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RESEARCH ARTICLE

IMPACT OF ELECTROPLATING INDUSTRIAL EFFLUENT ON THE HEMATOLOGY OF THE FISH *CATLA CATLA*

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ABSTRACT

Electroplating is considered a major polluting industry because it discharges toxic materials and heavy metals through wastewater (effluents) in environment. Hematological indices may be considered as stress indicators for estimation of the response reactions of the fish to various environmental conditions. The fish was exposed to different hours (24, 48, 72 and 96) in sub lethal concentration 5% electroplating effluent and parameters like RBC, WBC, HB, MCV, MCH MCHC, PCV has been analyzed. All hematological parameters except WBC were found to be decreased from control and the WBC was increased in all exposure periods indicating that test fish suffered hemolytic anaemia and leucocytosis. Increase in time produced a declining value of RBC, Hb, MCV, MCH and MCHC also exhibit the sub lethal concentrations of the effluent. The present study clearly suggests that the haematological values fluctuate in accordance with the change in the environment

INTRODUCTION

Pollution due to industrial waste is increasing and it is a problem throughout the world. The effluent contains various organic and inorganic contents in different concentration which are required by the plants (Andrino, 1986). Electroplating and metal finishing are widely practised and provide support to many major industries (Bhatt, 1998). Electroplating industries are using highly toxic and hazardous chemicals and metal ions which find their way into the effluent (Singh and Singh, 1997). Occupational exposure to many chemicals results in various forms of poisoning and other diseases (Sundararaju, 2003). Continuous discharge of industrial effluents into the aquatic environment can change both aquatic species diversity and ecosystems, due to their toxicity and accumulative behavior. Among industries, electroplating industries plays an important role in creating heavy metal pollution in water bodies through direct discharge of effluent in water bodies. Aquatic organisms including fish accumulate metals many times higher than in water or sediments (Madhusudan *et al.*, 2003; Surec, 2003 and Olaifa *et al.*, 2004). Hematology is used as an index of fish health status in a number of fish species to detect physiological changes following different stress conditions like exposure to pollutants, diseases, metals, hypoxia, etc. (Blaxhall *et al.*, 1972; Duthie *et al.*, 1985). The use of haematological technique in fish culture has made it possible for researchers to use it in environmental monitoring and fish health conditions (Mulcahy, 1975).

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The present study aim to gain insight into the changes induced in haematological parameters of *Oreochromis mossambicus* on chronic exposure to diethyl phthalate. Hematological indices are very indicators of changes in the internal and/or external environment of animals. In fish, exposure to chemical pollutants can induce either increases or decreases in haematological levels (Kori-Siakpere; Oboh, 2011). Metal contaminants are introduced into aquatic system through smelting process, effluents, sewage and leaching of garbage which cause serious damage to the aquatic fauna^{2,3}. When fish are exposed to elevated levels of metal in a polluted aquatic ecosystem, they tend to take these metals up from their direct environment. In recent years, hematological variables were used more when clinical diagnosis of fish physiology was applied to determine the external stressors and toxic substances as a result of close association between the circulatory system and external environment⁹.

Hence the objective of the present investigation is to determine the changes in different hematological parameters of the fresh water fish, *Labeo rohita* exposed to sublethal concentration of chromium. Chemical products discharged into the environment end up reaching aquatic systems, contaminating and/or affecting the aquatic biota, including fishes, through direct contact of the surface of the body and the gills of these animals with contaminated water, or else through their food. Once these products have penetrated the organism, their effect may be toxic (Erickson *et al.*, 2008). Exposure to contaminants can cause biological changes in organisms. These changes can be measured and used as indicators of exposure to or effects of environmental pollutants, which are called biomarkers.

These biomarkers enable the rapid assessment of the health of organisms and warn about possible environmental risks (Hugget *et al.*, 1992; Mayer *et al.*, 1992; Van der Oost *et al.*, 2003). Among biological changes, hematological parameters are considered potential biomarkers of exposure to chemical agents, since the latter can induce an increase or decrease in the various hematological components (Heath, 1995; Van der Oost *et al.*, 2003). Among the aquatic organisms, fishes occupy an important position in the field of aquatic toxicology (Di Giulio and Hinton, 2008). Toxicity tests with *P. lineatus* have shown that this species is sensitive to a variety of pesticides, and it is currently considered a potential bioindicator species (Langiano and Martinez, 2008; Pereira Maduenho and Martinez, 2008; Modesto and Martinez, 2010a). A fair amount of the heavy metals released in industrial effluents find their way into the aquatic bodies.

Metals like nickel, chromium, zinc, mercury and manganese tend to accumulate in the organisms living therein even when present at levels considered safe for survival. Chemical products discharged into the environment end up reaching aquatic systems, contaminating and/or affecting the aquatic biota, including fishes, through direct contact of the surface of the body and the gills of these animals with contaminated water, or else through their food. Once these products have penetrated the organism, their effect may be toxic (Erickson *et al.*, 2008). Among the aquatic organisms, fishes occupy an important position in the field of aquatic toxicology (Di Giulio and Hinton, 2008). Aquatic pollution has become a global problem in recent years. Extensive industrialization has measurably influenced the quality of water of lakes, ponds and rivers all over the world. Natural waters contaminated by untreated wastes of industrial, technological and agricultural origin often contain various metallic compounds.

MATERIALS AND METHODS

Test organisms

Bulk of sample fishes, catla catla ranging in weight from 4-5 gms and measuring 5-7 cm in length were procured from Aliyar Dam. Fishes were acclimatized to laboratory conditions for 2 weeks in a large Syntax tank. The water was changed twice in a day to maintain the oxygen content and to remove the excreta of fishes. The fishes were fed regularly with conventional diet rice bran and oil cake 1:1 ratio. Feeding was stopped two days prior to the experiment in order to keep the animal more or less in the same state of metabolic requirement. Fish were not fed during the toxicity tests. Fishes about the same size irrespective of sexes were selected for the experiment

Acute toxicity tests

The undiluted electroplating industrial wastewater collected was considered as 100% solution. From this, the selected concentrations of the waste water for the experiments were obtained by diluting it with clean non-chlorinated ground water. In acute bioassay studies, the determination of LC50, the lethal concentration at which 50% of fish dies, is of prime importance to derive the sub-lethal concentrations so as to treat the organisms. The LC50 values for 24, 48, 72 and 96h exposure to electroplating industrial wastewater were obtained using Probit Analysis (Finney, 1978). Fish were exposed to

5% electroplating industrial wastewater in 96h were taken as the sub lethal concentrations.

Sampling

After the 96-h experimental period, the fish were anesthetized with benzocaine (0.1 g L⁻¹) and blood was drawn from the caudal vein, using heparinized syringes. Blood samples were stored refrigerated (4 °C) for the subsequent analysis of the hematological parameters.

Hematological analyses

Hematocrit (Hct) was determined by blood centrifugation (5 min, 1400 g) in heparinized glass capillaries, using a micro hematocrit centrifuge (Luguimac S.R.L., Model LC 5, Argentina). The hemoglobin (Hb) concentration was estimated by the cyanmet hemoglobin method, using a commercial kit (Labtest Diagnóstica, Brazil) and a spectrophotometer (Libra S32, Biochrom, U.K.) at 540 nm. The number of red blood cells per cubic millimeter of blood (RBC) was determined from samples of blood fixed in formalin citrate buffer (sodium citrate diluted in 0.4% formalin solution), using a Neubauer chamber and an optical microscope (400× magnification). These blood parameters were then used to derive the following hematimetric indices: mean corpuscular volume (MCV, fl), calculated as (Hct×10)/RBC; mean corpuscular hemoglobin (MCH, pg), calculated as (Hb × 10)/RBC; mean corpuscular hemoglobin concentration (MCHC, %) calculated as (Hb × 100)/Hct.

Statistical analysis

All measurements were performed in average of three replicates. Data obtained was analyzed using the SPSS/PC+ Statistical package (ver.11.5). Significant difference between control and experimental groups were determined using Duncan's test for multiple range comparisons (Duncan D.B., 1955)

RESULTS

The amount of RBC in the blood of the fishes exposed to 5% electroplating effluent for 24, 48, 72 and 96hrs was found to contain 2.77, 2.47, 2.24, 1.86 x 10⁶/mm³ and mean control was found to be 2.98x 10⁶/mm³. The amount of WBC were found to be increased from the control. The values of WBC were 20.32, 21.52, 23.70, 26.82, 29.44 x 10⁶/mm³ in control, 24, 48, 72 and 96hrs respectively. The level of hemoglobin in the fish *Catla catla* exposed to 24, 48, 72 and 96hrs was found to contain 2.81, 2.02, 1.90, 1.65 % and mean control was found to be 3.06 g %. The value of MCV in fishes exposed to 4% electroplating effluent for 24, 48, 72 and 96hrs was found to contain 43.35, 41.10, 39.40, 27.60 and mean control was found to be 46.10µm³. The amount of MCH in the blood of the fishes exposed to electroplating effluent was recorded as 23.10, 22.20, 21.60, 21.40 and the control was found to be 26.40 Pg. The amount of MCHC recorded as 20.50, 17.41, 15.80, 14.90, 13.24 g/dL in control, 24, 48, 72 and 96hrs exposures respectively. The amount of PCV in the blood of the fishes exposed to electroplating effluent for 24, 48, 72 and 96hrs was found to contain 10.00, 9.04, 8.88, 8.24 % and mean control was found to be 10.25%.

Table I. Effect of Electroplating Effluent on Haematological Parameters in Blood of the Fish *Catla Catla*

parameters	Electroplating effluent exposed				
	Control	24 Hrs	48 Hrs	72 Hrs	96 Hrs
RBC(10^6 cu/mm)	2.98±0.12	2.77±0.07	2.47±0.09	2.24±0.13	1.86±0.08
't' value		2.13*	2.88**	4.20**	9.49**
% change		-7.04	-17.11	-24.83	-37.58
WBC(10^6 cu/mm)	20.32±0.12	21.52±0.12	23.70±0.10	26.82±0.11	29.44±0.13
't' value		-10.04	-30.61	-55.12	-72.70
% change		5.90	-16.63	31.98	44.88
HB(gms/dl)	3.06±0.28	2.81±0.30	2.02±0.07	1.90±0.33	1.65±0.34
't' value		1.35 ^{NS}	5.46**	5.93**	7.01**
% change		-8.16	-33.98	-37.90	-46.07
MCV(μm^3)	46.10±0.41	43.35±0.08	41.10±0.10	39.40±0.45	27.60±0.47
't' value		10.37**	18.50**	24.32**	65.90**
% change		-5.96	-10.84	-14.53	-40.13
MCH(pg)	26.40±0.10	23.10±0.09	22.20±0.13	21.60±0.11	21.40±0.08
't' value		34.69**	36.21**	45.66**	55.22**
% change		-12.5	-15.90	-18.18	-18.93
MCHC	20.50±0.12	17.41±0.11	15.80±0.10	14.90±0.07	13.24±0.13
't' value		18.45**	33.02**	45.31**	48.17**
% change		-15.07	-22.92	-27.31	-35.41
PCV(%)	10.25±0.66	10.00±1.84	9.04±0.69	8.88±0.71	8.24±0.72
't' value		0.28 ^{NS}	2.81**	3.41**	4.56**
% change		-2.43	-11.80	-13.38	19.60

Results are mean (\pm SD) of 5 observations % = Parenthesis denotes percentage increase/decrease over control.

** - Significant at 1% level, NS- Non Significant, *- Significant at 5% level

DISCUSSION

The exposure of fish, *Catla catla* to sublethal concentration of electroplating effluent for 96 hours caused significant alterations in hematological parameters are represented in Table. The results reveal that the Red blood corpuscles (RBC), Haemoglobin (Hb), Haematocrit (PCV), MCH, and MCHC values were significantly decreased after 96 hours of exposure periods when compared to control, On contrast to this, the White blood cell (WBC) were found statistically significant increase in experimental fish when compared with control. The fishes exposed to sublethal concentrations of electroplating effluent in the present investigation showed remarkable hematological alterations. Hematology is used as an index of fish health status in number of fish species to detect different stress conditions like diseases, hypoxia, and exposure to metals and pollutants etc. (Blaxhall 1972; Duthie *et al.*, 1985).

The general reduction in blood parameters is an indication of anaemia. The heavy metal induced significant decrease in RBC, Hb and PCV. The RBC count coupled with low haemoglobin content may be due to destructive action of pollutants on erythrocytes (Karuppasamy, 2000; Bela Zutshi *et al.*, 2010). The effluent induced significant decrease in RBC, Hb and PCV. The RBC count coupled with low haemoglobin content may be due to destructive action of pollutants on erythrocytes. The decrease in haemoglobin concentration indicates the fish inability to provide sufficient oxygen to the tissues (Nusse G., 1995). A specific toxic effect on fish blood and tissues occurs due to various heavy metals and toxins which enters the aquatic environment. Prolonged reduction in haemoglobin content is deleterious to oxygen transport and any blood dyscrasia and degeneration of the erythrocytes could be ascribed as pathological conditions in fishes exposed to toxicants (Shalaby, 2001).

Our results are in good concurrence with the earlier works of (Buckley *et al.*, 1996; Palanisamy *et al.*, 2011). The alterations in these hematological indices may be due to a defence reaction against toxicity through the stimulation of erythropoiesis.

The anemic conditions in fish may be detected using haematocrit (Adakole, 2012). The PCV values always decrease when a fish loses appetite or become diseased or stressed. At present, the distinct decrease in the level of Haemoglobin and PCV after exposure to electroplating effluent clearly suggests a haemo dilution mechanism possibly due to gill damage or impaired osmoregulation. The haemo dilution has been interpreted as a mechanism that reduces the concentration of the irritating factor in the circulatory system (Blaxhall *et al.*, 1973). The increase in WBC in the present study has been attributed to several factors like increase in thrombocytes, lymphocytes or squeezing of WBC's in peripheral blood. Increase in WBC count can be correlated with an increase in antibody production which helps in survival and recovery of fishes exposed to toxicant. High WBC counts indicate damage due to infection of body tissues, severe physical stress as well as Leukemia. Similar increase was reported by (Banerjee and Banerjee, 1988) in *Channa punctatus* due to Copper sulphate and Potassium dichromate induced toxicity (Singh M., 1995; Anand kumar, 2006).

In *Channa punctatus* exposed to Copper. The erythrocyte constants MCV, MCH, and MCHC offer relationship on size, form and Hb constants of erythrocytes. They allow the determination of morphological anaemia that whether Normocyte, Macrocyte or Microcytic anaemia. The alterations in the haematological indices i.e. increase in MCV and decrease of MCH and MCHC in the present study may be due to a defense against the toxic effect of chromium and in turn due to decrease in RBC's, Hb and PCV and the disturbances occurred both in metabolic and haemopoietic activities in fish. Increase in WBC count suggests that the anemia is of macrocytic type. (Sheiq Afaq, 2009). The decrease in MCH and MCHC in the present study clearly indicates that the concentration of hemoglobin in RBC is reduced. The MCH is a good indicator of Red Blood Cell swelling (Wepener, 1992). The significant decrease in the MCHC values in the present study may be due to swelling of RBC or decrease in hemoglobin synthesis. Increase in PCV shows the magnitude of shrinking of cell size (Shakoori *et al.*, 1996 and Abdul

RAUF *et al.*, 1996) reported that the acute exposure to LC50 values of diazinon induced haematological alterations in Indian carp and offers a tool to evaluate toxicity derived alterations.

Conclusion

The hematological parameters except WBC were found to be decreased from control and the WBC has increased in all exposure periods. From the above investigation it can be inferred that the aquatic animals are affected by the Electroplating effluent discharge via effluents into aquatic environments caused severe anemia and alterations in hematological indices in the fresh water fish, catla catla.

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