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Effect of heavy metal model mixture on haematological parameters of *Labeo rohita* from Gharni Dam Nalegaon, Latur

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Abstract

The present study of heavy metal model mixture on fish experimental changes in blood haematological parameters (*Viz*. WBC, RBC and Haematocript) of the laboratory acclimatized fish *Labeo rohita*, exposed to different heavy metals mixture model were studied. Erythrocyte count decreased significantly ($p < 0.005$) in the blood of fish exposed to almost all Heavy metal model mixture concentrations studied while alterations of haematocrit level depended on Heavy metal model mixture concentration. The highest concentration of Heavy metal model mixture (21.79%) induced a drop in haematocrit level meanwhile the level of haematocrit in blood of fish exposed to all lower Heavy metal model mixture concentrations was higher as compared to control. Erythrocyte count in the blood of Heavy metal model mixture-exposed fish was significantly lower as compared to control, even in fish exposed to the lowest concentration of Heavy metal model mixture (1.1%).

Keywords: Metal model mixture, Haematological parameters and *Labeo rohita*.

INTRODUCTION

Heavy metals are diluted and affected by various surface water components (carbonate, sulphate, organic compounds - humic, fulvic, amino acids) after entering natural water bodies which induce formation of insoluble salts or complexes. These salts and complexes are predicted to be not harmful to aquatic organisms (Eisler 1998 [3]). Part of them sink and are accumulated in bottom sediments. In the majority of ecotoxicological studies, effects of single metals on fish have been evaluated, while studies of biological responses of fish to the impact of a mixture of heavy metals (more than three components) are scarce (Larsson *et al.*, 1987 [4]; Kloepper-Sams *et al*, 1994 [5]; Kazlauskiene *et al.*, 1996 [6]; Kazlauskiene, Burba, 1997 [7]). However, these studies have demonstrated that the effects of metal mixtures can differ in their toxicity on living organisms from the effects of single components. The toxicity of heavy metal mixtures depends on their concentrations, specific composition, and duration offish exposure (Kazlauskiene *et al.*, 1996 [6]; Kazlauskiene, Burba, 1997[7]; Vosyliene, *et al...*, 2003). The present study is a first step in evaluating the possible toxic impacts of some traffic emission pollutants on aquatic animals.

MATERIAL AND METHODS

The fish were collected from the Gharni Dam Nalegaon, Latur district M.S. India and kept in tanks of about 3000 L capacity and feed FEED available in market daily as a diet, The weight of fish in the study was ranged from 12.9 to 14.4 g. The effect of a model mixture consisting of four heavy metals was investigated. The formation of the model mixture was based on available analytical data on the amounts of representative heavy metals in soil near the highway of Solapur-Aurangabad. The determined concentrations of metals were as follows Cd 40-60 mg/kg, Zn 20-50 mg/kg, Pb 160-200 mg/kg and Hg 20-30 mg/kg. The soil samples for pollution assessment were collected at a distance of 1-100 m from both roadsides and from a soil layer 0-15 cm deep. The stock solution of Heavy Metal Model Mixture was prepared in distilled water using the following chemically pure substances. CdSO4 × 2.5H2O, Zn (NO3)2 × 6H2O, HgSO4 + 7 H2O, Cr (NO3)3 × 9H2O and Pb (NO3) 2,4H2O. The final concentration being recalculated according to the amount of heavy metal ions. Heavy Metal Model Mixture concentrations were artificially decreased -50 tunes with the aim to be closer to LC50 levels of copper to rainbow trout. The Heavy Metal Model Mixture solution considered to be equal to 100% contained Cd 0.874, Zn 0.934, Pb 4.7 and Hg 0.66. The determination of LC50 of Heavy Metal Model Mixture started from a 180% concentration of the mixture. The different Heavy Metal Model Mixture concentrations applied in the study are presented in Table 1

Table 1. Concentration of metals in Heavy Model Mixture tested and their MPC accepted for Lithuanian inland waters (Valstybes zinios, 2002, No.62-2533)

Heavy Metal Model Mixture Concentration %	Heavy Metal Model Mixture Concentration (mg/l)			
	Cadmium (Cd)	Zinc (Zn)	Lead (pb)	Mercury (Hg)
180	1.58	1.27	6.50	0.84
162	1.43	0.88	5.74	0.77
108.97	0.96	0.77	3.91	0.52
100	0.88	0.8	3.6	0.47
21.79	0.826	1.46	0.756	0.0987
1.1	0.0094	0.008	0.037	0.005
MPC	0.01	0.1	0.005	0.01

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The system consisted of six 40 L aquaria. For the experiments, seven fish were transferred from holding tanks to each aquarium and kept in the new medium until acclimation, i.e. till they started to swim freely and feed well. Different amounts of stock solution were added to five aquaria except control. Each test was replicated twice. Every day the water was changed and the number of dead fish was recorded. Dead fish were weighed individually. No mortality was observed among control fish. The fish were transferred from holding tanks into five aquaria of 100L capacity. Control water and Heavy Metal Model Mixture solutions were renewed daily. During the tests fish were fed every day.

Blood samples were collected by punction of the caudal blood vessels. Heparin sodium salt was used for stabilization of the fish blood. Blood was sampled from 10 fish of each exposure group. Erythrocytes (RBC. 10" x mm"5), haemoglobin concentration (Hb, g/l), haematocrit level (Hot. l/l), leukocyte count (WBC, 10' * mm"5) were determined using routine methods. The median acutely lethal concentration (LC5 (1) values and their 95% confidence intervals were estimated by the trimmed Spearman-Karber method (Hamilton *et al...*, 1977 [11]). The statistical analysis of the data was performed with SPSS 10.0 software, and the significance of the data was determined by the one-way ANOVA test.

RESULTS AND DISCUSSION

Changes in blood parameters (erythrocyte count, haematocrit level, leukocyte count) revealed UMJMM toxicity to fish. Erythrocyte count decreased significantly ($p < 0.005$) in the blood of fish exposed to almost all Heavy metal model mixture concentrations studied while alterations of haematocrit level depended on Heavy metal model mixture concentration. The highest concentration of Heavy metal model mixture (21.79%) induced a drop in haematocrit level meanwhile the level of haematocrit in blood of fish exposed to all lower Heavy metal model mixture concentrations was higher as compared to control (Table 2).

Table 2. Effect of Heavy metal model mixture on *Labeo rohita* haematological parameters

Concentration %	RBC count,10^6×mm^3	Haematocript level, 1/1	WBC count, 10^3×mm^{-3}
21.79	0.64±0.02*	0.37±0.01*	32.7±2.7*
10.89	0.75±0.04*	0.46±0.02*	25.6±3.3
5.45	0.82±0.05	0.47±0.02	36.0±0.4*
1.1	0.61±0.04*	0.45±0.02	26.5±3.0
Control	0.85±0.04	0.43±0.01	22.1±1.8

The present study demonstrated obvious toxic effects of Heavy metal model mixture. On the red blood cell count of rainbow trout. Erythrocyte count in the blood of Heavy metal model mixture-exposed fish was significantly lower as compared to control, even in fish exposed to the lowest concentration of Heavy metal model mixture (1.1%). Though Cd, Zn, and Hg ion amounts in Heavy metal model mixture solution of this concentration were lower than MPC of these metals, Pb concentration was 7 times higher (Table 1). Probably the Heavy metal model mixture induced a specific haemotoxic effect on blood erythrocyte. Heavy metals such as zinc, cadmium, C and lead might alter the properties of erythrocyte membranes, rendering them more fragile and permeable, which probably resulted in cell swelling (indicated by an increase in haematocrit level), deformation and damage (Kazlauskiene, Vosyliene, 1999 [14]). This suggestion was confirmed in our studies by alterations in haematocrit level which decreased only in fish exposed to a 21% solution of Heavy metal model mixture. The exposure of fish to all lower concentrations of Heavy metal model mixture induced an increase in this parameter. Red blood cell count decrease in the blood of *Labeo rohita* previously determined when studying the long-term effect of the pond water on fish. It was supposed that various heavy metals that had entered the pond water exerted a specific haemotoxic effect on fish blood (Vosyliene, Kazlauskiene, 2004 [15]). The elevated leukocyte count determined in Heavy metal model mixture-exposed fish, as compared to the control, may indicate that Heavy metal model mixture induced a stress response in fish.

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