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Effect of Cadmium on Blood of Tilapia, *Oreochromis mossambicus* (Peters), During Prolonged Exposure

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Cadmium is recognized as one of the most hazardous environmental pollutants and is toxic to many living organisms. Experimental and environmental exposure to cadmium has been reported to cause disease in humans and other mammals (Friberg et al. 1986). Recent reviews on cadmium have reported on acute and subacute effects on fish, mechanisms of toxicity, the role of toxicity modifying factors and various sublethal effects, i.e. hematological and biochemical disorders (Sprague 1987). However, very little information is available on the subacute effects of cadmium on fish blood exposed in hard water. The present investigation, therefore, was carried out to observe the effects of cadmium on the blood of tilapia (Oreochromis mossambicus) in hard and alkaline water to determine whether cadmium causes changes in blood of fish kept in hard water in the same way it causes changes in blood of fish kept in soft water.

MATERIALS AND METHODS

Fish used in the experiments were collected from a local lake and were acclimatized in a PVC tank (238 cm x 82 cm x 61 cm; 1100 L water capacity) and in glass aquaria (92 cm x 38 cm x 37 cm; 120 L water capacity) for at least 30 days. Healthy and active fish were selected for the study having a mean weight of 103.59±3.66 gm and mean length of 19.9±0.25 cm. These fish were divided into four main groups and further into six subgroups having five fish in each. These groups were exposed to 0, 0.1, 1.0 and 10.0 ppm Cd for a period of 1, 3, 7, 14, 21 and 45 days. The nominal concentrations of cadmium were achieved by adding a suitable quantity of stock solution (600 ppm) to 60 L of water in the glass aquaria. Prior to the experiment and during experiment the fish were fed once daily with dry fish powder and on every alternate day with tubificid worms (Tubifex sp) ad libitum. The test medium was changed every day. The experiments were performed under natural light and ambient temperature. The quality of the test water (pH 7.97±0.013;temperature 29.77±0.049°C, alkalinity 333.56±

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1.538 ppm; total hardness177.55±2.94 ppm and dissolved oxygen 8.51±0.096 ppm) was studied as per standard methods (APHA 1985). Cadmium in each experimental aquarium was determined without any prior treatment to the water samples on an Atomic Absorption Spectrophotometer (Perkin-Elmer Model 373). The cadmium levels in liver and kidney were also determined after proper digestion (Reitz et al. 1960) using reference standard from pure cadmium metal prepared in 1 M. HNO_3.

Feeding was stopped 24 hr prior to each sampling interval. Five fish from each experimental and control aquarium were removed and anaesthetized with MS 222 (3-aminobenzoic acid ethyl ester) at each fixed exposure interval. Blood samples were collected in heparinized vials with a 1 ml disposable syringe equipped with a 22 guage hypodermic needle by puncturing the ventral aorta. Hemoglobin, hematocrit, red blood cell counts, red blood cell indices and differential white blood cell counts were measured (Blaxhall and Daisely 1973). The data were subjected to Student's 't' test for correlation and regression equation.

RESULTS AND DISCUSSION

The results of the present investigation showed various anomalies in the blood of tilapia, Oreochromis mossambicus, during prolonged exposure to cadmium (Tables 1 & 2).

Hemoglobin decreased significantly from the controls in fish exposed to cadmium for 3 days to 0.1 and 10.0 ppm, 7 days to 10.0 ppm, 14 days to 0.1 ppm and 45 days to 1.0 ppm. Hematocrits decreased significantly at 1 day to all three test concentrations, and at 3, 14 and 45 days to 10.0 ppm. Red blood cell counts also decreased compared to controls at 1 day to 0.1 ppm, 14 days to 1.0 and 10.0 ppm, 21 days to all cadmium concentrations and 45 days to 1.0 ppm.

Red blood cell indices such as MCV, MCH and MCHC showed certain changes in cadmium-exposed fish. Mean cell volume (MCV) increased significantly at 7 and 14 days to 10.0 ppm as well as 21 and 45 days to 1.0 ppm. The rise in MCH above the controls was significant at 3 days to 1.0 ppm and 10.0 ppm, 7 days to 1.0 ppm, 14 days to 1.0 and 10.0 ppm, 21 days to all concentrations of cadmium and 45 days to 0.1 and 10.0 ppm. However, MCHC was reduced significantly from the controls at 3 days to 0.1 ppm; 7 days to 10.0 ppm; 14 days to 0.1 ppm and 45 days to 1.0 ppm. The reduction in these variables was 70%, 63% and 56% of the control values.

Hemoglobin and hematocrit decreased in eels (Anguilla anguilla) and perch (Pleuronectes flesus) during short-term as well as long-term exposures to cadmium in sea water (Larsson 1975). Nine weeks exposure to sublethal levels of cadmium in brackish water reduced Hb, Hct and RBC counts in flounder, Pleuronectes flesus (Johansson-Sjobeck and Larsson 1978). The prolonged exposure to sublethal level of cadmium in soft water for 90 days caused reduction in Hb, Hct and RBC counts in fish Puntius conchonius (Gill and Pant

Table 1. Changes in hemoglobin, hematocrit and RBC count in tilapia, *O. mossambicus*, during prolonged exposure to cadmium

	Cadmium (Cd^{+2}) concentration (mg/l)	Exposure period (days)					
		1	3	7	14	21	45
Hb (g/dl)	0	5.88±072	5.82±0.33	6.36±0.18	6.48±0.32	6.68±0.37	6.77±0.37
	0.1	4.68±0.44	4.71±0.11*	5.60±0.37	4.06±0.27**	5.71±0.30	6.49±0.87
	1.0	4.88±0.30	5.01±0.53	5.87±0.69	5.90±0.72	6.18±0.18	4.13±0.13**
	10.0	5.64±0.41	4.81±0.36	4.90±0.23**	4.32±0.15**	4.78±0.36	6.08±0.86
Ht (%)	0	26.60±0.98	25.40±1.17	26.40±0.60	24.40±1.50	23.80±1.24	24.60±1.40
	0.1	19.60±1.33**	24.40±1.08	23.80±2.11	23.20±2.97	22.40±0.24	25.00±1.26
	1.0	22.60±0.68**	22.40±2.01	26.20±2.85	19.40±1.81	19.20±1.71	21.40±0.68
	10.0	19.80±1.07	19.60±2.01*	25.80±2.29	17.00±1.30*	22.40±1.36	19.20±1.36*
RBC x $10^6/mm^3$	0	1.31±0.04	1.26±0.03	1.20±0.02	1.24±0.06	1.21±0.06	1.26±0.05
	0.1	1.01±0.07 **	1.24±0.04	1.27±0.06	1.25±0.07	0.68±0.04**	1.15±0.15
	1.0	1.24±0.02	1.13±0.12	1.07±0.05	0.56±0.06**	0.77±0.03	0.88±0.03**
	10.0	1.25±0.07	1.13±0.06	1.07±0.07	0.96±0.03 **	0.88±0.03**	1.22±0.13
MCV (um^3/cell)	0	189.20±1.46	197.12±3.46	198.82±3.17	194.36±1.21	202.63±10.89	194.36±7.21
	0.1	194.93±3.18	200.56±4.22	185.72±10.32	182.34±15.34	208.57±10.19	180.82±5.00
	1.0	181.55±3.43	190.89±10.56	244.58±22.47	213.40±16.95	270.19±18.13*	239.67±14.01
	10.0	194.34±4.91	193.74±11.14	242.89±9.20 **	217.48±10.01*	254.15±13.67	163.18±17.64
MCH (pg/cell)	0	42.63±4.59	42.17±3.31	46.87±5.08	44.11±5.36	40.19±1.05	42.88±2.95
	0.1	46.22±1.83	39.19±0.80	43.87±1.53	34.85±1.51	67.54±3.92**	64.50±6.76**
	1.0	40.20±2.60	47.18±2.11**	65.66±3.47**	108.87±14.21**	67.00±2.68 **	46.65±2.68
	10.0	41.96±2.92	46.87±0.50**	46.30±1.66	52.49±3.68 **	53.72±5.10**	49.26±2.61 **
MCHC (g/100 ml)	0	26.10±2.72	24.44±1.41	25.45±0.62	24.27±1.31	24.07±1.87	26.08±1.21
	0.1	23.79±1.29	19.56±0.46*	23.81±1.05	19.46±1.16 *	23.99±0.81	26.79±0.59
	1.0	22.38±1.14	22.35±0.97	21.23±2.15	24.27±1.31	24.79±0.88	19.33±0.21**
	10.0	21.10±1.17	21.92±1.10	19.57±1.27**	23.11±1.67	24.58±1.78	31.81±4.43

N = 5   * $p < 0.01$ ** $p < 0.01$

Table 2. Differential white blood cells count in *O.mossambicus* during prolonged exposure to cadmium

	Cadmium (Cd^{+2}) concentration (mg/l)	Exposure period (days)					
		1	3	7	14	21	45
Thrombocyte	0	35.20±1.53	35.60±1.78	37.80±2.20	39.20±0.58	39.60±2.54	36.00±1.79
	0.1	43.60±1.21**	44.80±1.48 **	43.60±1.12*	40.80±1.46	39.00±2.60	40.40±2.62
	1.0	43.60±5.13	35.20±4.03	35.00±3.55	23.40±5.30	41.40±5.30	47.20±3.61
	10.0	32.00±3.03	29.60±2.40	30.80±6.90	37.00±2.66	35.40±1.16	35.40±1.49
Small lymphocyte	0	50.60±1.07	50.20±2.26	48.80±1.69	47.20±2.13	50.60±2.18	51.20±1.32
	0.1	46.00±1.48**	47.60±0.68	47.80±1.16	48.60±1.86	51.80±0.97	50.40±2.69
	1.0	36.80±2.91**	49.40±4.94	47.60±1.54	51.80±3.65	37.00±4.49*	38.00±3.16*
	10.0	48.60±2.42	48.20±2.63	53.20±6.59	37.40±2.85	48.40±1.32	44.40±1.36**
Large lymphocyte	0	2.80±0.86	3.00±0.55	2.40±0.81	2.80±1.16	2.40±0.71	2.80±0.37
	0.1	1.20±0.58	2.40±0.40	0.80±0.58	2.60±0.51	3.00±0.54	3.80±0.70
	1.0	5.20±2.40	4.40±0.87	3.00±0.97	3.80±0.24	2.60±0.40	4.80±0.44 **
	10.0	3.60±0.31	3.40±0.24	3.80±0.91	5.60±0.96*	3.60±1.51	3.00±0.30
Neutrophil	0	8.60±0.24	8.20±0.73	8.20±0.86	8.60±0.93	7.00±1.07	7.20±0.58
	0.1	7.60±0.40	6.20±0.58	6.40±0.51	6.80±0.66	5.20±0.66	5.80±0.58
	1.0	11.40±1.47	8.60±0.98	11.60±1.96	12.60±1.21*	16.60±1.65**	8.60±0.93
	10.0	13.60±1.16**	16.80±0.80**	6.60±0.67	11.00±1.04**	10.80±0.97*	13.60±0.81**
Monocyte	0	2.80±0.80	3.00±0.83	2.80±1.07	2.20±0.20	2.40±0.49	2.80±0.58
	0.1	1.60±0.33	1.00±0.31 *	1.40±0.51	1.20±0.37*	1.00±0.32 *	1.60±0.14
	1.0	2.40±0.80	2.40±0.50	3.40±0.25	3.40±0.58	2.40±0.60	2.00±0.40
	10.0	2.20±0.31	2.00±0.31	2.60±0.68	2.00±0.68*	1.80±0.37	1.60±0.51

N = 5 * $p < 0.01$ ** $p < 0.01$

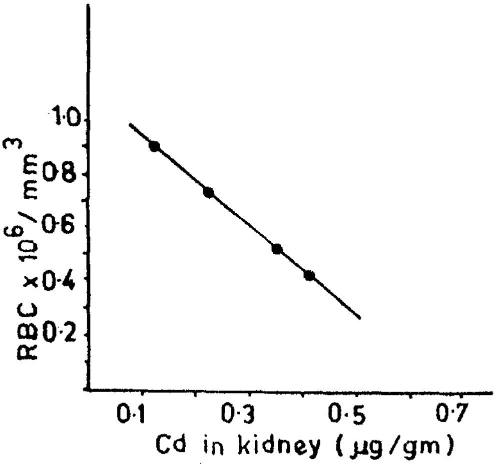

Figure 1. Correlation between cadmium accumulation in kidney and red blood cells (RBC) in fish exposed to 0.1 ppm cadmium.

RBC Count = 1.114-1.650 (Cd kidney)
r = 0.473 (N=18) $p < 0.05$
N = Number of samples.

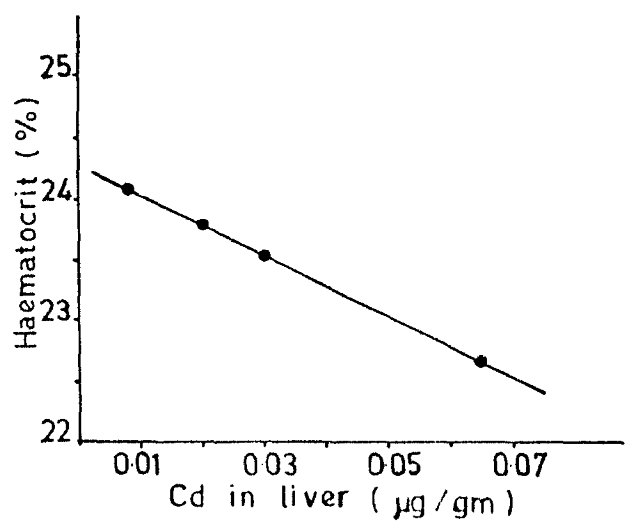

Figure 2. Correlation between cadmium accumulation in liver and hematocrit (Hct) in fish exposed to 10.0 ppm cadmium.

Hct = 24.294-25.257 (Cd liver)
r = 0.489 (N=18) $p < 0.05$
N = Number of samples.

1985). These reports revealed that the high level of cadmium in test water causes hematological changes much earlier than the lower ones. However, in contrast to the above mentioned result, Smith et al. (1976) and Calabrese et al. (1975) did not observe any statistically significant changes in these three variables in catfish (Ictaluras punctatus) and flounder (Pseudopleuronectes americanus), respectively, when exposed to cadmium in tap water and sea water. The present study differs little from the above mentioned report and indicates the reduction in Hb, Hct and RBC at many exposure intervals.

Red blood cell indices MCV, MCH and MCHC exhibited alteration during prolonged exposure to cadmium. The increase in MCV and MCH was 133% and 247% of the control values, while MCHC did not show much change. Increased MCV and MCH were observed in rainbow trout (Salmo gairdneri) and in Puntius conchonius during 30 days exposure to sublethal level of lead and cadmium (Johanson-Sjobeck and Larsson 1979; Gill and Pant 1985), respectively. Thus anemia developed by tilapia in this study could be regarded as macrocytic anemia.

The probable mechanism for developing anemia in tilapia could be due to the loss of erythrocytes as compensatory erythropoiesis could not be observed, which was reflected in the absence of immature erythrocytes in the peripheral blood. A negative correlation between cadmium accumulation in kidney and liver with lower number of red blood cells and hematocrit values was found in this investigation (Figs. 1 & 2). A similar negative correlation was observed between selenium accumulation in hepatopancrease and Hct in redear sunfish (Lepomis microlophus) by Sorensen and Bauer (1983). Thus a reasonable assumption could be made that in this study cadmium accumulation in internal organs such as kidney and liver has a profound effect on hematology of fish.

The proportion in differential white blood cell counts was altered in exposed fish. Thrombocytes increased over the controls significantly at 3 and 7 days to 0.1 ppm. Small lymphocyte counts decreased significantly at 1 day to 0.1 and 1.0 ppm, 21 days to 1.0 pm and 45 days to 1.0 and 10.0 ppm. The large lymphocyte population increased insignificantly at the two lower concentrations in the initial phase of study. There was a significant difference at 14 days to 10.0 ppm and 45 days to 1.0 ppm. The neutrophil number increased significantly at 1 and 3 days exposure to 10.0 ppm cadmium, 14 and 21 days to 1.0 and 10.0 ppm and 45 days to 10.0 ppm. Monocytes showed a reduction in number at 3 days to 0.1 ppm, 14 days to 0.1 and 10.0 ppm and 21 days to 0.1 ppm.

Very few reports are available on the study of differential white blood cell counts in fish which are affected upon exposure to chemical pollutants (Johansson-Sjobeck and Larsson 1978; Lowe-Jinde and Niimi 1986). The present investigation reveals a conspicuous fluctuation in the proportion of different white blood cells under experimental cadmium exposure. Cadmium produced thrombocytosis, neutrophilia and cytological shift in lymphocytes during prolonged exposure. Thrombocytosis was reported in fish (Colisa faciatus) during its exposure to cadmium (Srivastava and Mishra 1979). The cytological shift observed in this study was similar to the shift observed between small and large lymphocytes in cadmium-treated rats and mice (Oshawa and Kawai 1981). The responses of different white blood cells in this study might be secondary responses and were expected partly to be mediated through the increase pituitary inter-renal activity as reported by Donaldson (1981) during stress condition of the fish.

Table 3. Cadmium concentration (ppm) in test medium.

Nominal concentration		Measured concentration
0.1	Mean±SD	0.0783±0.0078
	Range	(0.019-0.130)
1.0	Mean±SD	0.7250±0.0548
	Range	(0.250-1.350)
10.0	Mean±SD	6.4635±0.4036
	Range	(2.100-9.850)

The present study observed macrocytic normochromic anemia with thrombocytosis, neutrophilia and cytological shift between large and small lymphocytes in fish during prolonged exposure to cadmium in alkaline and hard water. However, this study fails to observe any specific trend in hematological parameters as observed by other investigators (Larsson 1975; Johansson-Sjobeck and Larsson 1978; Gill and Pant 1985). The probable reason for non linear alteration in three hematological parameters could be due to the large variation in availability of cadmium ion in the test medium (Table 3), and also because of difference in sensitivity of test species used in the study. The study thus proves that cadmium, even in extremely alkaline and hard water, produces toxic effects in fish blood.

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REFERENCES

Agrawal VP, Goel KA, Sharma SD (1985) Hematological characteristics of Clarias batrachus under metallic stress of hexavalent chromium. Indian J Fish 32: 272-278

APHA (1985) Standard methods for the examination of water and waste water. American Public Health Association, Washington

Blaxhall PC, Daisely KW (1973) Routine hematological methods for use with fish blood. J Fish Biol 5: 771-781

Calabrese A, Thurberg FP, Dawson A, Wenzloff DR (1975) Sublethal physiological stress induced by cadmium and mercury in the winter flounder (Pseudopleuronectes americanus). In: Koemann JH, Strik JJTWA (ed) Sublethal Effects of Toxic Chemicals on Aquatic Animals. Elsvier Scientific Publishing Company, Amsterdam, pp 15-21

Donaldson EM (1981) The pituitary-interrenal axis as an indicator of stress in fish. In: Pickering AD (ed) Stress and Fish. Academic Press, New York, pp 11-47

Friberg LT, Piscator M, Nordberg G, Kjellstrom T (1986) Handbook on the toxicology of metal. Vol.II, Amsterdam

Gill TS, Pant JC (1985) Erythrocytic and leucocytic responses to cadmium poisoning in fresh water fish, Puntius conchonius. Environ Res 36: 327-337

Johansson-Sjobeck ML, Larsson A (1978) The effect of cadmium on the haematology and on the activity of delta-amino levulinic acid dehydratase (ALA-D) in blood and haematopoietic tissues of the flounder (Pleuronectes americanus). Environ Res 17:191-204

Johansson-Sjobeck ML, Larsson A (1979) Effect of inorganic lead on delta-amino levulinic acid dehydratase activity and hematological variables in rainbow trout (Salmo gairdneri). Arch Environ Contam Toxicol 8:419-431

Larsson A (1975) Some biochemical effects of cadmium on fish. In: Koemann JH, Strik JJTWA (ed) Sublethal Effects of Toxic Chemicals on Aquatic Animals. Elsevier Scientific Publishing Company, Amsterdam pp 3-13

Lowe-Jinde L, Niimi AJ (1986) Hematological characteristics of rainbow trout (Salmo gairdneri-Richardson) in response to cadmium exposure. Bull Environ Contam Toxicol 37: 375-387

Oshawa M, Kawai K (1981) Cytological shift in lymphocytes induced by cadmium in mice and rats. Environ Res 24:192-200

Reitz LL, Smith WH, Plumlee MP (1960) A simple wet oxidation procedure for biological materials. Anal Chem 32: 1728

Smith BP, Hejtmanick E, Camp BJ (1976) Acute effect of cadmium on catfish (Ictaluras punctatus). Bull Environ Contam Toxicol 15:271-277

Sorensen EMB, Bauer TL (1983) Haematological dyscrasia in teleosts chronically exposed to selenium laden effluent. Arch Environ Contam Toxicol 12:133-141

Sprague JB (1987) Effect of cadmium on fresh water fish. In: Niriagu JO, Sprague JB (ed) Cadmium in the Aquatic Environment. John Wiley and Sons, Inc. pp 139-169

Srivastava AK, Mishra G (1979) Blood dyscrasia in a teleost fish (Colisa faciatus) associated with cadmium poisoning. J Comp Pathol 89: 609-613

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