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Study of haematological changes and accumulation of cadmium chloride in the vital organs of the fresh water fish *Labeo Rohita*

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Abstract
The problem of appearance of toxic materials in water ecosystem is closely connected with increased concentration of different types of pollutants, which enter water bodies with industrial and communal waste waters. The extensive use of cadmium in industry and the degree of its toxicity indeed pose an environmental problem. The objective of the present work is to study the haematological changes in the fresh water fish *Labeo rohita* treated with sub lethal doses of cadmium chloride and to estimate the accumulation of the metal in the vital organs like gill, liver and kidney. The LC50 at 96 hrs was determined by the Probit analysis method. The experiment was designed to expose the fish to sub lethal concentrations of cadmium chloride - 500µg/l, 700µg/l, and 900µg/l. The haematological profile of the control and treated fish included Haemoglobin, total RBC and WBC count, platelet count and the erythrocyte indices of fish, MCV and PCV. The heavy metal cadmium was analysed by Atomic Absorption Spectroscopy. A significant decrease in the RBC, WBC, Platelet count, Haemoglobin, MCV and PCV was observed in different concentrations. A significant increase in cadmium chloride was observed in the gill, liver and kidney of the fish. Haematological and biochemical profile in fish is proved to be a sensitive index for the evaluation of fish metabolism under metallic stress.

Keywords: toxicity, cadmium chloride, Probit analysis, haematological profile, heavy metal analysis, sub lethal concentration

1. Introduction
The problem of appearance of toxic materials in water ecosystem is closely connected with increased concentration of different types of pollutants, which enter water bodies with industrial and communal waste waters. Metals are redistributed naturally in the environment by both geologic and biologic cycles. Many metals whether organically-complexed or not are known to accumulate in plant and animal tissues to a very high level, posing a potential toxic hazard to the organisms themselves, or organisms higher in the food chain including humans, which may consume them (Abel, 1998). Evidence of toxic effect of heavy metals has been reported on fishes and populations eating contaminated food (Chang, 1996) [2].

Cadmium is a rare element and is usually found as an impurity in ores of other metals principally those of zinc. It is obtained as a by-product in the refining of zinc and copper but small quantities can remain as impurities in these and other metals. It is present in low concentrations in soils, sandstones and shale's from which it is leached only very slowly into surface water (Bowen, 1966) [3] and is also present in some phosphate fertilizers. Because of its many industrial applications, chief among which is electroplating, it is often present in manufacturing industrial discharges.

Other principal uses of cadmium in industry are in paint pigments and as stabilizing material for polyvinyl plastics, batteries and alloys. Cadmium chemicals, especially pigments and nickel-cadmium alloys, account for 7% of total use. Cadmium alloys are used in bearings, solders, low-melting alloys and silver brazing. The extensive use of cadmium in industry and the degree of its toxicity indeed pose an environmental problem.

Scientific evidence indicates that cadmium is toxic to humans. The average daily intake of cadmium by humans is approximately 4-60mg, depending on the foods chosen (Schroeder, 1961). The body burden of cadmium in an adult is estimated to be about 30 mg. In the newborn it is 1mg, indicating that there is an increase in accumulation with age. Of the total body burden, 50%-75% is in the liver and kidney, with one third in the kidney.

Water born metals may alter the physiological and biochemical parameters in fish blood and tissues. The reaction and survival of aquatic animals depend not only on the biological state of the animals but also on the toxicity and type and time of exposure to the toxicant (Brungs, 1977). Haematological and biochemical profile in fish is proved to be a sensitive index for the evaluation of fish metabolism under metallic stress (Kishore Dhara *et al*., 2014, Al-Attar, 2005, Satheeshkumar *et al*., 2011) [6, 7, 8].

The objective of the present work is to study the haematological changes in the fresh water fish *Labeo rohita* treated with sub chronic doses of cadmium chloride and to estimate the accumulation of the metal in the vital organs like gill, liver and kidney.

2. Materials and Methods
Fresh water fish *Labeo rohita (rohu)* were collected from Aliyar dam near Pollachi, Coimbatore district, Tamilnadu. The healthy fingerlings of *Labeo rohita* ranging in length 10-12 cm and weighing about 12-14g were used for the experiment. Fishes were acclimated for 2-3 weeks in a large plastic trough containing plain tap water. The physico-chemical characteristics of water were analyzed. Fish was stocked in a big trough containing 1000 liters of water and

acclimatized for fifteen days. Fishes were fed with artificial feed twice a day.

The toxicant used in the static bioassay was cadmium chloride in tap water. One plastic trough served as the control and the other troughs were provided with different concentrations of cadmium chloride namely 4000µg/l, 4250 µg/l, 4500 µg/l, 4750 µg/l,5000 µg/l, 5250µg/l. Ten fishes were placed in each trough and mortality was recorded after 24 hrs, 48hrs, 72hrs, and 96hrs. The LC50 at 96 hrs was determined by the Probit analysis method (Finney 1971).

The experiment was designed to expose the fish to sub lethal concentrations of cadmium chloride - 500µg/l, 700µg/l, and 900µg/l. One trough served as the control. Each trough contained ten fishes and the experiment was conducted in triplicate. The duration of the experiment was 30 days.

The haematological profile of the control and treated fish included Haemoglobin, total RBC and WBC count, platelet count and the erythrocyte indices of fish, MCV and PCV.

Fish samples were descaled and rinsed with ultrapure water before dissection for the isolation of tissues of test sample. USEPA (United Sates Environmental Protection Agency) methodology was followed for metal extraction. A known weight of tissue sample was dried in a hot air oven at 100^0C. 1 gram of powdered tissue sample was taken in a 100 ml beaker and treated with100 ml nitric acid and 5ml hydrochloric acid and was left over night. Next day they were dried at 150^0C in a hot air oven for 30 minutes. The digestible was cooled and made to 100ml and filtered through whatman No.1 filter paper. The heavy metal cadmium was analysed by Atomic Absorption Spectroscopy (AAS). Metal concentration was calculated in micro grams per gram dry weight ($\mu g/g^{-1}$).

Students't' test and Chi-square test was done for the analysis.

3. Results and Discussion

Cadmium compounds pose toxic effects on *Labeo rohita* which is evident by the findings of the present investigation and the calculated LC50 value observed in the present study confirmed with the reports of Tripathi, 2014. 1/10th of the LC50 value at 96 hours was found to be 500µg/l (Fig 1) (Table 1 and 2).

The RBC of the control was 0.02 ± 0.01. Changes were not observed in 700µg/l and 900µg/l treated fishes. In 500µg/l treated fishes the value was 0.01 ± 0.00 which are not significant (Table 3).

The WBC count in the control fish was 90.00 ± 46.71. A significant decrease was observed in 900µg/l treated fishes 31.80 ± 10.3 ($P < 0.01$). In the 700µg/l treated fish the WBC count decreased to 86.40 ± 53.53 which was not significant. In the 500µg/l treated fish a slight increase was observed 93.50 ± 25.92, but was not significant (Table 4).

The platelet count in the control group was 11.30 ± 4.14. A significant decrease was observed in 700µg/l treated fishes 8.00 ± 1.94 ($P < 0.05$). An increase was observed in 500µg/l treated fishes 13.70 ± 4.62 which was not significant and decrease was observed in 900µg/l treated fishes 8.30 ± 4.32 (Table 4).

The haemoglobin content in the control group was found to be 0.53 ± 0.09. A significant decrease was observed in all the groups, 0.43±0.08 ($P<0.05$), 0.19 ± 0.07 ($P<0.01$) and 0.21 ± 0.07 ($P<0.01$) of 500µg/l, 700µg/l and 900µg/l treated fishes respectively (Table3).

The PCV value in the control group was found to be 0.33 ± 0.13. A significant decrease was observed in all the treated groups, 0.16 ± 0.05 ($P<0.05$), 0.11 ± 0.03 ($P<0.01$) and 0.13 ± 0.05 ($P<0.01$) of 500µg/l, 700µg/l and 900µg/l treated fishes respectively (Table3).

The MCV value in the control group was found to be 75.60 ± 21.81. A significant decrease was observed in 700µg/l treated fishes 47.10 ± 5.40 ($p<0.01$) and significant increase in 900µg/l treated fishes 84.00 ± 2.67 which is not significant. In 500µg/l treated fishes, reduction in MCV 66.50 ± 21.39 was not significant (Table 4).

The slight reduction in RBC count after 30 days treatment and significant reduction in haemoglobin concentration, PCV and MCV have been reported by Beena and Viswaranjan, 1987 [11]; Johansson- Sjobeck and Larsson, 1978 [12]. The reduction in RBC values may be due to haemolysis or due to haemodilution. Metal exposure induces changes in haematological values generally because of changes in blood water content (Tort and Torres, 1988) [13]. Exchange of water ions also increase resulting in water uptake in freshwater (haemodilution) and water loss in salt water (haemoconcentration), (Dick and Dix, 1985) [14].

Houston and Keen, (1984) [15] and Kori-siakpere and Ikomi, (2011) suggested that cadmium causes reduction in erythropoiesis and impeded formation of red blood cells. The decrease in mean cell volume is the result of immature red blood cells from hematopoietic tissues. Immature cells are released to compensate the loss of red blood cells.

Brandao *et al.*, (2009) [17] and Oliveira Ribeiro *et al.,* (2002) [18] found a reduction in immunological parameters (platelet, leucocyte and lymphocyte count) and an increase in neutrophil and monocyte percentages were demonstrated in mercuric chloride exposed fishes. It is known that metals can induce abnormal responses in the immune system. According to Wedemeyer *et al*., (1990) [19], the suppression of the immune system increases the susceptibility of fish to diseases.

Geode *et al.,* (1990) found an extremely low WBC count indicating either suppression of circulating lymphocytes, a characteristic acute stress response, or that of an active bacterial infection which induced leucocytolysis (Murad and Houston, 1998, Ghazyly, 1991 and Sexena *et al*., 1992) [21]. Increased leucocyte count was also reported in carps exposed to acute sub lethal concentration of cadmium nitrate and cadmium chloride (Schmidt and Picos, 1980) [24].

The decrease in PCV may be attributed either to decreased cellular content and increased plasma content mainly water (Lohner *et al.,* 2001) [25]. The decrease in MCV after short term exposure coupled with low hemoglobin content indicates that the red blood cells have shrunk, either due to hypoxia or microcytic anemia.

Hemoglobin is the oxygen carrying component in the blood of fish and its significant decrease at all sublethal concentrations is a good indicator of anemia (Dhanapakium *et al*., 2001; Sjobeck *et al*., 1984; Nanda *et al.,* 1996 and Bersenyi *et al*., 2003) [27, 28, 29].

Cadmium content in the gill of control fish is 0.75 ± 0.03. A significant decrease was observed in 500µg/l treated fishes 0.47 ± 0.02 ($P>0.01$). In the 900µg/l treated fish the cadmium content showed an increase of 0.95 ± 0.02 ($P>0.01$). The 700µg/l treated fish showed a slight increase 0.74 ± 0.02 which was not significant (Table 5).

Cadmium content in the liver of the control fish is 0.19 ± 0.02. A significant increase was observed in all the three concentrations 0.44 ± 0.03 (P<0.01), 0.71 ± 0.02 (P<0.01) and 0.91 ± 0.02 (P<0.01) in the 500µg/l, 700µg/l and 900µg/l treated fishes respectively.

Cadmium content in the kidney of the control group was found to be (0.58 ± 0.02). A significant decrease was observed in 500µg/l treated fishes 0.53 ± 0.02 (P<0.01). A significant increase was observed in 700µg/l 0.74 ± 0.03 (P<0.01) and 900µg/l 0.88 ± 0.02 (P<0.01).

Protasowicki and Chodyniecki, 1992 [30]; Narayanan *et al.*, (1997) [31] and Smith and Bell, 1976 [32] have observed higher rates of heavy metal accumulation, especially in the posterior kidney, a tissue primarily involved in the excretory function.

One of the main reasons attributed to the increased presence of heavy metals in these organs is their capacity to accumulate cadmium brought by blood from other parts of the body and induce the production of the metal binding protein, metallothioneins, which is believed to play a crucial role against the toxic effects of heavy metals by binding with them (Bhattacharya *et al.*, 1985) [33].

In the present study in fishes treated with 500µg/l the gill showed decrease in cadmium content. According to Kent, 1998 the liver and kidneys are involved in detoxification and removal of toxic substances circulating in the blood stream. Cadmium might also be transported into these organs from other tissues, including gills and muscle, for the purpose of subsequent elimination. Such transportation might lead to higher rates of accumulation in these two organs. The possibility of such detoxification/elimination-related mobilization of accumulated cadmium may be one reason for the intermittent reduction in the quantity of accumulated cadmium in gills, at various stages of exposure (Kumada *et al.*, 1980; Giles *et al.*, 1988 and Clinier *et al.*, 1997) [35, 36, 37].

Table 1: Physicochemical characteristics of tap water during the study period

Parameters	Mean ± SE
Temperature (°C)	27 ± 36.231
pH	8.4 ± 0.052
Dissolved oxygen (mg/L)	0.28 ± 0.10
Salinity (ppt)	0.946 ± 0.086
Alkalinity (mg/L)	49.5 ± 0.35

Table 2: Percentage (%) Mortality in *Labeo rohita* treated with different concentrations of Cadmium chloride.

S. No	No. of fishes	Toxicant concentration in µg/l	Mortality in Test Animals	% Mortality
			96Hrs	%
1	10	4000	0	0
2	10	4250	1	10
3	10	4500	2	20
4	10	4750	3	30
5	10	5000	5	50
6	10	5250	7	70

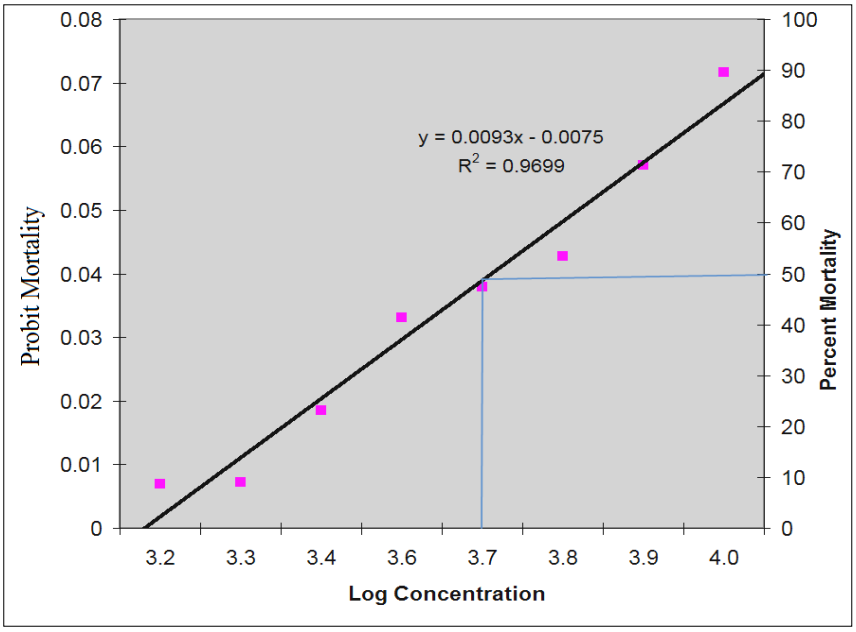

Fig 1: Regression graph showing LC50 for fishes treated with different concentrations of cadmium chloride.

Table 3: RBC, Haemoglobin and PCV content of *Labeo rohita* after exposure to cadmium chloride.

Concentrations	RBC in millions/cu mm		Haemoglobin in gm %		PCV in gm %	
	Mean	't' value	Mean	't' value	Mean	't' value
Control	0.02 ± 0.01	-	0.53 ± 0.09	-	0.33 ± 0.13	-
500µg/l	0.01 ± 0.00	1.059^{NS}	0.43 ± 0.08	2.388*	0.16 ± 0.05	3.767*
700 µg/l	0.02 ± 0.01	0	0.19 ± 0.07	8.487**	0.11 ± 0.03	5.112**
900 µg/l	0.02 ± 0.01	0	0.21 ± 0.07	7.988**	0.13 ± 0.05	4.472**

Values are expressed as mean ± SD

Comparison between Control vs different concentrations

** - Significant at 1% level; * - Significant 5% level NS–Not significant

Table 4: WBC, Platelet count and MCV of *Labeo rohita* after exposure to cadmium chloride.

Concentrations	WBC in No. of cells/cu mm		Platelet Count in No. of cells/cu mm		MCV in microns	
	Mean	't' value	Mean	't' value	Mean	't' value
Control	90.00 ± 46.71	-	75.60 ± 21.81	-	11.30 ± 4.14	-
500	93.50 ± 25.92	0.197^{NS}	66.50 ± 21.39	0.894^{NS}	13.70 ± 4.62	1.161^{NS}
700	86.40 ± 53.53	0.152^{NS}	47.10 ± 5.40	3.855^{**}	8.00 ± 1.94	2.166*
900	31.80 ± 10.39	3.649**	84.00 ± 2.67	1.148^{NS}	8.30 ± 4.32	1.504^{NS}

Values are expressed is mean ± SD
Comparison between Control vs different concentrations
** - Significant at 1% level; *-Significant 5% level NS–Not significant

Table 5: Accumulation of cadmium chloride in (ppm) in the organs of fish as shown by Graphic Furnace Atomic Absorption Spectrophotometer (GFAAS) after 30 days of exposure

Concentrations	Gill		Liver		Kidney	
	Mean	't' value	Mean	't' value	Mean	't' value
Control	0.75 ± 0.03	-	0.19 ± 0.02	-	0.58 ± 0.02	-
500 µg/l	0.47 ± 0.02	20.75**	0.44 ± 0.03	21.922**	0.53 ± 0.02	5.659**
700 µg/l	0.74 ± 0.02	0.331^{NS}	0.71 ± 0.02	54.117**	0.74 ± 0.03	14.852**
900 µg/l	0.95 ± 0.02	14.765**	0.91 ± 0.02	79.542**	0.88 ± 0.02	29.04**

Values are expressed as mean ± SD
Comparison between Control vs different concentration
** - Significant at 1% level; *-Significant 5% level NS–Not significant

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