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Effects of chromium (VI) on haematological parameters in catfish, *Clarias batrachus* (Linnaeus, 1758) (Actinopterygii: Siluriformes)

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Abstract

Heavy metals have been recognized as serious pollutants in the aquatic environment. They cause serious impairment in metabolic, physiological and structural systems when present in high concentrations in the milieu. Heavy metals may affect organisms directly by accumulating in their body or indirectly by transferring to the next trophic level of the food chain. The present study is aimed to investigate hematologicalof fresh water fish *Clarias batrachus* exposed to sublethal concentrations of chromium (VI). On the various exposures, various haematological parameters showed a significant result in the red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), haematocrit (Ht), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell hemoglobinconcentration(MCHC) were assessed.

Keywords: *Clarias batrachus*, chromium, Haematology, RBC, WBC, Haemoglobin, Mean cell volume (MCV), Mean cell Haemoglobin (MCH), Mean cell haemoglobin concenmtration (MCHC).

Introduction

Heavy metal contamination severely interferes with ecological balances of an ecosystem and produces devastating effects on environmental quality anthropogenic inputs like waste disposal directly adds to the burden of environmental degradation (Farombi *et al* 2007). Toxicity tests will reveal the organism's sensitivity to a particular toxicant that would help us to determine the permissible limit of a toxicant in an ecosystem.Heavy metals such as chromium have gained wide interest in the scientific community in recent years due to their potential human health hazards (Shuhaimi-Othman *et al.,* 2010). Physiological responses like rapid opercular movement andfrequent gulping for air due to respiratory rate impairment, darkening of the body, sudden and quickmovement, rolling movement was observed during the initial stages of exposure after which it becameoccasional. All these observations can be considered to monitor the quality of aquatic ecosystems andseverity of pollution (Sentamilselvan *et al.,* 2015).

Cr is a common element in the environment and has several oxidation states; two of those states are Cr(III) and Cr(VI). Cr(VI) is known to be a toxic chemical that has been listed as one of the 18 hazardous air pollutants (HAPs) according to the United States Environmental Protection Agency (Ho Yu *et al.,* 2014). Cr(VI) is extremely harmful and considered not only a health concern but an environmental concern as well due to its high solubility, mobility, and toxicity, and since there are several effects that relate to humans beings (by ingestion, dermal contact, and inhalation), plants, aquatic animals, and microorganisms (Xu and Wang 2012).

It is well-known to be toxic to living organisms due to their bioaccumulation and non-biodegradable properties. According to Indian standards, the maximum tolerance of total Cr for public water supply is 0.05 mg/L. Chromium (VI) salts have several applications in diverse industries and their indiscriminate introduction into the aquatic ecosystem pose a serious threat to the growth and survival of the aquatic fauna including the fish populations (Mishra and Mohanty, 2008).

The toxicity of any pollutant is either acute or chronic. Although the toxicant impairs the metabolic and physiological activities of the organisms, physiological studies alone do not satisfy the complete understanding of pathological conditions of tissues under toxic stress. Inorganic mercury, have been reported to develop a disorder called acrodynia or “pink disease”. Symptoms includes leg cramps, irritability, redness and peeling of the skin of hands, nose, and soles of the feet. Itching, fever, sweating, salivating, rashes (including “baboon syndrome” rashes in the buttocks, anal and genital regions), sleeplessness and/or weakness. Additional reports indicate the effects of inorganic mercury in children and adults include kidney damage and digestive tract problems including diarrhea, nausea, and ulcers.(Thangam *et al*., 2016)Acute toxicity test constitute only one of the many tools available to the aquatic toxicologists but they are the basic means of provoking a quick, relatively inexpensive and reproducible estimate of the toxic effects of a test material (Spacie and Hamelink, 1985). Contamination of aquatic ecosystems (lakes, rivers, streams, etc.) with heavy metals has been receiving increased worldwide attention due to their harmful effects on human health and other organisms in the environment. Fish have been largely used as bioindicators for environmental pollutants (Saiki and Jennings, 1993; Kock and Triendl, 1996)and have been used to estimate the influence of environmental pollution due to the sensitivity of their biochemical and hematological parameters under such conditions (Lopes et al., 2001)One of the most important properties of a toxic pollutant is its ability to accumulate in the tissues of organisms. Over a long period, the pollutants present in the environment at very low levels may accumulate within the body of aquatic species by various mechanisms to the extent that they exert toxic effects (Palaniappan and Karthikeyan, 2009). Chromium is ubiquitous traces metal and occurs in soil, water, air, and in the biosphere. It is emitted into the environment from both natural and man-made sources. Once released to the environment, chromium readily forms complexes with many ligands, making it more mobile than most heavy metals (Nanda and Behera, 1996). Hematological parameters have been included, as they are measurable in blood and circulating levels of hormones can be altered by exposure to xenobiotic chemicals. Pollution of the environment is a serious and growing problem.(Thangam *et al*., 2016)

Materials and Methods

Experimental fish

The *Clarias batrachus* were collected from the fish farm located at Kolathur, near Chennai, 17 km away from the campus. The fish were brought to the laboratory and transferred to the rectangular cement tanks (125X100X75cm) of 1000liters capacity containing chlorine free aerated well water and acclimatized to the food and laboratory conditions with 12 hr dark and 12 hr light cycles, pH range of 6.95 to 7.20 and temperature ranging from 16 to 24 °C for 15 days.

Experimental design

The experimental fingerlings were exposed to sublethal concentrations of chromium for the period of 28 days. The control and experimental fingerlings were dissected at the end of 7, 14, 21 and 28 days of exposure and the blood samples were also collected for determining the haematological parameters . Experiments were done on the control, earlier (7days) and final exposure days (28 days).

Haematological studies

Collection of Blood

Blood samples were collected from the control and experimental fingerlings from the ductus Cuvier with the help of 24 guage needle and stored in heparinized glass tube. The haematological parameters such as total Red blood corpuscles (RBC), White blood corpuscles (WBC), Haemoglobin (Hb), Haematocrit (Ht), Mean corpuscular haemoglobin (MCH) and Mean corpuscular haemoglobin concentration (MCHC) were determined by adopting the method of Dacie and Lewis (1984).

Enumeration of Red Blood Corpuscles (RBC)

Blood samples were slowly sucked up by means of the Haemocytometer pipette till that mark 0.5 is reached (marked 0.5, 1.0 and 101). Then the diluting fluid was sucked as far as the mark 101. This produced a dilution of 1 in 200. While this was done, the pipette was gently rotated so as to start the mixing. The pipette was firmly sliced by its ends between the forefinger and thumb and shaken thoroughly for about one minute. The finger was then removed from the pipette and the diluting fluid in the capillary tube blown out. After a few drops of the diluted blood have been shaken out, a small drop was transferred to the counting slide.

For enumeration of red blood corpuscles, at least five sets of sixteen squares were counted. The squares in each set should be gone over systematically in horizontal rows of four at a time and only those on the upper and on the left-hand lines were counted.

Calculation

$$\text{Number of RBC/Cu.mm} = \frac{\text{Total No. of cell counted}}{\text{Total No. of small squares counted}} \text{x Dilution}$$

Enumeration of White Blood Corpuscles (WBC)

The total white blood corpuscles count was made with the help of Haemocytometer’s Neuberger counting chamber. The blood samples were drawn up to the 0.5 mark in WBC pipette and diluted up to the Mark 11 with diluting fluid (turk’s fluid = Gention violet, glacial acetic acid 3 ml and distilled water 97 ml).This produced a dilution of 1 in 20. The remaining procedures were the same as above for the RBC counting.

For enumeration of leucocytes four sets of sixteen squares were counted out of nine squares. Instead of going over the squares in rows of four, a whole set of sixteen can easily be counted at one time.

Calculation

$$\text{Number of WBC/Cu.mm} = \frac{\text{Total No. of leucocytes counted}}{\text{Total No. of large squares counted}} \text{x Dilution}$$

Estimation of Haemoglobin (Hb) content

Haemoglobin content of blood was estimated using Haldane’s Haemoglobinometer (Superior, Germany) with permanents coloured glass comparison standards and expressed in gm/100ml of blood.

Determination of Haematocritvalue (Ht) or Packed Cell Volume (PCV)

Haematocrit value of blood was estimated by centrifuging blood in heparinized haematocrit tubes (Germany) at 7,000 rpm/min for 30 minutes. Packed cell volume or haematocrit per cent was calculated after centrifugation from the volume of blood taken.

Determination of Mean Corpuscular Haemoglobin (MCH)

The mean corpuscular haemoglobin (MCH) content was computed from the values of haemoglobin content and erythrocyte count using the formula and expressed as pictograms.

$$\text{MCH} = \frac{\text{Haemoglobin (gm/100ml)}}{\text{Erythrocyte count (Million cells/ cu.mm blood)}} \text{X100}$$

Determination of Mean Corpuscular Haemoglobin Concentration (MCHC)

Estimation of mean corpuscular haemoglobin concentration (MCHC) was computed from the values of haemoglobin and the haematocrit percentages using the formula and expressed as percentage.

$$\text{MCHC} = \frac{\text{Haemoglobin (gm/100ml)}}{\text{Haematocrit percentage}} \text{x 100}$$

Results

Count of red blood cells (RBC)

The red blood cells of fingerlings *Clarias batrachus* exposed to sub lethal concentration of chromium and control was given in the Table-1. The RBC counting in control group was $1.69X10^6$ cells per cubic mm of blood. In chromium treated fingerlings, they were 1.52, 1.41, 1.18 and 1.03 million/blood Cu mm for the 7, 14, 21 and 28 days of exposure periods. The RBC count was decreased during chromium exposed fingerlings. The decreased percentages were -10.06,-16.57, -30.18 and -39.05. The decreased count of RBC at all the exposure periods were statistically significant at $p< 0.05$ level (Fig.1 A).

Table 1. Changes in the various hematological parameters of fish, *Clarias batrachus* exposed to sub lethal concentration of chromium (VI).

Exposure periods	RBC (X 10^6/Cu mm^3)	WBC (X 10^3/Cu mm^3)	Haemoglobin (8/100 ml)	Haematocrit (%)	MCV (μm^3)	MCH (Pg)	MCHC (%)
Control	1.69±0.08	8.25±0.05	15.05±0.51	32.16±0.10	19.56±0.17	89.05±0.17	46.80±1.52
7 Days	1.52±0.05 (-10.06) 0.0026*	6.86±0.20 (-16.85) 2.2618*	14.25±0.43 (-5.32) 0.0147*	29.85±0.67 (-7.18) 4.9547*	19.12±0.07 (-2.25) 0.0004*	93.75±0.21 (-5.09) 0.1127*	47.74±2.27 (-1.61) 0.2738*
14 Days	1.41±0.07 (-16.57) 0.0002*	6.28±0.22 (-23.88) 2.6756*	13.12±0.16 (-12.82) 2.2008*	28.3±0.39 (-12.00) 1.8978*	18.75±0.15 (-4.14) 2.5892*	93.05±0.08 (-4.42) 0.1498*	46.36±1.07 -1.36 0.2345*
21 Days	1.18±0.06 (-30.18) 2.1498*	5.95±0.40 (-27.88) 7.1991*	12.85±0.09 (-14.62) 6.8712*	27.1±0.25 (-15.73) 7.5862*	17.03±0.19 (-12.93) 9.8951*	108.89±0.19 (-22.22) 0.0004*	47.42±0.67 (-0.87) 0.2956*
28 Days	1.03±0.05 (-39.05) 2.5436*	4.65±0.33 (-43.64) 4.9265*	10.28±0.10 (-31.69) 1.8192*	19.16±0.27 (-40.42) 6.3769*	14.86±0.14 (-24.03) 2.3680*	99.80±0.27 (-12.06) 0.0119*	53.65±1.10 (-14.14) 2.3465*

The values are mean ± S.E of six individual observations. Parentheses holds percent change over the control values. *Significance ($p<0.05$) of student't' test.

Count of white blood cells (WBC)

The white blood cells of fingerlings *Clarias batrachus* exposed to sub lethal concentration chromium and control was given in the Table-1. In the control group of fingerlings the WBC count was 8.25 X10^3 per cubic mm of blood. In chromium treated fingerlings, the WBC was 6.86, 6.28, 5.95 and 4.65X10^3 Cu mm for the 7, 14, 21 and 28 days of exposure. The WBC count was decreased in the chromium exposed fingerlings. The decreased percentages were -16.85, -23.88, -27.88 and -43.64. The decreased number of WBC at all the exposure periods were statistically significant at $p<0.05$ level (Fig.1 B).

Amount of Hemoglobin (Hb)

The level of hemoglobin was given in the Table 1. The hemoglobin level in control group was 15.05 gm/100 ml. In chromium treated fingerlings, the haemoglobin was 14.25, 13.12, 12.85 and 10.28 for the 7, 14, 21 and 28 days of exposure during chromium treatment. The hemoglobin level was decreased in all the exposure blood of fingerlings. The decreased percentages were -5.32, -12.82, -14.62 and -31.69. The decreased level of hemoglobin at all the exposure periods were statistically significant at $p<0.05$ level (Fig.1 C).

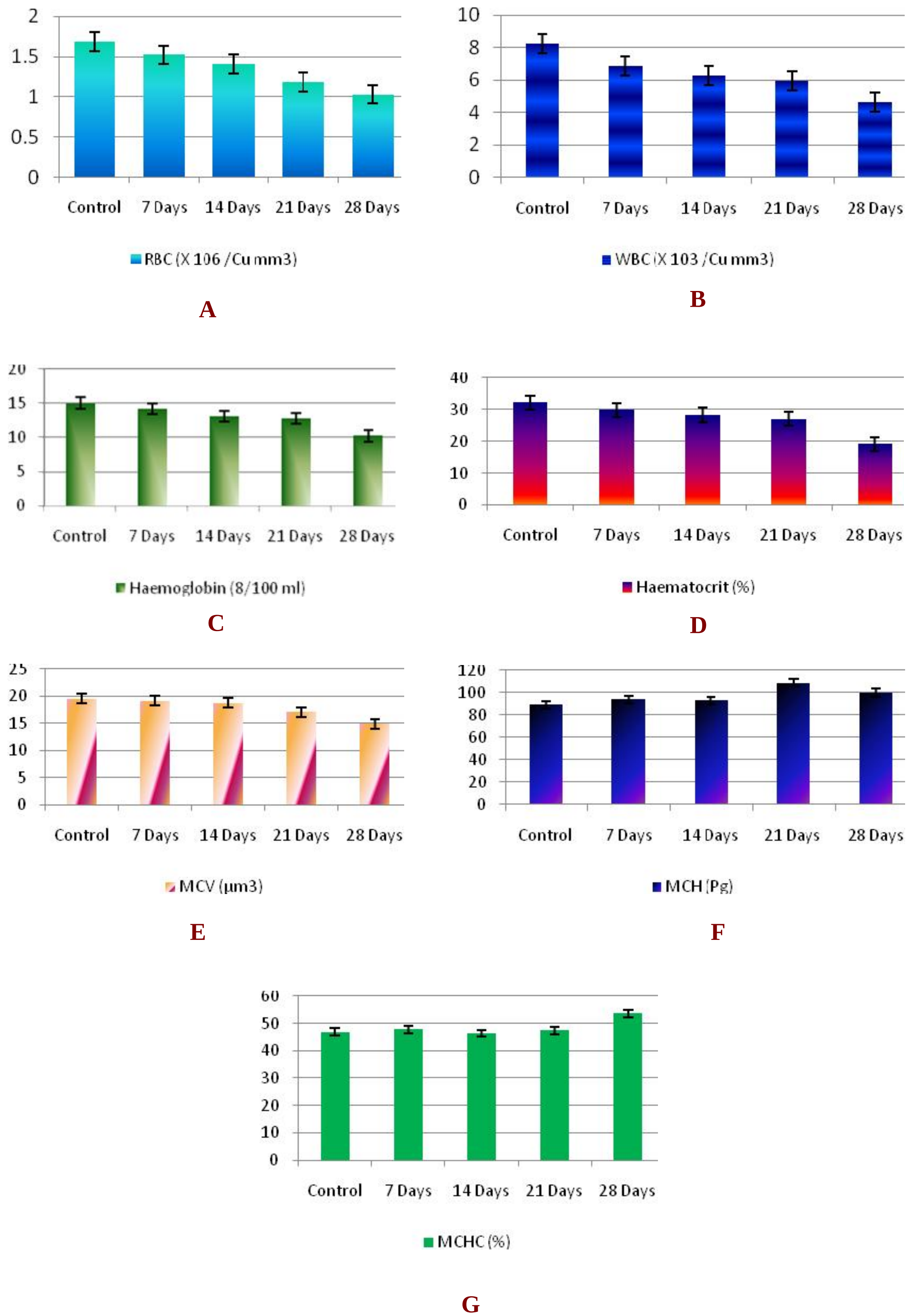
Haematocrit (Ht)

The Haematocrit values of fingerlings *Clarias batrachus* exposed to sub lethal concentration of chromium was decreased in all the exposed periods. The haematocrit in control group was 32.16 %. In chromium treated fingerlings, haematocrit values were 29.85; 28.3; 27.1 and 19.16 % for the 7, 14, 21 and 28 days of exposure periods respectively. The decreased percentages were -7.18; -12.00; -15.73 and -40.42. The decreased haematocrit at all the exposure periods were statistically significant at$p<0.05$ level (Table 1; Fig.1 D).

Mean cell volume (MCV)

The Mean cell volume of *Clarias batrachus* exposed to sub lethal concentration chromium and control was given in the table 1. The MCV in control group was 19.56 μm^3. In chromium treated fingerlings they were 19.12; 18.75; 17.03 and 14.86 μm^3 for the 7, 14, 21 and 28 days of exposure periods respectively. The decreased percentages were -2.25; -4.14; -12.93 and -24.03. The decreased MCV were statistically significant at all the exposure periods at $p<0.05$ level (Fig. E).

Figure 1. Effect of Chromium (VI) on various haematological parameters of freshwater fish *Clarias batrachus*



Mean cell haemoglobin (MCH)

The Mean cell haemoglobin of control fingerlings was 89.05 Pg. In chromium treated fingerlings, the MCH were 93.75; 93.05; 108.89 and 99.80 Pg for the 7, 14, 21 and 28 days of exposure of periods. The MCH were increased in 7 days exposure and then decreased in 14 days exposure. Same as again increased in 21 days exposure then decreased in 28 days exposure. The increased and decreased percentages were -5.09; -4.42; -22.22 and -12.06. The increased MCH were statistically significant at $p<0.05$ level at all the exposure periods (Table 1; Fig. F).

Mean cell haemoglobin concentration (MCHC)

The Mean cell haemoglobin concentration of fingerlings *Clarias batrachus* exposed to sub lethal concentration chromium and control was given in the Table-5.2. The MCHC in control group was 46.80 %. In chromium treated fingerlings, the MCHC were increased 47.74 and 53.65 % for the 7 and 28 days of exposure of chromium. likewise decreased 46.36; 47.42% for the 14 and 21 days of exposure. The increased percentages were -1.61; -14.14. decreased percentages 1.36 and -0.87 The increased and decreased MCHC at all the exposure periods were statistically significant at $p<0.05$ level (Table 1; Fig. G).

Discussion

The blood parameters have been used as sensitive indicator of stress in fingerlings exposed to different water pollutants and toxicants, such as metals, biocides, pesticides, chemical industrial effluents, etc. Haematological variables remain veritable tools in deter mining the sublethal concentration of pollutants such as heavy metals in fingerlings. The most common hematological variables measured during stress included red and white blood cells count, hemoglobin content, and hematocrit value and red blood cells indices. Fingerlings hematological parameters are often determined as an index of their health status (Oshode *et al.*, 2008). The blood parameters have been used as sensitive indicator of stress in fingerlings exposed to different water pollutants and toxicants, such as metals, biocides, pesticides, chemical industrial effluents, etc. These metallic ions are the probable major cause of the physiological abnormalities in fingerlings. A fall in RBC count, Hb % and PCV %, in the fingerlings, *Channa punctatus* upon treatment with both copper and chromium was noticed along with acute anemia (Singh, 1995). The metal entering into fingerlings system are slowly eliminated (James *et al.,* 1996), hence the blood parameters get affected on account of metal toxicity. Blood cell indices like MCV, MCH and MCHC seem to cause changes that are more sensitive and can cause reversible changes in the homeostatic system of fingerlings. Fluctuations in these indices correspond with values of RBC count, hemoglobin concentration and PCV. A similar response was noted in common carp and other freshwater fingerlings exposed to acute toxic level of pesticides (Rao, 2010). The exposure of *Channa punctatus* to sub-lethal concentration of copper significantly decreased Hb%, RBC count and PCV% values leading to anemia. The anemia might have led to a fall in the red blood cell count, haemoglobin concentration, and haematocrit volume. Anemia, under copper induced stress, may also be due to blood cell injury and disrupted heamoglobin synthesis (Mckim *et al*., 1970; Gross *et al*., 1975; Pamila *et al*., 1991).

In the present study, the white blood cell count, mean cell hemoglobin and mean cell hemoglobin concentration have increased at sub lethal concentration of chromium in *Clarias batrachus* for 7, 14, 21 and 28 days. This result may be due to a compensatory erythropoisis due to stimulatory effects. MCHC was found to be increased slightly after chromium treatment might be due to decrease in PCV. This elevated MCHC is merely a reflection of young erythrocytes were synthesized in blood (Muniyan, 1999). Saraswathi *et al.* (2002) have observed the haematological response of *Cyprinus carpio* to sublethal sodium nitrate exposure. It has been reported that cadmium, lead and mercury caused anemia in fingerlings (Fletcher and White, 1986; Tewari *et al.,* 1987, Houston *et al.,* 1993).These observations are in agreement with those of Acharya *et al.* (2005) who have been observed that the changes in the shape and size of RBCs of fingerlingses after nitrite treatment and suggested that the degeneration of red cells structural and functional properties may cause an increased removal of red cells from the circulation and contribute to the low update of oxygen ultimately leading to the lethal effect. Sawhney and Johal, (2000) who have studied erythrocyte alterations in *Channa punctatus* induced by Malathion.

A significant decrease in erythrocyte (RBC) counts, haemaglobin (Hb), an increase of White Blood Corpuscles (WBC), in the fresh water fingerlings *Channa punctatus* from polluted waters can definitely be related to the pollution due to slaughter house wastes (Rao and Hymavathi, 2000). The reduction in

RBC and Hb, in *Labeo rohita* after exposure to arsenic trioxide has been suggested by Pazhanisamy, (2002). A point of interest noticed in the present investigation is the increase in WBC count after chromium treatment. Such an increase in WBC might indicate a condition of stress and/or a need for removal of cellular debris, as has been suggested by Garg *et al.* (1989). Similar type of reports were also noticed in fingerlings, *Channa punctatus* exposed to cadmium (Karuppasamy *et al.*, 2005).

Erythrocyte morphology is one of the most sensitive indicators of toxic impact of various environmental factors on fish (Seema Tripathi *et al.*, 2003; Shanthi *et al*., 2003; Anupama Tyagi and Neera Srivastava, 2005; Meena, 2005; Shanthi *et al.*, 2005; Paul and Ramanujam, 2016).

In the present study, the significant decrease in RBC counts during sublethal study may be due to anemic condition and haemolyis caused by chromium. Earlier, heavy metals such as lead and copperwere known to have multiple haematologicla effects such as haemoloysis and anemia (Arjun *et al.*, 2002;Sevcikova *et al*., 2016).

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