



Studies on Metallic Salt Intoxication on Blood Parameters of Two Fishes, *Channa gachua* and *Channa reba*

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ABSTRACT

The values of haematocrit, haemoglobin and erythrocytes percentage of untreated *Channa gachua* in 24, 48, 72 and 96 hr of exposure were in the range of 42.38 to 42.79; 13.95 to 14.35; 3.14 to 3.52, and that of *Channa reba*, in the range of 42.63 to 43.75; 13.95 to 14.72 and 3.18 to 3.65 respectively. After treatment with two metallic salts i.e., CuSO_4 and $\text{K}_2\text{Cr}_2\text{O}_7$ in different concentrations at these exposures these parameters exhibited a of range of 40.12 to 41.2 and 40.25 to 42.25 (haematocrit); 14.12 to 14.84 and 13.89 to 14.25 (haemoglobin) and 3.06 to 3.26 and 3.05 to 3.22 (erythrocytes) in case of *C. gachua* while the values were 41.85 to 42.00 and 41.25 to 42.87 (haematocrit); 14.12 to 14.65 and 14.01 to 14.65 (haemoglobin) and 3.10 to 3.55 and 3.12 to 3.62 (erythrocytes) respectively in case of *C. reba*.

INTRODUCTION

It is very pertinent to focus our attention to the global problem of aqua pollution owing to various types of effluents and pesticides which reach to aquatic systems from several sources. These effluents are unknown to aquatic biota when they arrive in the aquatic systems. Some of these effluents have such ingredients which are nonbiodegradable causing immense effects on various physiological activities of the aquatic biota especially the fish and haematological arena is one of them. Piscine blood physiology has now become a hazardous problem for ichthyobiologists which requires immediate attention. Sariswa river, one of the most polluted rivers, has twin problems for both India and Nepal which needs haematological studies regularly for fish health and its production due to immense menace of aqua pollution. It is rather more relevant to study the various haematological parameters of the fishes because it is the indicator of fish health and the pathological status of tissues and organs.

One such hazardous effluent, which this river receives, is growing volume of various metallic salts in the water which are being expelled by a large number of sugar mills, paper mill, leather mill, soap factories, detergent factory, etc. The metallic salts are known to be highly toxic to fishes which become one of the immediate target of various pollutants as they are comparatively more susceptible to such pollutants than other aquatic vertebrates.

MATERIALS AND METHODS

Mature specimens of living and healthy *Channa gachua* commonly known as "Changa" and *Channa reba* commonly known as "Reba" of 36.5 ± 3.5 g weight of both the sexes were collected from Sariswa river and brought to the laboratory, where they were washed for few minutes in 0.1% aqueous potassium permanganate solution followed by several changes of freshwater to remove any dermal

infection. They were acclimatized to the laboratory conditions for one week before being used for various experiments. The fishes were kept at natural photoperiod and temperature.

During acclimation and experimental periods, the fishes were provided with "Fish-tone" (an artificial food), tubifex and chopped goat's liver on alternate days, two to three hours prior to change of water and during chronic exposures except bioassay test, during which fishes were not provided food 20 hours prior to start of the experiment to the end of the experiment (i.e., up to 96 hours).

At selected hours of exposure (i.e., 24, 48, 72 and 96 hrs) blood was collected from the fishes separately by severing the caudal peduncle and/or by direct heart puncture only in morning hours between 7:00 and 9:00 A.M. to avoid diurnal variations in blood parameters. Anaesthesia and anticoagulant were not used to the fish and blood respectively, but heparinized capillary tubes were used for determining haematocrit value by microhaematocrit centrifuge, in which blood filled capillaries were centrifuged for 15 minutes at 8000 rpm and packed cell volume was noted. Prior to collection of blood, blood films were prepared to study the changes in erythrocytes.

RESULTS AND DISCUSSION

Blood is a good bioindicator to determine the health of an organism. The percentage occurrence of various constituents of blood indicates the changes in the quality of the environment and, therefore, blood parameters are important in diagnosing the functional status of the animal exposed. The effect of treatment of both the heavy metals as well as in normal condition on the percent haematocrit, haemoglobin and erythrocytes in the blood of *C. gachua* and *C. reba* exposed for 24, 48 72 and 96 hrs are given in Tables 1 and 2. The result was summarised after 5 observations with standard error.

Under normal conditions, in case of *C. gachua*, the value of haematocrit percentage in 24, 48, 72 and 96 hrs of exposure was found to be in the range of 42.38 to 42.79. When this fish was treated with CuSO_4 in different concentrations at different exposure times, it was noticed to be in the range of 40.12 to 43.12 (Table 1), while the range of its percentage was found to be between 40.25 and 42.25 when it was treated with $\text{K}_2\text{Cr}_2\text{O}_7$ under similar exposures. The percent alteration was found to be in the range of 0.91 to 3.65 and 0.79 to 3.65 respectively (Figs. 1 and 2).

The haemoglobin percentage in case of the test fish *C. gachua* under normal condition was in the range of 13.95 to 14.35. When the fish were treated with CuSO_4 in the different concentrations at different exposures it was found to be between 14.12 and 14.84. The fish when treated with $\text{K}_2\text{Cr}_2\text{O}_7$ under similar conditions, it was found to be in the range of 13.89 to 14.25. The % alteration in this case was noticed to be in the range of 0.42 to 1.41 and 0.42 to 3.65 respectively (Figs. 1 and 2).

The percent number of erythrocytes in case of normal *C. gachua* at different exposures was in the range of 3.14 to 3.52. When the fish was treated with CuSO_4 in different concentrations at different exposures, it was in the range of 3.06 to 3.26. Its percent alteration was found to be in the range of 0.25 to 5.29 (Figs. 1 and 2).

In case of another test fish, *C. reba*, the haematocrit percentage in the normal condition in different exposures was between 42.03 and 43.75. When the fish was treated with CuSO_4 in different concentrations at different exposures, the range was found from 41.85 to 44.18 (Table 2). The percent alteration was noticed in the range of 1.62 to 3.58. Again, the same fish when treated with $\text{K}_2\text{Cr}_2\text{O}_7$ the range was found to be from 41.25 to 42.87. The percent alteration was from 1.10 to 3.82 (Figs. 3 and 4).

The haemoglobin percent was examined in the same fish under normal condition and at different

Table 1: Haematocrit, haemoglobin and total number of RBC in *C. gachua* exposed to selected concentration of pollutants at selected period of exposure (\pm standard error of five observations).

Toxicant	Concentration	Exposure (hr)	Haematocrit %	% Alteration	Haemoglobin (g/L)	% Alteration	Erythrocytes no. ($\text{mm}^3 \times 10^6$)	% Alteration
Test	0	24	42.59 \pm 1.24		13.95 \pm 0.36		3.52 \pm 0.18	
CuSO ₄	32.7		43.12 \pm 0.85	2.45	14.84 \pm 0.38	0.95	3.14 \pm 0.16	3.56
	21.8		41.85 \pm 1.12	0.91	14.25 \pm 0.34	0.82	3.16 \pm 0.14	0.25
K ₂ Cr ₂ O ₇	15.7		40.25 \pm 1.04	3.65	14.10 \pm 0.30	0.50	3.05 \pm 0.18	5.62
	10.5		42.25 \pm 1.25	1.89	14.21 \pm 0.38	1.10	3.15 \pm 0.18	0.89
Test	0	48	42.61 \pm 1.25		14.35 \pm 0.24		3.14 \pm 0.12	
CuSO ₄	32.7		40.12 \pm 0.95	3.62	14.12 \pm 0.24	1.41	3.12 \pm 0.40	4.56
	21.8		41.25 \pm 1.14	1.95	14.05 \pm 0.28	0.89	3.12 \pm 0.40	5.29
K ₂ Cr ₂ O ₇	15.7		41.25 \pm 1.12	1.62	14.12 \pm 0.60	3.65	3.24 \pm 0.16	0.82
	10.5		41.12 \pm 1.4	2.35	13.89 \pm 0.48	0.20	3.06 \pm 0.70	3.95
Test	0	72	42.79 \pm 1.12		13.96 \pm 0.22		3.22 \pm 0.16	
CuSO ₄	32.7		41.16 \pm 0.92	1.42	14.26 \pm 0.75	1.26	3.06 \pm 0.16	3.15
	21.8		41.95 \pm 1.14	1.95	14.25 \pm 0.82	0.42	3.05 \pm 0.16	4.72
K ₂ Cr ₂ O ₇	15.7		41.79 \pm 1.14	0.79	14.05 \pm 0.42	1.26	3.22 \pm 0.12	0.82
	10.5		41.25 \pm 1.25	2.95	14.25 \pm 0.75	1.25	3.08 \pm 0.10	1.42
Test	0	96	42.38 \pm 1.16		14.29 \pm 0.72		3.14 \pm 0.10	
CuSO ₄	32.7		41.24 \pm 1.32	1.89	14.12 \pm 0.60	0.75	3.15 \pm 0.14	4.89
	21.8		42.16 \pm 1.26	20.59	14.15 \pm 0.48	1.65	3.26 \pm 0.18	0.82
K ₂ Cr ₂ O ₇	15.7		41.36 \pm 0.94	0.92	14.22 \pm 0.68	3.35	3.18 \pm 0.18	0.60
	10.5		41.25 \pm 1.08	1.26	14.15 \pm 0.76	3.45	3.05 \pm 0.16	5.29

Table 2: Change in haematocrit, haemoglobin and total number of RBC in *C. reba* exposed to selected concentration of pollutants at selected period of exposure (\pm standard error of five observations).

Toxicant	Concentration	Exposure (hr)	Haematocrit %	% Alteration	Haemoglobin (g/L)	% Alteration	Erythrocytes no. ($\text{mm}^3 \times 10^6$)	% Alteration
Test	0	24	43.12 \pm 1.23		14.12 \pm 0.35		3.65 \pm 0.16	
CuSO ₄	32.7		44.18 \pm 0.89	3.05	41.26 \pm 0.36	0.98	3.55 \pm 0.18	2.65
	21.8		42.12 \pm 1.18	1.82	14.55 \pm 0.38	0.94	3.29 \pm 0.15	0.38
K ₂ Cr ₂ O ₇	15.7		41.25 \pm 1.18	3.82	14.26 \pm 0.34	0.65	3.12 \pm 0.14	4.89
	10.5		42.13 \pm 1.04	1.95	14.46 \pm 0.36	0.95	3.17 \pm 0.16	1.10
Test	0	48	42.63 \pm 1.18		14.72 \pm 0.38		3.18 \pm 0.18	
CuSO ₄	32.7		41.85 \pm 1.25	3.58	14.65 \pm 0.20	1.20	3.22 \pm 0.22	4.25
	21.8		41.85 \pm 0.95	1.72	14.15 \pm 0.26	1.11	3.25 \pm 0.35	6.12
K ₂ Cr ₂ O ₇	15.7		42.12 \pm 1.14	1.42	14.35 \pm 0.30	2.95	3.62 \pm 0.18	1.10
	10.5		42.18 \pm 1.12	2.45	14.01 \pm 0.60	0.12	3.12 \pm 0.16	4.12
Test	0	72	43.75 \pm 1.14		13.95 \pm 0.48		3.29 \pm 0.20	
CuSO ₄	32.7		42.12 \pm 1.12	1.62	14.12 \pm 0.22	1.35	3.10 \pm 0.18	3.85
	21.8		42.25 \pm 0.95	2.12	14.65 \pm 0.75	0.52	3.18 \pm 0.16	5.12
K ₂ Cr ₂ O ₇	15.7		42.25 \pm 1.15	1.12	14.65 \pm 0.82	0.35	3.42 \pm 0.18	0.95
	10.5		42.18 \pm 1.14	2.18	14.46 \pm 0.42	2.25	3.12 \pm 0.12	2.15
Test	0	96	42.79 \pm 1.18		14.38 \pm 0.75		3.29 \pm 0.12	
CuSO ₄	32.7		42.12 \pm 1.16	2.05	14.46 \pm 0.72	0.78	3.18 \pm 0.12	5.18
	21.8		43.15 \pm 1.32	2.78	14.29 \pm 0.60	2.12	3.19 \pm 0.18	0.98
K ₂ Cr ₂ O ₇	15.7		42.87 \pm 1.26	1.18	14.26 \pm 0.48	2.98	3.28 \pm 0.18	0.60
	10.5		42.18 \pm 1.26	1.10	14.26 \pm 0.68	2.15	3.12 \pm 0.12	0.12

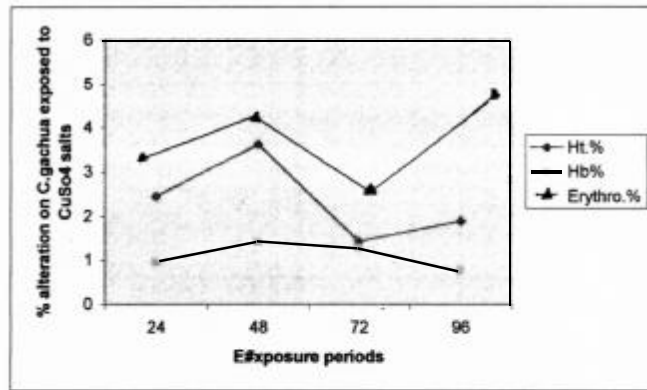


Fig. 1: Percent alteration on blood parameters of *C. gachua* exposed to CuSO_4 salt.

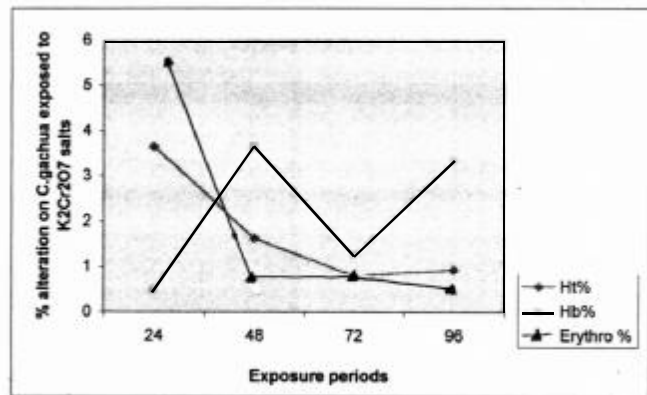


Fig. 2: Percent alteration of blood parameters of *C. gachua* exposed to $\text{K}_2\text{Cr}_2\text{O}_7$ salt.

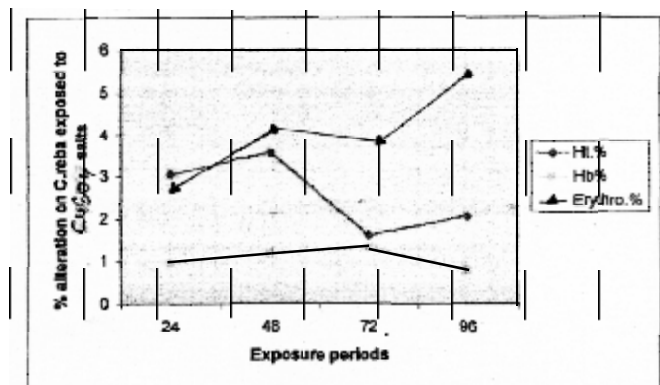


Fig. 3: Percent alteration on blood parameters of *C. reba* exposed to CuSO_4 salt.

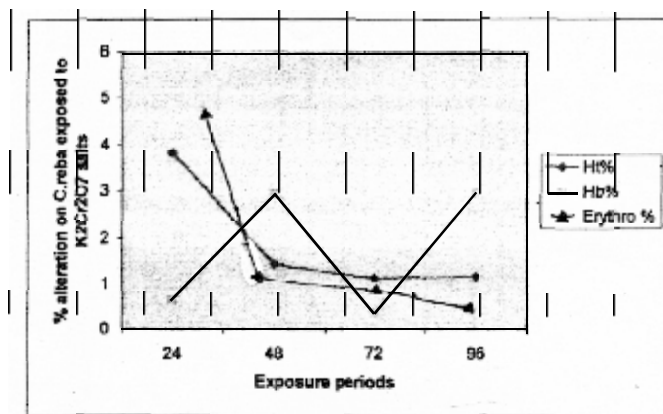


Fig. 4: Percent alteration on blood parameters of *C. reba* exposed to $K_2Cr_2O_7$ salt.

exposures. It ranged from 13.95 to 14.72. When the fish was treated with $CuSO_4$ under similar conditions, the range was noticed between 14.12 and 14.65. The range was between 14.01 and 14.65 when the test fish were treated with $K_2Cr_2O_7$ under the same exposures. The percent alteration was found from 0.52 to 2.12 and 0.35 to 3.45 respectively after treatment with these metallic salts (Figs. 3 and 4).

The total percentage of erythrocytes count in the normal state of fish *C. reba* was observed to be in the range of 3.18 to 3.65. After treatment with $CuSO_4$ and $K_2Cr_2O_7$ under different conditions it was in the range of 3.10 to 3.55 and 3.12 to 3.62 respectively. The % alteration exhibited from 0.38 to 5.12 and 0.60 to 6.12 respectively in these salts (Figs. 3 and 4).

The gradual increase in the haematocrit value in both the test fishes to various concentrations of the two heavy metals is concentration dependent and the depression was more in higher concentration and at longer period of exposures. Dalela et al. (1981) and Raizada et al. (1984) while working on the effect of pollutants on *M. vittatus* and *C. mrigala* observed the similar results, which are in conformity with the present findings.

However, Das & Mukharjee (2000) found that the haematocrit level of *L. rohita* increased haematocrit value due to cellular swelling, but Dhanapakian & Ramaswamy (2001) reported significant depression in haematocrit level while working on *C. carpio* which were treated with Cu and Zn mixture. Subba Rao & Behra (1973) did not find any consistent difference in the haematocrit level and presumed that increase in metabolic rate might be caused by a generalized non-specific stress which is also in quite conformity with the findings of Srivastava & Agarwal (1979) in *C. fassiatatus* and Siddiqui & Sidhiqui (1973) in *C. batrachus*.

In the present findings, the haemoglobin concentration exhibited an inconsistent trend in both the test fish exposed to various concentrations of the metallic salts. Kumari (1990) reported similar findings while working on *H. fossilis* when treated with the lethal and sublethal concentrations of zinc. These observations indicate that the exposure to different concentrations of these heavy metals to the fish bring anaemic condition as suggested by Larson et al. (1972) and Hilmy et al. (1980).

The erythrocytes level in the present study was noticed to be swelled *in vitro* to a remarkable extent and also due to the adverse disturbance by depressing anticoagulative mechanism. The

surface area of erythrocytes gradually increased in both the fishes exposed to these metallic salts depending upon the concentration of pollutants as well as exposure period, but comparatively higher level was noticed in case of treatment with $K_2Cr_2O_7$. This observations is in conformity with the findings of Mishra & Srivastava (1979) in *C. fassiatatus*, Das & Mukherjee (2000) in *L. rohita* fingerlings, and Hymavathi & Rao (2000) in *C. punctatus*. Yamashita (1968) reported that various heavy metals adversely affect fibrinolysis by depressing anticoagulative mechanism. The disturbance of coagulative mechanism may result in the alteration of coagulation and also in the abnormal fibrinolytic activity of the plasma as also reported by Haniffa et al. (1980) in *H. fossilis*.

REFERENCES

- Dalela, R.C., Rani, S., Kumar, V. and Verma, S.R. 1981. *In vivo* haematological alternations in a freshwater teleost, *Mystus vittatus* following sub-acute exposure to pesticides and their combinations. *J. Environ. Biol.*, 2(2): 79-86.
- Das, B.K. and Mukherjee, S.C. 2000. Sub-lethal effects of quinolophos on selected blood parameters of *Labeo rohita* fingerlings. *Assam Fisheries Science T.*, 3: 225-235.
- Dhanapakian, P. and Ramaswamy, V.K. 2001. Toxic effects of copper and zinc mixtures on some haematological biochemical parameters in common carp, *Cyprinus carpio* (Linn.). *J. Environ Biol.*, 22(2): 105-111.
- Haniffa, M.A., Murugeson, A.G. and Porchelvi, M. 1980. Haematological effects of distillery and paper mill effluents on *Heteropneustes fossilis* (Block) and *Saotherodon mossambicus* (Peters). *Proc. Indian Acad. Sci., (Anim. Sci.)* 95: 155-61.
- Hilmy, A.M., Shabana, M.B. and Said, M.M. 1980. Haematological responses to mercury toxicity in the marine teleost, *Aphanius dispar* (Rupp.) *Comp. Biochem. Physiol.*, 67: 147-158.
- Hymavathi, V. and Rao, J.M. 2000. Effects of sublethal concentration of lead on the haematology and biochemical constituents of *Channa punctatus*. *Bull. Pure and Applied Sci.*, 199(1): 1-5.
- Kumari, R. 1990. Effects of some toxic solutions on the respiration of an air breathing catfish, *Heteropneustes fossilis* (Block). Ph.D. Thesis, Bihar Univ., Muzaffarpur, India.
- Larson, et al. 1973. Metabolic effects of epinephrine and norepinephrine in the cell *Anguilla anguilla* L. *Gen. Comp. Endocrinol.*, 20:155-167.
- Mishra, S. and Srivastava, A.K. 1979. Haematology as an index of sublethal toxicity of zinc in a freshwater teleost. *Bull. Environ. Contam. Toxicol.*, 22(4-5): 695-698.
- Raizada, M.N., Jain, K.K. and Razada, K.S. 1984. A study of the biochemical constituents of blood of a freshwater teleost, *Cirrhinus mrigala* (Ham.) *Comp. Physiol. Ecol.*, 2: 146-148.
- Siddiqui, A.O. and Siddiqui, N. 1973. Effect of asphyxiation on the chemical constituents of blood plasma of *Clarias batrachus*. *Ind. J. Zool.*, 1(1): 47-48.
- Srivastava, A.K. and Agrawal, S.J. 1979. Haematological anomalies in a freshwater teleost, *Colisa fasciatus* on acute exposure to cobalt. *Acta Pharmacol. Et. Toxicol.*, 44: 197-199.
- Subha Rao, M.V. and Behera, M.K. 1973. A haematological study on the freshwater teleost. *Catla catla*. *Curr. Sci.*, 42: 612-613.
- Yamashita, H. 1968. Haematological studies of a species of rock fish. II. Changes of moisture content of blood specific gravity serum protein, haematocrit value and urea nitrogen level of serum in the specimen affected by ulcer. *Bull. Jap. Soc. Sci. Fish.* 33(1): 995-1001.