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Alteration in hematology of *Labeo rohita* under stress of pollution from Lakes of Bangalore, Karnataka, India

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Abstract Blood is an indicator of physiological condition of an animal. Therefore, a field study was conducted to investigate the hematological parameters of wild population of rohu, *Labeo rohita* (Ham). The following aspects were evaluated in blood: hemoglobin content, red blood cell (RBC) and white blood cell (WBC) count, packed cell volume (PCV), and mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) values, and in plasma: cholesterol, protein, and glucose levels. For this purpose, rohu fish of varying sizes and weights were sampled from Hebbal (receiving a storm water drain) and Chowkalli lake (received domestic sewage and industrial effluents from various sources and was more polluted than Hebbal lake). It revealed noticeable differences in hemoglobin content, RBC and WBC count, and PCV and MCHC values. Severe anemia can be marked by a significant decrease in RBC count ($p < 0.5$), hemoglobin content, and PCV and MCHC values, whereas an increase in leukocyte count and MCV values were observed in fish from Chowkalli lake. Fish from lake B had fewer RBC and low concentration of serum protein and cholesterol. Serum concentration of glucose showed initial higher levels and then low concentration (900–1,500 g) in fish from lake B when compared to lake A. The variation in values of different parameters can be attributed to exposure of fish to various types of pollutants present mainly in the Chowkalli lake which receives heavy metals, synthetic detergents, petroleum products, and other acid and alkali substances from the nearby local industries. Other observations of these fish include dark body color and aggressive nature of fish.

Keywords Hematology · RBC · WBC · Hemoglobin · PCV · Serum parameters protein · Glucose · Cholesterol · Rohu

Introduction

Blood is known to exhibit pathological changes before the onset of any external symptoms of toxicity. Fish blood is a pathophysiological indicator of the whole body function and therefore blood parameters are important in diagnosing the structural and functional status of fish exposed to a toxicant (Sampath et al. 1998). Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes

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in fishery management and disease investigations (Mulcahy 1975; Bansal et al. 1980).

Hematological abnormalities have also been studied in various toxicants-exposed fish: *Channa punctatus* to lead (Hymavathi and Rao 2000); *Cyprinus carpio* to carbofuran (Chandra et al. 2001); *C. punctatus* to cadmium (Karuppasamy et al. 2005); and *Labeo rohita* to synthetic detergents and sublethal levels of nitrite (Chellan et al. 1999; Acharya et al. 2005). Changes in hemoglobin content under toxic stress are reflected on the oxygen consumption and metabolism. Since oxygen transport in blood depends upon the hemoglobin contents of erythrocytes in blood of fish, the erythrocyte count (TEC) and hemoglobin (Hb) content are taken as reflection of the pollution stress. 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 hematological parameters (Van Vuren 1986). McLeavy and Brown (1974) reported leukocytosis in zinc-treated fish, *Oncorhynchus kisutch*, due to tissue damage and subsequent removal of debris.

Results from hematological studies in laboratory animals may be used to predict possible chemical toxicity in human beings because human hematopoietic cell renewal systems are similar in their principal structure and function to those of laboratory animals, and the study of circulating blood cells will reflect direct chemical effects on these cells (Awasthi et al. 2003).

A number of studies have been carried out to determine the mode of toxicity of potentially dangerous chemicals in the wastewater by exposure of fish to these chemicals in laboratory conditions. In addition, factors such as temperature of the environment, age, sex, and nutritional and reproductive status of the fish can modify the expression and activity of the detoxification enzymes, which could complicate the interpretation of induction responses (Jiménez and Stegeman 1990). Alternatively, measurements of physiological indices for assessing the effects of different stressors on fish are extremely valuable because they incorporate several levels of biological organization.

Limited studies are available on the hematological parameters of wild population of fish. Due to the availability of freshwater bodies in abundance in and around Bangalore city, localities prefer to rear food fish like rohu, catla, and other major and minor carps in them. The fish are also considered to be very tasty and are a source of cheap nutrition for the poor. Recent reports on sudden mass mortality of these fishes reared in the lakes have been a cause of growing concern. The field studies on fish captured in a site polluted by sewage and other types of stress responses demonstrated by various workers (Cazenave et al. 2005; Cech et al. 1996) showed negative effect on its physiological functions.

This study presents my findings on the effect of various types of pollutants from sewage and from treated and untreated effluents discharged from various industries into the two lakes, Hebbal and Chowkalli. Therefore, considering the importance of hematological parameters as indicators of fish health, the present work dealing with the toxic stress and synergetic effect of various pollutants on fish reared in the two lakes was investigated.

Materials and methods

Analysis of water and fish sample for the field study

The physico-chemical parameters of water samples of the two lakes, Hebbal (A, sewage polluted) and Chowkalli (B, highly polluted), and Hessarghatta fish farm (control) were assessed Table 1 on a monthly basis by adopting standard methods (APHA 1995) for a period of 1 year from 2005 to 2006. Hebbal lake (lake A) receives overflowing storm water drain containing only the domestic sewage. Chowkalli lake (lake B) water receives effluents from industries like Toyota Kirloskar, paint and dye industries, plastic, rubber pharmaceutical, and carbon monoxide-producing factories as well as domestic sewage from storm drain water pipes containing all types of heavy metals, acids, petroleum products, synthetic detergents, etc. Approximately ten fish with a body weight varying from 200 to 1,500 g were collected randomly with minimum stress from contaminated site of Hebbal and Chowkalli lake and also from the reference site of Hessarghatta fish farm. They were immediately anesthetized using MS222 at 100 mg/l.

Table 1 Seasonal changes in physico-chemical parameters of two lakes Hebbal (A) and Chowkalli (B)

Parameters	Standard	Winter		Summer		Rainy	
	Maximum limits in mg/l	A	B	A	B	A	B
Temperature (°C)	22–28	22	21	26	25	22	23
Color	25 (Pt–Co scale)	7.6	7.8	6.8	8.6	9.5	7.2
Turbidity (NTU)	05 to 25 NTU	11	12	6	8	17	58
pH	06.50 to 08.50	7.2	7.5	7.2	8.5	7.1	7.5
TDS	1,500	577.2	1,694.2	680	1,800	380	490
Conductivity (μS/cm)	200	660	980	756	2,800	799	1,037
Total alkalinity	200	312	839	354	499	235	379
Sodium	–	169	191	121	181	112.5	142.7
DO	4.0	4.8	3.1	4.2	1.4	6.3	4.9
BOD	6.00	5.5	3.2	6.3	5.2	2.8	5.2
COD	2.00	27	53	14	103	25	43
Nitrates	10.0	13.3	29.2	12.5	34.5	15.5	17.1
Phosphate	2.0	1.5	1.05	0.59	0.55	0.39	0.52
Sulfates	20.0	25.58	32.82	14.8	24.68	49.36	74.05
Iron	0.10	0.39	0.42	0.06	0.13	0.46	0.98
Zinc	0.15	0.09	0.15	0.02	0.20	0.20	0.32

Except temperature, pH, conductivity, and turbidity, all values are in milligrams per liter

The fish was washed with tap water and dried using blotting paper before collecting the blood sample. Blood was obtained by cardiac puncture using 3-cm^3 syringes and 18 gauge hypodermic needles and immediately transferred to 2-ml glass vials containing 1% EDTA solution. Red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), and Hb were estimated at the sampling site. The methods employed for determination of various hematological parameters were RBC and WBC, counted by Neubauer's improved hemocytometer using Hayem's and Tuerk's solution as a diluting fluid, respectively (Samuel 1986), the packed cell volume (PCV) or hematocrit values (measured by Wintrobe's method), and Hb (treated by N/10 HCl and the color of the acid hematin was matched with given standards using Sahli's hemoglobinometer). Mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular volume (MCV) were calculated by the following standard formulae (Dacie and Lewis 1991).

$$\text{MCV} = \frac{\text{PCV}/1{,}000\ \text{ml blood}}{\text{RBC in millions/mm}^3} = \text{fl}$$

$$\text{MCH} = \frac{\text{Hb in g}/1{,}000\ \text{ml blood}}{\text{RBC in millions/mm}^3} = \text{pg}$$

$$\text{MCHC} = \frac{\text{Hb in g}/100\ \text{ml blood}}{\text{PCV}/100\ \text{ml}} \times 100 = \text{g/dl}$$

Statistical significance of difference was tested by using analysis of variance. Remaining blood was centrifuged and serum sealed, stored in ice, and subsequently brought to the laboratory for further analysis. Serum biochemical analysis of the total protein, glucose, and cholesterol of blood plasma were determined following the procedures of Lowry et al. (1951), Seifter et al. (1950), and Zlatkis et al. (1953) respectively.

Note that experimental results have not been detailed since there is enough work done on the fishes exposed to various types of heavy metals and effluents. Therefore, the present study is based on the synergetic effect of polluted water parameters of lake on freshwater fish, *L. rohita.*

Results

Hematology parameters

Site-related results of hematological studies from the two lakes are summarized in Figs. 1, 2, 3, 4, 5, 6, and 7). Blood forms a unique compartment between external and internal environments and any agent including toxic substances that causes

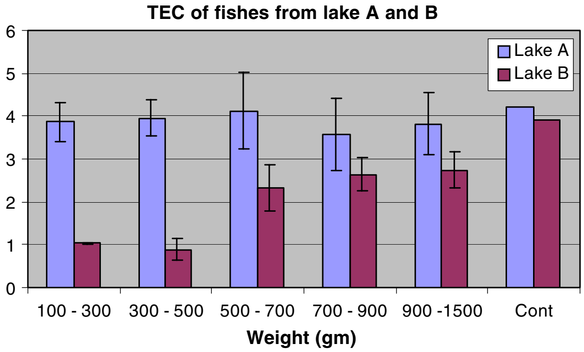

Fig. 1 Comparative TEC of *Labeo rohita* from Hebbal (**a**) and Chowkalli Lake (**b**)

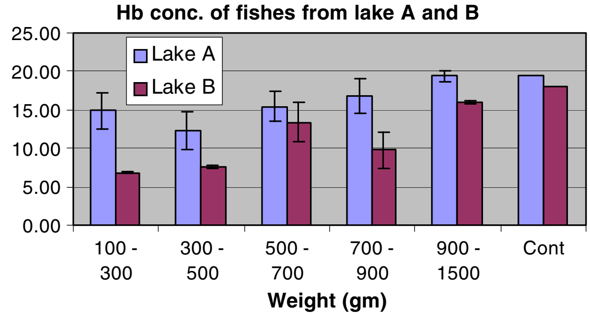

Fig. 3 Comparative Hb of *Labeo rohita* from Hebbal (**a**) and Chowkalli Lake (**b**)

stress can alter blood composition either directly or indirectly by altering osmotic and ion regulation. In the present study, the fishes weighing 200 to 500 g of lake B showed an increase in MCV, MCHC, and MCH values, but these values showed a significant decrease in the fishes weighing above 700 g. This can be corroborated to the time of exposure of the fishes in that polluted environment of lake B. The present investigation showed a significant decrease in TEC count, Hb content, and PCV of the blood in fish sampled from Chowkalli lake (lake B) (Figs. 1, 3, and 4). Variation in hemoglobin content and fish weight was also noticed in these fish when compared to fish sampled from sewage-polluted lake A and the controls. Therefore, a positive correlation was observed between Hb content, TEC count, and PCV but it does not appear to be related to sex of the fish.

TLC showed great sensitivity to the changes in the environment, and the most important of leukocytes were lymphocytes. Leukocytosis is evidenced in the present studies by the increase in the total leukocyte count in the fish of lake B during its growth and maturation till it gains 300–500 g ($26.15 \pm 3.450 \times 10^3/mm^3$), after which there was a decrease and then constant increase observed till it weighed 1,500 g ($25.68 \pm 2.68 \times 10^3/mm^3$). Continuous fluctuations in TLC and Hb were noticed in these fish due to the pollutants they were exposed to. Since fish from lake A were not exposed to pollutants like in B, they showed an increase in TLC count till they reached maturation phase weighing 900–1,500 g ($27.69 \pm 3.0 \times 10^3/mm^3$) but the values reduced constantly thereafter (Fig. 2).

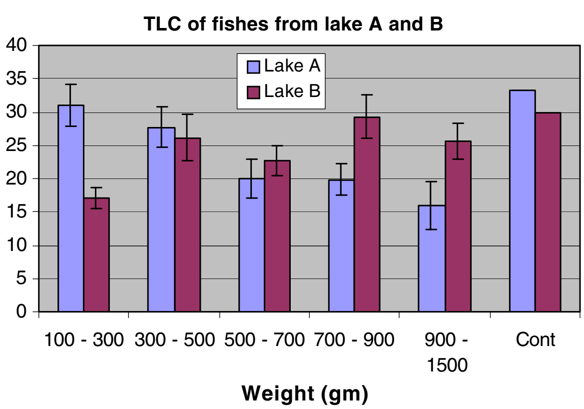

Fig. 2 Comparative TLC of *Labeo rohita* from Hebbal (**a**) and Chowkalli Lake (**b**)

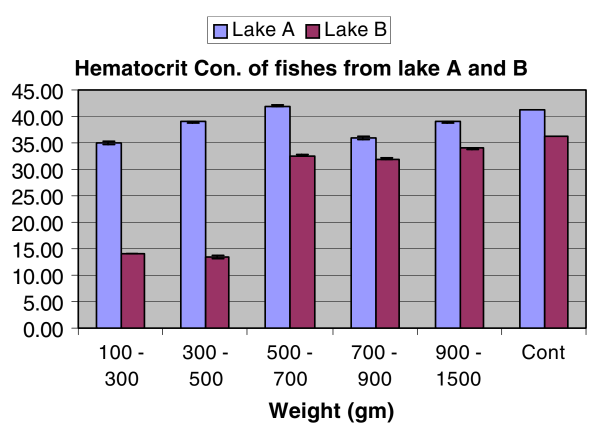

Fig. 4 Comparative PCV of *Labeo rohita* from Hebbal (**a**) and Chowkalli Lake (**b**)

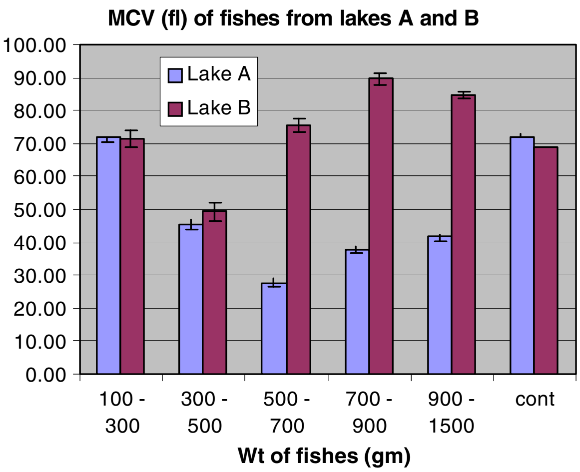

Fig. 5 Comparative MCV of *Labeo rohita* from Hebbal (**a**) and Chowkalli Lake (**b**)

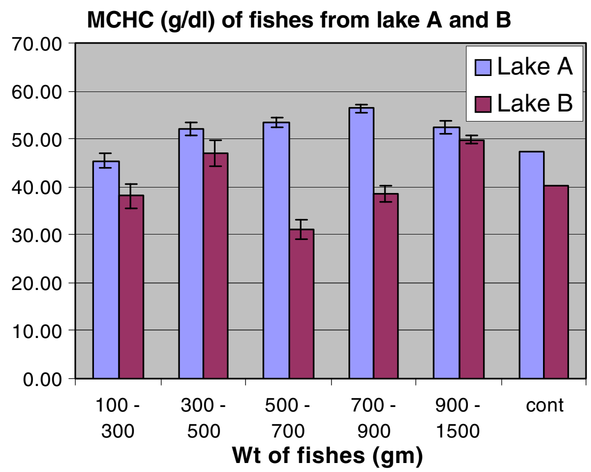

Fig. 7 Comparative MCHC of *Labeo rohita* from Hebbal (**a**) and Chowkalli Lake (**b**)

Fish reared in lake B reveal alterations in MCV and MCH and decreased values of PCV, indicating a condition of anemia. PCV and MCH values of fish showed fluctuating results with an increasing trend during short-term (700 g) exposure in polluted water and decreasing trend during further growth (1,500 g). But they showed a significant rise in the fish from lake B when compared to those of lake A. PCV values of contaminated site showed an initial reduction (32.60 ± 0.20) in the fish from lake B attained 500 g (32.60 ± 0.20 fl) but a significantly sharp rise in their values was noticed after they attained 900–1500 g (34.00 ± 0.13 fl) as compared to those from lake A (Fig. 4).

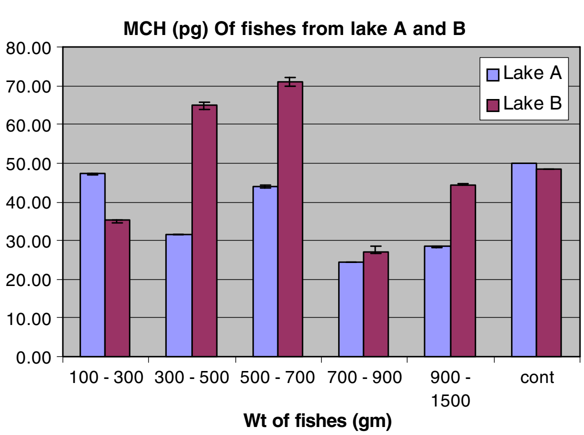

Fig. 6 Comparative MCH of *Labeo rohita* from Hebbal (**a**) and Chowkalli Lake (**b**)

Similarly, a significant rise in MCV values, with an intermittent rise and fall in the MCH values in the fishes from lake B, was observed. But the values remain constantly high in lake B. MCHC values also showed significant reduction in the fish from lake B during maturation (31.25 ± 2.05 g/dl) when compared to those of lake A with a sudden rise till it attained 1,500 g (Figs. 5 and 7). However, the variations observed in MCHC were statistically insignificant.

Serum biochemical studies

Biochemical assay indicates the impact of stress through the changes in metabolic substances from their normal threshold to stressed level. Pollutants bind to the blood protein and induce biochemical changes in blood glucose, serum protein, and cholesterol level (Figs. 8, 9, and 10).

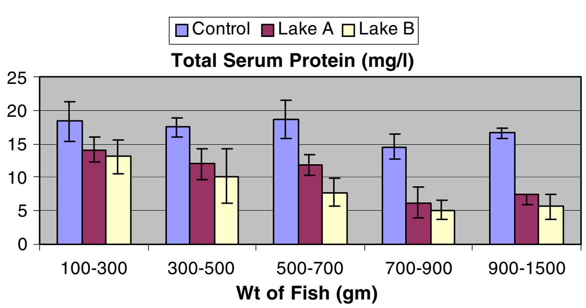

Fig. 8 Comparative total serum protein of *Labeo rohita* from Hebbal (**a**) and Chowkalli Lake (**b**)

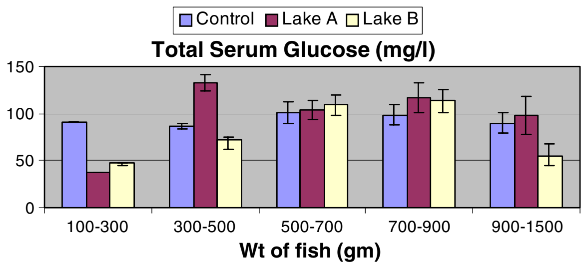

Fig. 9 Comparative total serum glucose of *Labeo rohita* from Hebbal (**a**) and Chowkalli Lake (**b**)

Blood serum total cholesterol, glucose, and protein were estimated from the blood of the fishes sampled from the two freshwater lakes A and B. The cholesterol content shows a high level (239.23 mg/l) in the control fish but a sudden decrease was seen when they were initially exposed to the water for rearing in lake A and B. A significant decrease was noticed in fishes weighing 900–1,500 g of lake A when compared to that of lake B. The blood serum cholesterol showed a significant increase as the fish reached 900 g and above (136.4 and 157.73 mg/l) and sudden decrease (56.26 and 76.87 mg/l) as soon as the fish reached 1,500 g and above in both lakes A and B, respectively. A decrease in cholesterol level (Fig. 10) of fish collected from lakes A and B causing hypo- or hypercholesterolemia can be correlated to alteration in cholesterol synthesis and nutritional stress.

Serum glucose level was observed to increase in the fishes exposed to polluted lake B when compared to those of lake A. An increment in the serum glucose levels without any decrease in

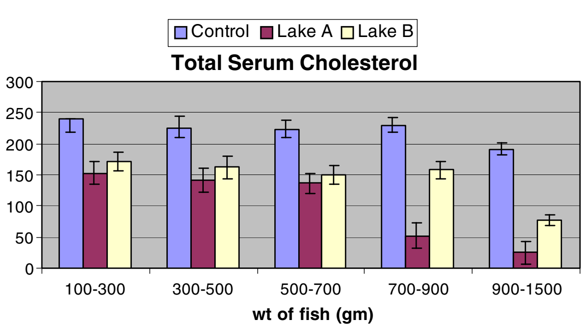

Fig. 10 Comparative total serum cholesterol of *Labeo rohita* from Hebbal (**a**) and Chowkalli Lake (**b**)

glycogen reserves in fish exposed to pollutants as heavy metals indicates the involvement of gluconeogenic events in this process (Figs. 8, 9, and 10). A significant drop in glucose level was noted during long-term exposure to pollutants when fishes reached 1,500 g weight group.

In the present investigation, the serum protein level showed a gradual decrease in the polluted lake fishes. Since any toxic substance or heavy metal is known to depress blood protein in fish, it is possible that the pollutant stress influences the conversion of tissue protein into soluble fractions reaching the blood for utilization. Total serum protein level showed a consistent reduction in fish weighing 1,500 g in lake B. The result showed that the protein level shows the least concentration when compared to glucose and cholesterol in lake B (cholesterol > glucose > protein).

Discussion

Water parameters are one of the major factors responsible for individual variation in fish hematology. Since hematological parameters are necessary for clinical diagnosis of a disease and pathological conditions in human, these criteria should receive enough attention in assessing the health of the fish with regard to aquatic pollution and has been accepted by many workers such as McCarthy et al. (1973) and Christensen et al. (1978). Lethtinum et al. (1990) reported alterations in PCV, MCV, and MCH values due to exposure of fish to effluents from mills with six different bleaching processes. While evaluating the total effect of zinc and lead on the hematological indices of carp, a synergetic effect of these metals was found on the erythrocyte count, concentration of hemoglobin, and the present leucocytes (Vosylienë 1999). Similar changes have been reported in the present study on the polluted lake B containing zinc as a metal. This study on hematological changes in fish serves as an effective tool in the diagnosis of the extent of environmental pollution and also the abiotic fish diseases. Hypoxia, anemia, and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient oxygen unless otherwise compensated by erythropoiesis or suitable physiological adjustments. Decreased availability of oxygen generally causes increased synthesis of hemoglobin, release of blood cells from storage sites, and enhanced erythropoiesis. This condition is clearly evident in the initial decrease and then increase in the PCV content of our present study which could be attributed to swelling of RBCs as a result of disturbed ion regulatory mechanisms caused by the effluent (Vosylienë 1999).

Lowering of TEC count coupled with low Hb content here may be due to destructive action of pollutants on erythrocytes and as a result of which the viability of the cells may be affected as was also reported by Karuppasamy (2000). Buckley et al. (1976) also showed that prolonged reduction in Hb content is deleterious to oxygen transport in catfish exposed to cassava mill effluent. This could be ascribed as pathological condition in fish exposed to toxicants. Multiple form of hemoglobin allows fish to adjust more efficiently to physiological stress such as varying water temperature and oxygen concentration (Hochachka and Somero 1973). Hemolysis occurs in response to toxicity that leads to alteration in the selective permeability of the membrane (Das et al. 1987). All these reports are in agreement with the present study of reduction in TEC count and Hb content of fish from polluted lakes due to the inhibition of aerobic glycolysis curtailing synthesis of iron and hemoglobin via the lowered energy status in fish. Joshi et al. (2002) and Banerjee and Banerjee (1988) have suggested that heavy metal exposure decreases the TEC count, Hb content, and PCV value due to impaired intestinal absorption of iron. PCV values always decrease when a fish loses appetite or become diseased or stressed.

Increase in TLC in the present study was a result of direct stimulation for its defense from diseases due to the presence of polluted substances. Progressive increased levels of TLC have been reported in *C. punctatus* exposed to lead (Hymavathi and Rao 2000) and *Clarias batrachus* exposed to mercuric chloride (Joshi et al. 2002). Leukocytosis is directly proportional to severity of stress condition in maturing fish and is a result of direct stimulation of immunological defense due to the presence of pollutants in these lakes. This is in correlation with the report by Saravanan and Harikrishnan (1999) in freshwater fish, *Sarotherodon mossambicus*, when exposed to sublethal concentration of copper and endosulfan and in *Heteropneustes fossilis* during nickel intoxication by Nanda (1997). This may be attributed to alteration in blood parameters and direct effects of various pollutants. These observations are also in good agreement with those of Karuppasamy et al. (2005) and Hardikar and Gokhale (2000). The variation in MCH and MCHC in the present study clearly indicates that the concentration of hemoglobin in the RBCs is reduced in fish from lake B compared to lake A thereby indicating anemic condition. MCHC is a good indicator of RBC swelling (Wepener et al. 1992). The MCHC, which is the ratio of blood hemoglobin concentration as opposed to the PCV, is not influenced by the blood volume or by the number of cells in the blood but can be interpreted incorrectly only when new cells, with a different hemoglobin concentration, are released into the blood circulation (Soivo and Nikinmaa 1981). In our study, MCH increased over lake A during long-term exposure indicating low hemoglobin content in the shrunk RBCs. Similarly, MCHC showed fluctuating results.

Various workers such as Larsson et al. (1980) have observed elevation in blood glucose levels in flounders exposed to titanium effluent which significantly enhanced their blood glucose levels due to the enhanced breakdown of liver glycogen. Increases in ammonium ion concentration and decreases in oxygen saturation of water produce increases in the blood sugar concentration (Hattingh 1976). Initial increase in glucose level was due to glycogenolysis, but a sudden reduction can be correlated to utilization of stored glycogen in order to meet the energy requirements of the fish. Stress-induced release of catecholamines and glucocorticoids from adrenal tissues of fish (Wedemeyer and Yasutake 1977) are known to cause elevated blood glucose levels in fish. According to Ramakritinan et al. (2005), in *C. carpio*, glycogenolysis resulting from chronic exposure to distillery effluent may be due to a stress-induced increase in circulating catecholamines.

Fish sampled from lake B showed a decrease in the level of serum protein with an increase in weight due to continuous exposure to pollutants of the lake. This decrease was significantly more than in lake A. Bhatia et al. (2002) reported an insignificant increase in protein content in endosulfan-treated *H. fossilis* on short-term exposure of 5 days. However, prolonged exposure of 15 days resulted in its decrease which the authors attribute to its utilization as an energy source during stress condition due to depletion of immediate source of energy upon chronic exposure to endosulfan. Similarly, the depletion was observed in protein level in *Barbus stigma* (Manoharan and Subbaiah 1982) and in *C. carpio* (Maruthanayagam and Sharmila 2004), which may be due to the diversification of energy to meet the impending energy demand caused by toxic stress. On the contrary, Jana and Bandyopadhyaya (1987) have stated that any toxic substance like pesticide or heavy metal is known to depress blood protein in fish either by inhibiting the protein synthesis activity or somehow affecting the uptake of amino acids in the polypeptide chain. All these reports are equally in confirmation with the present study on fishes of polluted lakes. Biochemical changes resulting in altered physiology are known to develop more quickly in response to toxicants than any apparent morphological changes

Hence, the present investigation results confirm that stress due to various pollutants present in the lakes does create hematological disturbances, erythrocyte destruction (hemolysis), and leukocytosis in fish population, affecting the immune system and making the fish vulnerable to diseases.

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