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Lead-induced effects on hematological parameters and red cell indices of *Cirrhinus mrigala* (Hamilton, 1822) and *Ctenopharyngodon idella* (Steindachner, 1866)

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ABSTRACT

The function of blood is to maintain tissue stability by keeping the internal environment of the body constant. However, changes in the values of blood parameters take place in fish inhabiting water polluted with heavy metals. The objective of this study was to evaluate the sublethal effect of lead on red blood cell (RBC) and white blood cell (WBC) counts, hematocrit, hemoglobin, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC) of *Cirrhinus mrigala* and *Ctenopharyngodon idella* exposed to 0.06 mg/l Pb and 0.04 mg/l Pb, respectively, during 28 days exposure. Static bioassay method was employed for the study. Fingerlings were sampled for the selected parameters, on the 7th, 14th, 21st, and 28th days. Lead induced changes in the present study were a reduction in the RBC, WBC counts, hematocrit, and hemoglobin, and fluctuating MCV coupled with an increase in MCH and MCHC in both the species. MCV values of *C. mrigala* have showed an increase during the 7th and 14th days and subsequent decrease during the 21st and 28th days, while *C. idella* exhibited an intermittent rise and fall till the end of the exposure period. MCH and MCHC have increased in both species at all exposure periods.

1. INTRODUCTION

Metals are one of the pollutants which pose a potential hazard to the water bodies, and aquaculture ponds are no exception. The elements which pose the greatest risk are those that accumulate in the body. Fish have been used as bio-indicators of metal pollution since a long time, as their biochemical and hematological parameters are sensitive to heavy metals [1]. Lead is a non-nutritive trace metal. It is released into the aquaculture ponds by neighbouring chemical and fertilizer industries, ore refineries, the electroplating process, and fuel containing lead that leaks from fishery boats [2]. Lead ions enter the body of fish through gills, after binding to the mucous layer. It is also ingested along with food and water and is finally absorbed in the intestine [3]. After absorption, it is distributed particularly to the heart, liver, and kidneys. It also affects the immune system [4]. Studies with various fish species revealed that lead is neurotoxic, since it passes the blood–brain barrier. It causes changes in hematologic parameters, and structural deformations of tissues such as bones [5]. The concentration of metals in water and time of exposure, determines the alterations in hematological parameters causing both reversible and irreversible changes in the homeostasis of fish. Blood cell responses are important indicators of changes both within the body, as well as in the external environment of fishes. These changes depend on fish species, age, the cycle of sexual maturity of spawners, and diseases [6]. The primary stress responses, i.e., release of adrenalin and cortisol, trigger biochemical and physiological alterations called secondary stress responses [7]. The secondary responses of these early stages to lead can be evaluated by the measurement of secondary biochemical indicators such as variations in hematological parameters and red cell indices [8], which are non-invasive and permit regular check-up. A number of studies have been undertaken to investigate the effects of nutritive metals such as copper in carps, but only a few have been carried out with non-nutritive metals as lead, in carp fingerlings or juveniles under sublethal conditions. Studies on the effect of lead in fishes are restricted to the hematological parameters in tench *Tinca* on short-term exposure to lead [8], hematological changes in common carp after short-term lead exposure [9], and biochemical effects of sublethal lead concentrations in common carp [10].

Cirrhinus mrigala and *Ctenopharyngodon idella* are commercially important carp fishes widely cultured in India. Hence, an attempt has been made to study the sublethal effect of lead on the red blood cell (RBC) and white blood cell (WBC) counts, hematocrit, hemoglobin, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), and MCH concentration (MCHC) in fingerlings of both these species.

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2. MATERIALS AND METHODS

2.1. Experimental Fish, Design, and Conditions

Fingerlings of *C. mrigala* and *C. idella*, measuring 3 ½" to 4" and weighing 8 ± 0.5 g and 8.5 ± 1 g, respectively, were procured from a private fish farm in Kaikaluru, Andhra Pradesh, India. After conditioning, the fingerlings were transported in polythene bags filled with water and oxygen. On arrival, they were emptied into separate, 200 l, rectangular, fiber glass tanks, filled with tap water, and pond water in the ratio 1:1, with continuous aeration. The fingerlings were allowed to stay thus overnight, and water exchange carried out every alternate day with fresh tap water, during the week-long acclimation period. Fingerlings were fed with rice bran and oil-cake. Lead in water and feed were below detectable level. Fingerlings were observed for parasites, disease, and recovery from transport stress. Tanks and equipment were cleaned thoroughly to avoid algal growth and wastes. Uneaten food was removed within 24 h. Hand nets were cleaned between uses and hands cleaned before touching the fingerlings, whenever necessary. Tanks were covered with nets to protect the fingerlings from nearby movements and noise. Temperature during acclimation was 28 ± 2°C. Dead and abnormal individuals were periodically removed. Feeding was terminated 24 h before experimentation. The water quality was maintained with the following physicochemical characteristics during the experiment – temperature: 28 ± 2°C; dissolved oxygen: 2.2–5.23 ppm; pH: 6.5–7.2; alkalinity: 160–230 ppm; and total hardness: 200–270 ppm.

Static test method was employed for carrying out the sub-lethal toxicity experiments. The test organisms were introduced into 500 l fiber-glass tanks, stocking density not exceeding 1 g fingerlings/l of water containing the toxicant. Fingerlings of *C. mrigala* and *C. idella* were exposed to 1/5 of LC 50 calculated, i.e., 0.06 mg/l Pb and 0.04 mg/l Pb, respectively. Aeration was ceased, to avoid alteration in test results [11]. Fish were maintained at 28 ± 2°C and fed once a week, during the 28-day long experiment. Lead nitrate was the lead agent employed. Control fish were maintained under similar conditions, in lead-free water.

Every 7th, 14th, 21st, and 28th days, control fish and fish from lead concentration exposure were sampled, anaesthetized with 1.5 ppm. Quinaldine for 30 min, and blood withdrawn from their heart into K3 EDTA vials by piercing 1 ml disposable insulin syringe. The analysis of blood hematocrit, RBC count, WBC count, and hemoglobin concentration was done immediately.

2.2. Hematological Parameters and Red Cell Indices

The RBC and WBC counts were carried out in a modified Neubauer chamber after saline (0.9% NaCl solution) dilution of blood [12]. Hematocrit was determined by spinning the blood sample contained in hematocrit capillary tubes in a microhematocrit centrifuge [12] and hemoglobin by the cyanmethemoglobin method [13] using a commercial kit. The absorbance was measured on GENESYS 10 UV Spectrophotometer in triplicate, and concentration of the test sample read on the plotted calibration. The red cell indices, namely, MCV, MCH, and MCHC were calculated using the obtained blood measurements, based on the formulae given below [12],

$$MCV = Hct \times 100 / RBC$$

$$MCH = Hb \times 10 / RBC$$

$$MCHC = Hb \times 100 / Hct$$

2.3. Statistical Analysis

All results in this study were expressed as mean ± standard error of three replicates. The comparison of the control and treatment groups was statistically analyzed by student's "*t*" test, and the validity of the investigation was expressed as probability (*P*) values. Values of $P < 0.05$ were considered significant and $P > 0.05$ not significant.

3. RESULTS AND DISCUSSION

3.1. Hematological Parameters

The hematological parameters of the control and treatment groups of *C. mrigala* and *C. idella* are presented in Figures 1-4.

The minimum RBC counts of the control groups of *C. mrigala* and *C. idella* recorded values 1.54 ± 14.32 million cells/mm^3 and 1.63 ± 17.43 million cells/mm^3, respectively, and those of the treatment groups 1.12 ± 24.3 million cells/mm^3 and 1.12 ± 43.24 million cells/mm^3, respectively. While there was no significant ($P > 0.05$) decrease in RBC values at any exposure period in *C. mrigala*, the decrease was significant ($P < 0.05$) on the 28th day of exposure in *C. idella* [Figure 1].

The sublethal exposure of *C. mrigala* and *C. idella* to lead for 28 days induced a gradual reduction in RBC from the 7th to the 28th day of exposure, in comparison with the control group. RBC count is a stable index, and the body of fish tries to maintain it within the limits of certain physiological standards, with the help of various compensatory mechanisms. However, lead causes early mortality of mature RBC and

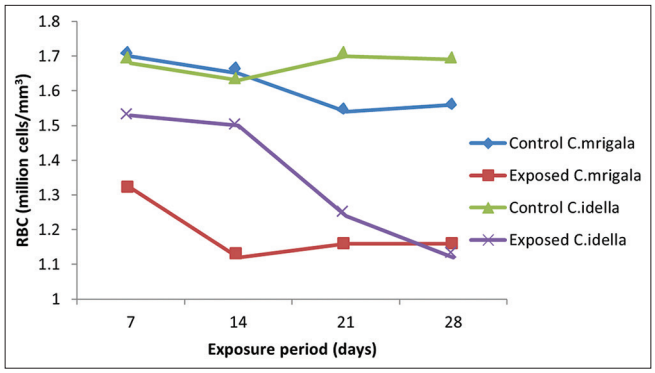

Figure 1: Sublethal effect of lead on the red blood cell counts of control and treatment groups of *Cirrhinus mrigala* and *Ctenopharyngodon idella*

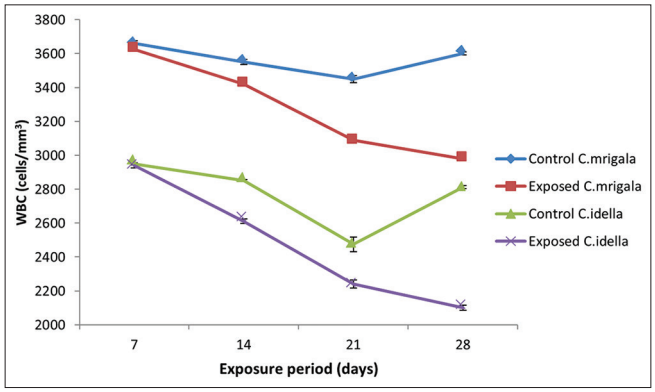

Figure 2: Sublethal effect of lead on the white blood cell counts of control and treatment groups of *Cirrhinus mrigala* and *Ctenopharyngodon idella*

inhibition of erythrocyte alpha-levulinic acid dehydratase (ALA-D), which is responsible for heme synthesis [14]. High lead exposures result in anemia and low exposures in compensating erythropoiesis [4]. The result in the present study can probably be explained by compensating erythropoiesis, as it has been carried out under sublethal conditions. Adeyemo has reported increased erythropoiesis and an increase in mortality of mature RBCs to compensate for inhibition of hemoglobin production, in lead-exposed *Salmo gairdneri* [14]. Dutta *et al.* observed a similar time-dependent response in *Channa punctatus* exposed to 1/5 of LC 50 of lead for 96 h [15].

The minimum WBC counts of the control and treatment groups of *C. mrigala* and *C. idella* recorded values 3450 ± 20.2 cells/mm^3, 2475 ± 43.3 cells/mm^3, and 2980 ± 8.66 cells/mm^3, 2100 ± 14.43 cells/mm^3, respectively. The decrease in WBC values was significant ($P < 0.05$) from the 14th day of exposure in *C. mrigala*, whereas, except on 7th day, there was a significant ($P < 0.05$) decrease in WBC in *C. idella* [Figure 2] compared to the control group. Similar observations were seen in *Prochilodus lineatus* by Martinez *et al*. The reduction in WBC count of both *C. mrigala* and *C. idella* might be due to a compensatory reaction, i.e., secretion of high level of cortisol as part of non-specific stress changes, leading to low WBC count. The primary stress responses, i.e., release of adrenalin and cortisol, trigger biochemical and physiological alterations called secondary stress responses [7]. Jezierska and Witeska observed an increase in cortisol concentration of plasma in fish exposed to copper and lead [16].

The hematocrit of the control groups of *C. mrigala* and *C. idella* recorded least values of 20.0 ± 0.05% and 18.10 ± 0.01%, and those of the treatment groups recorded 10.2 ± 0.17% and 11.70 ± 0.11%, respectively. In the present study, hematocrit of the lead treatment groups of both *C. mrigala* and *C. idella* decreased significantly ($P < 0.05$) relative to that of the control [Figure 3]. Shah reported low hematocrit in *T. tinca* exposed to long-term sublethal levels of lead [8]. A similar response was reported by Martinez *et al*. in *P. lineatus* exposed to sublethal concentration of lead [7]. On the contrary, Adeyemo *et al*. reported that hematocrit levels of *A. anguilla* increased after exposure to lower sublethal concentration (0.06 ppm Pb) in 15 and 30 days, whereas, higher sublethal concentration (0.12 ppm Pb) did not alter the hematocrit levels [17]. While the response to the lower sublethal concentration has been explained by release of RBC by erythropoietic tissues, response to higher sublethal concentration has been attributed to hormonal mechanisms.

The minimum hemoglobin values of the control groups of *C. mrigala* and *C. idella* recorded were 7.25 ± 0.01 g% and 6.50 ± 0.02 g%, and those of the treatment groups were 6.80 ± 0.05 g% and 6.30 ± 0.01 g%, respectively. There was a slight increase on the 7th day, followed by a significant ($P < 0.05$) decrease on the 28th day of exposure in *C. mrigala* [Figure 4]. In *C. idella,* hemoglobin decreased non significantly ($P > 0.05$) to a level equal to that of the control group on the 14th day of exposure [Figure 4]. Alkahemal *et al*. and Kim and Kang (2015) reported similar significant reduction in RBC, hematocrit, and hemoglobin in *C. gariepinus* and juvenile rock fish *Sebastes schlegelii* exposed to sublethal concentrations of water-borne and dietary lead, respectively [18,19]. These probably might be compensatory responses. Hemoglobin concentrations reflect the supply of an organism with oxygen, and the organism itself tries to maintain them as much stable as possible. Hence, this can be interpreted as a compensatory response that improves the oxygen carrying capacity, and subsequently the gaseous exchange [18]. The gradual reduction of hemoglobin content in the present study from the 14th to the 28th days in both the species may

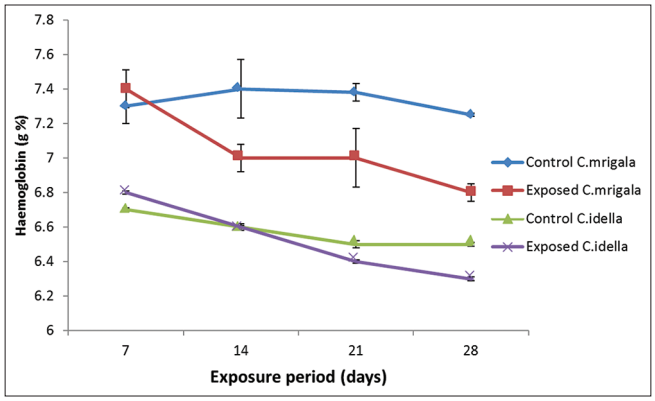

Figure 3: Sublethal effect of lead on the hemoglobin content of control and treatment groups of *Cirrhinus mrigala* and *Ctenopharyngodon idella*

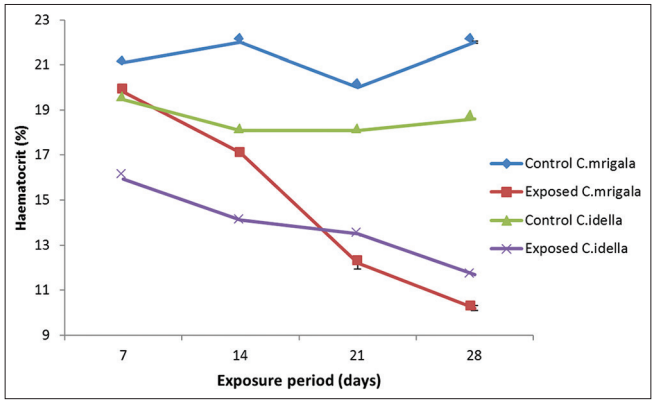

Figure 4: Sublethal effect of lead on the hemotocrit level of control and treatment groups of *Cirrhinus mrigala* and *Ctenopharyngodon idella*

be due to inhibition of heme synthesis. The most well-known effect of lead is the inhibition of α-aminolevulinic acid dehydratase activity (ALA-D). Lead reduces activity of ALA-D, one of the key enzymes participating in heme synthesis [19]. Time-dependent decrease in hemoglobin has also been reported in *C. punctatus* [15]. The sensitivity of hematological parameters to various environmental factors and chemicals is different [4]. Changes in RBC count, hemoglobin, and hematocrit have been understood to indicate secondary responses of an organism to irritants [20].

3.2. Red Cell Indices

The results of red cell indices, namely, MCV, MCH, and MCHC of the control and treatment groups of *C. mrigala* and *C. idella are* presented in Figures 5-7.

The minimum mean cell volume (MCV) of the control groups of *C. mrigala* and *C. idella* recorded was 124.11 ± 0.01 µm^3 and 106.40 ± 0.34 µm^3, respectively. On the other hand, minimum MCV of treatment groups was 87.9 ± 1.5 µm^3 and 94.00 ± 0.01 µm^3 for *C. mrigala* and *C. idella,* respectively. MCV in the treatment group of *C. mrigala* recorded a significant increase ($P < 0.05$) in the first half of the exposure period, and a decrease during the second half against the control group [Figure 5]. The increase in MCV may be attributed to the swelling of erythrocytes resulting in hypoxic condition or impaired osmoregulation.[21] Higher MCV has been reported by Ciftci *et al*. and Adeyemo *et al*. in *Oreochromis niloticus* and *C. gariepinus* exposed to sublethal concentrations of lead [21,22]. While the response in

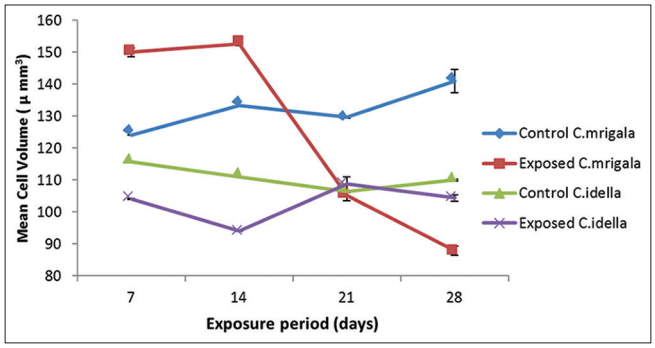

Figure 5: Sublethal effect of lead on the mean cell volume of control and treatment groups of *Cirrhinus mrigala* and *Ctenopharyngodon idella*

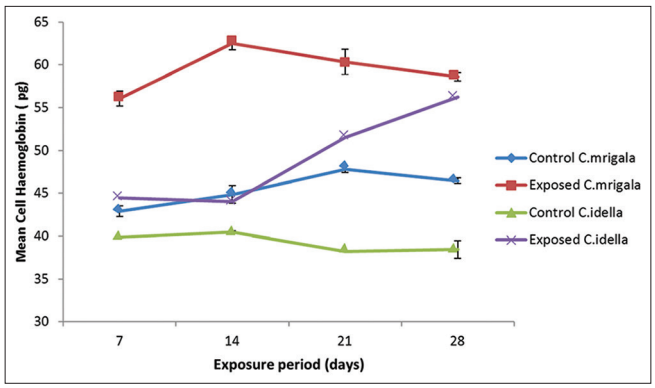

Figure 6: Sublethal effect of lead on the mean cell hemoglobin of control and treatment groups of *Cirrhinus mrigala* and *Ctenopharyngodon idella*

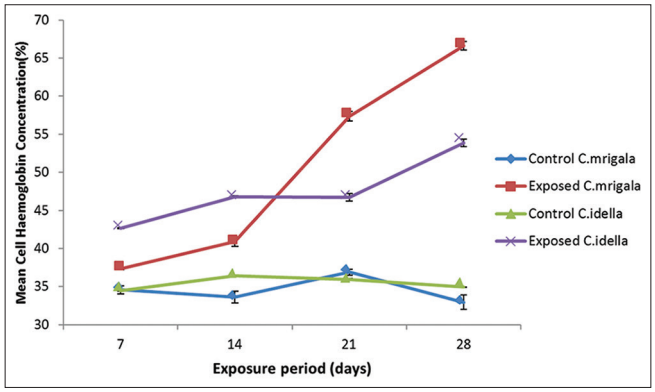

Figure 7: Sublethal effect of lead on the mean cell hemoglobin concentration of control and treatment groups of *Cirrhinus mrigala* and *Ctenopharyngodon idella*

O. niloticus has been attributed to hemoconcentration or stimulation of erythropoiesis by feedback mechanisms, that in *C. gariepinus* has been explained by direct or feedback responses of damage to RBC membranes, leading to hemolysis, defective hemoglobin synthesis, stress related release of red blood corpuscles from spleen, and lead-induced hypoxia. In the present study, MCV in *C. idella* of the treatment group recorded intermittent rise and fall up to the end of the exposure period [Figure 5]. The decrease was significant ($P < 0.05$) on the 7th and 28th days of exposure. The intermittent increase and decrease in MCV of *C. idella* can be explained by non-relevant relationship between RBC and blood indices. There has been found to be a non-relevant relationship between red cell indices and RBCs [23]. A slight increase and/or decrease in red cell indices may be due to disproportionality between the RBC count and hemoglobin concentration [19]. Intermittent rise and fall in MCV, coupled with low hemoglobin content, has been reported in *Oreochromis* hybrid exposed to sublethal dose of aluminum [24]. Lead causes morphological changes in the nucleus of red blood corpuscles, structural deformations and spreading in chromatin, probably resulting in distorted RBC and its nuclear membrane permeability or RNA synthesis [9]. The decrease in the level of hemoglobin content and increase in the MCV in *C. mrigala*, probably suggests hemodilution mechanism. The MCV indicates the status or size of the RBCs and reflects an abnormal/normal cell division during erythropoiesis. Kim and Kang suggested that lead might alter the properties of hemoglobin by decreasing its affinity towards oxygen, resulting in fragile and permeable RBCs, which probably lead to cell swelling and damage [19].

The minimum recorded mean cell hemoglobin (MCH) of the control groups of *C. mrigala* and *C. idella* was 42.93 ± 0.63 pg and 38.23 ± 0.03 pg, respectively, and those of the treatment groups were 56.05 ± 0.86 pg and 44.0 ± 0.14 pg, respectively. While, MCH concentration (MCHC) of the control groups of *C. mrigala* and *C. idella* recorded values 32.95 ± 0.95% and 34.44 ± 0.01%, respectively, those of the treatment groups recorded 37.37 ± 0.2% and 42.65 ± 0.05%, respectively. There was a significant ($P < 0.05$) increase in MCH and MCHC at all exposure periods in both the species, relative to control group [Figures 6 and 7]. Similar response has been reported by Adeyemo, in *C. gariepinus* exposed to sublethal concentrations of lead, and in Tench (*T. tinca*) on short-term exposure to lead [8,14]. The alterations in the latter study, were attributed to hemolysis and impairment in hemoglobin synthesis, stress-related release of RBCs from the spleen and hypoxia, in response to damage of RBC membranes, induced by exposure to lead. This has been explained by RBC shrinkage due to hypoxia or microcytic anemia, because of decrease in hematocrit during exposure. Structural changes in RBCs may result in an elevation in blood parameters [23].

4. CONCLUSION

Sublethal exposure of lead had a profound effect on the blood parameters of the selected species. Lead caused a reduction in RBC, WBC, hematocrit and hemoglobin, and elevation in MCH and MCHC, coupled with fluctuation in MCV. Hematological biomarker assays are ideal biomarker assays in being non-invasive and allow periodical monitoring of the individuals, so that routine blood tests can be performed to evaluate the health status of fingerlings and juveniles in culture ponds.

5. CONFLICTS OF INTEREST

Authors declared that they do not have any conflicts of interest.

6. FINANCIAL SUPPORT AND SPONSORSHIP

None.

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