in fishes exposed to all concentrations of copper and mercury.

The MCHC values in the metal-dosed fishes did not vary significantly from that of the controls.

DISCUSSION

In the present study, a distinct difference in Hb values between copper-dosed and mercury-dosed fishes in the initial stage (24 h) of the experiment was observed. Unlike fishes exposed to mercury, the copper-dosed fishes showed
Table 15. Haemoglobin values in *O. mossambicus* exposed to copper and mercury

<table>
<thead>
<tr>
<th>Concentration μg/l</th>
<th>24 h</th>
<th>Hb g/100 mL blood, ( \bar{x} \pm S.D. (N = 22) )</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu 100</td>
<td>8.59* ± 0.55</td>
<td>9.25** ± 0.69</td>
<td>9.92** ± 0.69</td>
<td>9.94** ± 0.63</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>8.28** ± 0.69</td>
<td>9.43** ± 0.45</td>
<td>10.19** ± 0.79</td>
<td>10.27** ± 0.71</td>
<td></td>
</tr>
<tr>
<td>Hg 100</td>
<td>8.88 ± 0.67</td>
<td>8.57 ± 0.88</td>
<td>9.15** ± 0.58</td>
<td>9.56** ± 0.67</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>8.76 ± 0.51</td>
<td>8.51 ± 0.73</td>
<td>9.34** ± 0.62</td>
<td>9.70** ± 0.57</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>9.01 ± 0.74</td>
<td>8.52 ± 0.84</td>
<td>8.19 ± 0.76</td>
<td>8.05 ± 0.53</td>
<td></td>
</tr>
</tbody>
</table>

Table 16. Haematocrit values in *O. mossambicus* exposed to copper and mercury

<table>
<thead>
<tr>
<th>Concentration μg/l</th>
<th>24 h</th>
<th>Haematocrit percentage, ( \bar{x} \pm S.D. (N = 12) )</th>
<th>72 h</th>
<th>120 h</th>
<th>168 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cu 100</td>
<td>34 ± 2.6</td>
<td>34* ± 2.9</td>
<td>35** ± 2.6</td>
<td>35** ± 3.1</td>
<td></td>
</tr>
<tr>
<td>200</td>
<td>34 ± 2.6</td>
<td>34* ± 3.4</td>
<td>38** ± 2.3</td>
<td>39** ± 2.7</td>
<td></td>
</tr>
<tr>
<td>Hg 100</td>
<td>34 ± 2.2</td>
<td>33 ± 2.9</td>
<td>34* ± 2.8</td>
<td>35** ± 2.9</td>
<td></td>
</tr>
<tr>
<td>150</td>
<td>33 ± 2.4</td>
<td>34* ± 2.8</td>
<td>36** ± 2.4</td>
<td>37** ± 2.3</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>33 ± 2.8</td>
<td>31 ± 2.7</td>
<td>31 ± 2.9</td>
<td>30 ± 2.4</td>
<td></td>
</tr>
</tbody>
</table>

* P < 0.05  ** P < 0.01
Table 17. Mean corpuscular haemoglobin concentration in *O. mossambicus* exposed to copper and mercury

<table>
<thead>
<tr>
<th>Concentration</th>
<th>MCHC g/100 ml packed RBC (x ± S.D. (N = 12))</th>
<th>Exposure time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>72 h</td>
</tr>
<tr>
<td>Cu 100 µg/l</td>
<td>26.30 ± 3.1</td>
<td>27.11 ± 2.4</td>
</tr>
<tr>
<td></td>
<td>25.49 ± 3.4</td>
<td>27.63 ± 3.3</td>
</tr>
<tr>
<td>Cu 200 µg/l</td>
<td>26.19 ± 2.5</td>
<td>27.12 ± 3.9</td>
</tr>
<tr>
<td></td>
<td>27.58 ± 3.7</td>
<td>25.38 ± 2.7</td>
</tr>
<tr>
<td>Hg 100 µg/l</td>
<td>26.19 ± 3.4</td>
<td>27.55 ± 2.2</td>
</tr>
<tr>
<td>Hg 150 µg/l</td>
<td>25.55 ± 2.2</td>
<td>26.72 ± 2.4</td>
</tr>
<tr>
<td>Control</td>
<td>26.19 ± 3.4</td>
<td>27.55 ± 2.2</td>
</tr>
</tbody>
</table>

The values are not significantly different from that of controls.
FIGURE 15. HAEMOGLOBIN VALUES IN O. MOSSAMBICUS
EXPOSED TO COPPER AND MERCURY

Hb gm/100 ml

0 24 48 72 96 120 144 168 192

Cu 100  Cu 200  Hg 100  Hg 150  Control
FIGURE 16. HAEMATOCRIT VALUES IN *O. MOSSAMBICUS* EXPOSED TO COPPER AND MERCURY

Haematocrit %

40 35 30 25 20 15 10 5 0

24 48 72 96 120 144 168 192 hours

Control

Cu 100

Hg 150

Hg 100

Cu 200
FIGURE 17. MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION IN O. MOSSAMBICUS EXPOSED TO COPPER AND MERCURY

MCHC gm/100 ml

hours

- Cu 100  - Cu 200  - Hg 100  - Hg 150  - Control
a significant decrease in the Hb content at 24 h, but there was no change in the Hct values of these fishes. This shows a haemodilution and the resultant swelling of the erythrocytes combined with the release of erythrocytes from the erythropoietic organs.

Haemodilution has been interpreted as a mechanism which reduces the concentration of an irritating factor in the circulatory system (Smit et al., 1979). Decreased osmoregulation is the consequence of copper toxicity in fish (Leland and Kuwabara, 1985). Haemodilution has been observed in *Colisa fasciatus* exposed to zinc by Mishra and Srivastava (1979, 1980). One of the consequences of haemodilution is the decrease in plasma osmotic pressure as observed in *Ictalurus punctatus* in response to zinc and copper (Lewis and Lewis, 1971) which would result in the swelling of RBC as reported in the dogfish *Scylorhinus canicula* in response to copper (Tort et al., 1987). Such an increase in the erythrocyte size is generally considered as a response against stress. The swelling would also be a consequence of factors like high pCO₂, high lactate concentration or low pO₂ in the blood, leading to low ATP concentration, which would increase the oxygen affinity of blood (Soivio and Nikinmaa, 1981). Since metals produce changes in blood gases and lactate, the swelling of red blood cells could be involved in the response of fish against heavy metal pollution (Tort et al., 1987). A decrease in Hct value and an increased RBC count were observed by Tort and Torres (1988) in the dogfish *S. canicula* exposed to cadmium. Abrahamsson and Nilsson (1975) observed that the contraction of spleen of cod exposed to a stress would release blood cells into the blood stream. A similar pattern has been detected in *Cyprinus carpio* after cadmium exposure (Koyama and Ozaki, 1984), in which haematocrit decrease, maintenance of RBC count and an increase of circulating reticulocytes were recorded.

Helmy et al. (1978) reported a decrease in RBC count, Hb, and Hct in Kuwait mullet exposed to copper and mercury. Similar effects were detected in flounder exposed to cadmium (Johansson-Sjöbeck and Larsson, 1978); in winter flounder exposed to mercury (Dawson, 1979); in *C. fasciatus* exposed to lead (Srivasthava and Mishra, 1979); in marine teleost, *Aphanius dispar* (Hilmy et al., 1980); and in *C. carpio* exposed to cadmium and mercury (Beena and Viswaranjan, 1987) and in *Sarotherodon mossambicus* treated with cadmium (Ruparelia et al.,
Panigrahi and Misra (1978, 1980) reported low Hb and RBC count associated with reduced respiratory rate in the freshwater fish *Anabus scandens* and *Tilapia mossambica* dosed with mercury.

Decrease in Hb was observed in perch in response to cadmium (Larsson, 1975); in *Pleuronectes flesus* exposed to cadmium (Larsson et al., 1976); in brooktrout exposed to lead (Christensen et al., 1977); in *A. scandens* dosed with mercury (Panigrahi, 1977); in *Labeo umbratus* treated with various pollutants (van Vuren, 1986); in *S. mossambicus* exposed to mercury (Aruna and Gopal, 1987); in *Clarias lazera* intoxicated with copper (El-Domiaty, 1987) and in the dogfish *S. canicula* exposed to copper (Tort et al., 1987).

There are reports that various other chemical substances, also cause a decrease in the Hb and Hct values in different fishes (McLeay, 1973; Buckley, 1976; Buckley et al., 1976, 1979; Oikari and Soivio, 1977; Anees, 1978; Koundinya and Ramamurthy, 1979; Dalela et al., 1981; Natarajan, 1981; Pandey et al., 1981; Verma et al., 1981 c; Goel et al., 1982; Mishra and Srivastava, 1983, 1984; Madhyastha and Nayak, 1984; Scarano et al., 1984; Torres et al., 1986).

At 72 h the copper-dosed fishes showed a reverse trend. There was a significant increase in Hb content and a corresponding increase in the haematocrit values. The body of the copper exposed fish might have adapted to the metal stress by this time. Haemodilution could be an initial reaction of the body to stress. Afterwards the living system rectified the imbalance by removing water from the blood. This could result in haemoconcentration. Hilmy et al. (1980) reported that values of Hct, Hb and RBC count returned to control levels after an initial decrease in marine teleost *A. dispar* in response to mercury toxicity. Buckley (1976) also observed a partial recovery of Hb after a decline in coho salmon exposed to treated water containing total residual chlorine (TRCl₂). He suggested (1) decreased haemolysis as a result of elimination of susceptible cells and decreased sensitivity of younger cells to oxidants and (2) compensatory erythropoiesis with the establishment of a balance between cell destruction and formation resulting in reduced number of circulating cells. But Tort and Torres (1988) ruled out the haemolysis or RBC destruction as the RBC count increased in the fish after cadmium exposure. They postulated that the RBC count elevation was due to consequence of blood cell reserve
release combined with cell shrinkage, probably due to osmotic alterations of blood by the action of the metal. In addition, haemoglobin measurements by Tort and Torres (1988) in plasma showed no increase of extracellular haemoglobin. Hence the alteration in the haematological parameters observed in copper-treated fishes at 24 and 72 h could be due to changes in blood water content, that is, a change from haemodilution to haemoconcentration. Torres et al. (1986) found that in fish subjected to confined stress condition, zinc treatment significantly decreased Hct and RBC count and the decrease was identical. Gluth and Hanke (1985) postulated a biphasic response to pollutants in *C. carpio* consisting of water loss followed by a water gain in the blood. But in the present study the biphasic response observed in the copper-dosed fish was just the reverse, that is, water gain followed by a water loss. Gill and Pant (1981) also obtained a biphasic response similar to the findings in the present study. They observed a fall in Hb, RBC following 1-3 weeks exposure to sublethal concentrations of mercury in the teleost *Puntius conchonius* but recorded an increase in Hb and RBC count after 8 weeks of exposure. Gill and Pant (1981) ascribed the initial fall in Hb to haemolysis by mercury whilst subsequent recovery and the rise to enhanced erythropoiesis was triggered by stress.

During the entire experimental period there was no significant variation in the mean corpuscular haemoglobin concentration (MCHC) in the fishes exposed to metals. Svobodova (1982) in *C. carpio* treated with copper and Gill and Pant (1985) in *P. conchonius* dosed with cadmium did not find any significant difference in MCHC values from that of control values. Because MCHC is the ratio of blood Hb concentration to the Hct, it is not dependent on the blood volume or on the number of red cells per unit volume. This clearly indicates that the decrease of Hb noted in the present study was not due to haemolysis or unusual RBC destruction but caused by haemodilution. Similarly the increase in Hb and a corresponding increase in Hct was due to either haemoconcentration or increased erythropoiesis or both.

The lack of decrease in Hb in mercury-treated fishes could be due to increased production of urine which might remove the excess water present in the blood as a result of haemodilution. Lock et al. (1981) observed that increased water uptake by the gills did not result in the decreased Haematocrit
values of mercury-treated rainbow trout and instead there was an increase in the haematocrit values. He suggested that the inflow of water is offset by an enhanced urine flow. The enzyme Na, K-ATPase appears to be involved in osmoregulatory transepithelial electrolyte transport in the gills, intestine and urinary bladder as well as in active sodium-potassium exchange across all cell membranes (Schmidt-Nielsen, 1974). In a wide variety of tissues this enzyme is sensitive to mercurials and other sulphydryl reagents (Schwartz et al., 1975). In fact, previous studies (Renfro et al., 1974) demonstrated mercurial inhibition of both active sodium transport and Na, K-ATPase in seawater flounder urinary bladder in vitro and of sodium transport by freshwater killifish gills in vivo. Hence mercury may prevent the reabsorption of water across kidney tubules, resulting in the increased urine flow and hence haemoconcentration.

The Hb content in both the copper and mercury-dosed fishes increased at 120 and 168 h. There was a corresponding increase in the haematocrit values as well. The effect of mercury in fish was not felt at 24 h. The significant increase of Hb and Hct observed in copper and mercury-treated fishes could have been due to an increased production of RBC by the erythropoietic organs along with haemoconcentration. McKim et al. (1970) in brook trout Salvelinus fontinalis and Svobodova (1982) in C. carpio exposed to copper reported a significant increase in RBC, Hct and Hb. The mean cell volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in C. carpio remained without change. This indicates that the increase in Hb and Hct is due to an increase in RBC number. Svobodova (1982) explained the changes in the haematological parameters in the intoxicated carp as disorders in the oxidation process in the fish.

There are many reports that both Hb and Hct in fishes increase after exposure to metals. An increase in Hb, Hct values were observed in S. fontinalis in response to copper (McKim et al., 1970); in Ictalurus nebulosus exposed to copper (Christensen et al., 1972); in C. fasciatus treated with nickel (Agrawal et al., 1979); in rainbow trout dosed with copper (Wotten and Williams, 1980) and in C. carpio exposed to copper (Svobodova, 1982).

Many other substances also elevate both Hb and Hct in fishes (Agrawal et al., 1978; Juelich, 1979; Natarajan, 1984; Ghosh and Chatterjee, 1986; Beevi
Haemoglobin levels were elevated in response to copper in brook trout (McFadden, 1965); in *S. fontinalis* in response to methyl mercury (Christensen *et al.*, 1977); in *Channa punctatus* in response to mercury (Chitra and Ramanarao, 1986) and in dogfish in response to cadmium (Tort and Torres, 1988). Similarly Hct values increased in rainbow trout exposed to methyl mercury and in *Mystus vitatus* in response to copper and zinc (Singh and Singh, 1982).

The increase in Hb and Hct observed in the present study in metal-dosed fishes may be an attempt by the body to counteract the low oxygen content of the blood. The low oxygen content may be due to the low oxygen carrying capacity of the blood or faulty gaseous exchange caused by damage to the gills.

It has been widely reported that many pollutants enter the RBC and either oxidise or denature the Hb by inhibiting the glycolysis or metabolism of the hexose monophosphate shunt (HMPS). Buckley (1976) found degenerative changes including formation of Heinz bodies in the erythrocytes of coho salmon *Oncorhynchus kisutch* exposed to chlorinated waste water containing total residual chlorine (TRCl\(_2\)). Fairbanks (1967) showed that copper penetrates the intact erythrocyte, inhibiting glycolysis, denaturing Hb and oxidising glutathione. Chlorine also seemed to diffuse readily through gills oxidising Hb to methaemoglobin and disrupting erythrocyte membrane resulting in haemolysis (Zeitoun, 1977). In a study on the mechanism of acute toxicity of monochloramine to fathead minnows (*Pimephales promelas*), Grothe and Eaton (1975) found a methaemoglobin (MHb) level of 30% of total Hb. Formation of methaemoglobin reduces the oxygen carrying capacity of the blood. Therefore, methaemoglobinemia and the resulting anoxia were considered as the basis for the toxicity of monochloramine under test conditions (Buckley, 1976). Scarano et al. (1984) observed a decrease in Hb and increase in methaemoglobin in seabass exposed to nitrite.

Asano and Hokari (1987) stated that toxic concentration of copper may cause a cytotoxicity by its oxidant action and can affect the functions of
erythrocytic enzymes leading to oxidation of Hb, a disulphide formation of the membrane proteins and a decrease in the intracellular concentration of glutathione. Boulard et al. (1972) also postulated that copper might inhibit erythrocytic enzymes. Cupric ions can bind to membrane protein sulphydryl groups and cause a disulphide formation of neighbouring sulphydryls (Salhany et al., 1978).

Johansson-Sjobeck and Larsson (1978) in Pleuronectes flesus detected a significant reduction in Hct, Hb and RBC count accompanied by a significant increase of aminolevulinic acid dehydratase, the enzyme necessary for erythropoiesis in renal tissue, indicating a compensatory stimulation of erythropoiesis so that the oxygen carrying capacity is increased.

Hodson et al. (1980) studied the effects of waterborne selenium on rainbow trout and found that eventhough the blood parameters decreased from the control levels by 30%, the fish appeared to be compensating for these changes by increased erythropoiesis. Sahib et al. (1981) found that the exposure of fish to a sublethal concentration of malathion showed a consistent increase in the oxygen consumption up to 24 h and later declined to 48% suggesting a reduction of oxidative metabolism at the end of 48 h. A decrease in the rate of oxygen consumption of the fish Labeo rohita was observed with an increase in the concentration of effluents (Hingorani et al., 1979). Panigrahi and Misra (1980) found that the uptake of oxygen decreased 27% in Tilapia mossambica exposed to mercury. Similarly chlorine produced oxidants (CPO) reduced oxygen carrying capacity of the fish Leiostomus xanthurus (Middaugh et al., 1980).

A decrease in the oxygen carrying capacity may stimulate erythropoiesis in fish so that blood carries enough oxygen to meet the requirements of the body. The increase in Hb and Hct in metal-exposed fishes of the present study may be due to this phenomenon. An increased erythropoiesis may result in an increase in RBC count, Hb and Hct. An increase in RBC count or polycythemia in fishes after exposure to various toxicants were reported by many authors (Buckley et al., 1976, 1979; Agrawal et al., 1979; Juelich, 1979; Verma et al., 1981 c; Singh and Singh, 1982; Juneja and Mahajan, 1983; Lal et al., 1986; Haniffa et al., 1986; and Pant et al., 1987). Increased
erythropoiesis to compensate for the inhibition of Hb by water-borne and dietary lead was recorded by Hodson et al. (1978). Along with stimulation of erythropoiesis, a reduction in plasma volume and a mobilization of new erythrocytes into circulation could also have contributed to the increase in Hb and Hct. Milligan and Wood (1982) observed a reduction in plasma volume and a mobilization of new RBC in rainbow trout associated with low pH. Plasma volume reduction reflected a general redistribution of body water from extracellular compartments in response to ionic disturbances. Erythrocyte recruitment was associated with depletion of splenic RBC reserves which may be reflected in the erythrocyte count (Milligan and Wood, 1982). Lal et al. (1986) found that increase in RBC count was followed by a reduction in spleeno-somatic index indicating a release of RBC from the spleen. Larsson (1973) also suggested that contracting and partly emptying spleen of RBC as a cause of polycythemia. Buckley (1976) and Buckley et al. (1979) had shown that there is an increase in the number of circulating immature erythrocytes when fishes were exposed to different pollutants. Overt increase of circulating immature erythrocytes can be used in monitoring lead poisoning in fish (Srivasthava and Mishra, 1979). Buckley (1976) postulated that increased number of RBC in the circulatory system was an attempt by the body to meet the elevated demands for O<sub>2</sub> or CO<sub>2</sub> transport as a result of increased metabolic activity during stress or by a destruction of gill membrane causing faulty gaseous exchange. Nayak and Madhyastha (1980) found an erythropoietic response as evidenced by a significant increase in the number of immature RBC.

Pollutants can influence the functioning of all parts of respiratory chain. Pollutants may not only restrict gas transfer, but their irritant effect can also interfere with ventilation (Hughes, 1981). Lindahl and Hell (1970) found that the gills from fishes exposed to mercurials show clear tissue injuries. The layer of epithelial cells is detached from the deeper layers. This causes faulty gaseous exchange. When gill from fish exposed to phenylmercurial were studied, a decrease in the circulation of blood was observed in the secondary lamellae. They also found that the oxygen content of blood collected from poisoned fish is greatly reduced. This may either be the effect of decreased circulation of the blood in the secondary lamellae or diminished exchange between water and blood in the secondary gill filaments or structural
change in the Hb molecule due to binding of phenylmercury ions. Diffusing capacity of the gill is reduced, following the action of pollutants and consequently there is a fall in oxygen supply to the tissues which become hypoxic. Pant et al. (1987) suggested that the polycythemia observed in Barbus conchonius exposed to aldicarb was related to enhanced erythropoiesis, and it is hypoxemia that triggers an exodus of erythrocytes from haemopoietic organs in an attempt to compensate for the reduced oxygen carrying capacity of the blood. Davis (1973) found an increase in the oxygen uptake, ventilatory water flow, cough, and buccal pressure in sockeye salmon (Oncorhyncus nerka) exposed to Bleached Kraft Pulp Mill Effluent (BKME). He also found that arterial oxygen tension decreases rapidly when exposed to BKME. On the average, this decline represented a 20% decrease in oxygen saturation of the blood. Increase in Hb content could be a mechanism by which the body tries to absorb more O₂ from the surrounding medium to meet the increased demand. Haniffa et al. (1986) observed that in fishes exposed to paper mill effluents the increase in RBC number was more in nonaerated fish than in aerated fish, indicating an association between RBC number and oxygen content of the blood. Interference with gas transfer will reduce oxygen levels within the blood circulating the brain where responses are initiated by the diffusely located respiratory centre (Ballintijn and Bamford, 1975).

Wedemeyer (1971) explained the increased pituitary activity in formalin treated rainbow trout on the basis of a chemical adversely affecting gill function. Such an interference with gill function can be expected to reduce its respiratory role so that the Hb was increased in the treated fish to compensate the loss. This sort of compensatory reaction is known to occur in fishes infected by certain parasites (Kabata, 1970).

So a faulty gaseous exchange of gases as a result of damage to the gills by the action of metals or oxidation of Hb to MHB by various toxicants lowers the oxygen carrying capacity of the blood. Reaction to such a situation would be by stimulating the erythropoietic tissue and increasing the Hb content of the blood. The increased Hb and Hct values observed in the metal treated fishes of the present study could be due to the increased erythropoiesis and Hb synthesis.