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Effect of Methyl Mercury Pollution in water on the Peripheral Blood of *Channa Punctatus* (Bloch)

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ABSTRACT: An experiment for a period of 60 days was carried out to evaluate the effect of organic mercury (mmc) pollution on *Channa punctatus*. Fishes were exposed to 0.004, 0.008, 0.013 and 0.017 ppm methyl mercury chloride for a period of 45 days followed by 15 days recovery study. Haematological effects on erythrocytic counts, haemoglobin, haematocrit, MCV, MCH, MCHC, and total leucocytes counts were studied. Haematological studies help in assessing physiological stress. Increase in RBC, leucocytes number, haemoglobin and haematocrit values were recorded. Leucocytosis mainly contribute due to thrombocytosis and characteristic neutropenia which were even more significant as indices of stress. Varying degrees of recovery signs could be observed within fortnight when the fishes were returned to mercury free water after an initial exposure of 45 days.

KEYWORDS: methylmercury chloride (mmc), Haemoglobin (Hb), Haematocrit (Hct), Red blood cell (RBC).

I. INTRODUCTION

A review of the available literature reveals that most of the experiments on water pollution are concerned with short term experiments (Mc Gear et al; 2000, Chandra et al; 2001, Idriss et al; 2015). The haematological parameters are used as an index to detect the stress conditions of fishes (Suvetha et al; 2010). The effect on growth and swimming behaviour in rainbow trout observed due to Zinc and Cadmium pollution (Sunita Rani et al; 2015) as observed in mercury also. The number of leucocytes was increased in the presence of methyl mercury (Robert S, 2010). The impact of mercury, lead and tributyltin chloride was studied on haematological parameters in neotropical fish (Oliveira C.A, 2006). Toxicity of heavy metal like Cadmium was observed on haematological parameters (Nussey G et al; 1995, Adlhikari S et al; 2004, Nasser et al; 2015) The effects on behaviour and haemoglobin contents were studied in marine teleost (Hilmy et al; 1980, Panigrahi et al; 1987, Gill et al; 1987).

In the present investigation general behaviour, haematological parameters as RBC, Hb, Hct, total leucocyte counts have been studied for long term experiment with recovery treatment.

II. EXPERIMENTAL PROCEDURES

Four Hundreds fishes (*Channa punctatus*) belonging to 1+ age group (av. weight 20.50 gm av. Length 123.0 mm) were selected from a population in a pond and acclimatised for fifteen days on pelleted diet in plastic pools (91 cm diameter and 91 cm length) in open air. They were fed at the rate of 3% body weight. After acclimatisation the fishes were weighed and arranged into five groups of 80 each.

Experiments were designed at sub lethal concentration of 1.12%, 2.9%, 4.6% and 6.2% of 48 hours LC 50 value (0.268 mg/l) for methyl mercury.

Group I Control (water free from mmc)

Group II 0.004 mg/l

Group III 0.008 mg/l

Group IV 0.013 mg/l

Group V 0.017 mg/l

The physico-chemical characteristics of water during experimental period were

Temperature	Total Alkalinity	Total Hardness	DO2	pH
24.5-28.0C	143-148mg/l	191-197mg/l	6.5-6.8mg/l	7.3-7.5

The fishes were exposed to pollutant water for 45 days. They were then transferred to normal water free from mercury (as in control) for 15 days .Day to day record of changes in general behaviour, feeding pattern , morphological changes, mortality etc. were maintained. Haematological changes were also studied including RBC, haemoglobin, haematocrit and absolute indices (MCH,MCV, MCHC)TLC and DLC.

III.RESULTS

A. General and morphological changes:

Fishes belonging to experimental groups G3, G4, G5 showed avoidance response evidenced by their disturbed swimming when they were transferred to test concentrations. These were accompanied by increased opercular movement. After 48 hours situation was almost normalised. In G4 and G5 disturbed swimming continued for few days after which fishes were seen to lethargic and settled down at bottom of pool. All treated groups showed varying degree of recovery when transferred to mercury free water after 45 days. Copious mucous secretion could be seen in G2 to G5 for first 15 days and then changes in peripheral blood.

B.RBC and Related values:

Observation on RBC, haemoglobin, haematocrit and related values of different time intervals and at different exposure levels are graphically presented. The figure(1.1) shows that methyl mercury pollution in water induced statistically significant increase in RBC, haemoglobin and haematocrit values. These changes in all were time and concentration dependent. Thus after 45days there was an increase of about 0.51 million RBC/ml of blood in fishes exposed to highest level0.017 ppm (G5)tested in experiment while there was increase of 0.02 million RBC/ml of blood at lowest level i.e. 0.004 ppm (G2).Haemoglobin and haematocrit showed similar trend. Thus after 45 days in G5, there was an increase of nearly 25% in haemoglobin content whereas it was about 12% in G2. During the recovery period of 15 days trend was reversal and reduction in RBC, haemoglobin and haematocrit values could be seen. Thus in G5 there was reduction of 0.23 million RBC/ml of blood while in G4, G3 and G2 reduction was 0.18,0.17 and 0.11 million RBC/ml respectively.

The Key data of significance at selected time intervals is shown in following table:

Days	Exposer (ppm)	Level	% Increase (RBC)	% increase (Hb)	% Increase Hct
15	0.004		4.73	7.82	8.48
	0.008		5.99	1	1
				12.17	11.33
	0.013		1	2	2
			8.20	14.72	14.70
45	0.017		2	2	3
			11.04	19.13	17.59
	0.004		6.98	1	2
				11.79	13.51
45	0.008		1	2	2
			8.57	17.90	15.48
	0.013		2	3	3



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		12.38	21.40	17.14
	0.017	3	3	3
		15.55	24.02	21.43
60(R)	0.004	3.22	8.69	2
				10.84
	0.008	6.75	1	13.72
			11.30	
	0.013	2	2	3
		8.39	16.17	15.40
	0.17	2	2	3
		10.00	18.43	18.04

1=P<0.05,2=P<0.01,3=P<0.001,(R)=Recovery

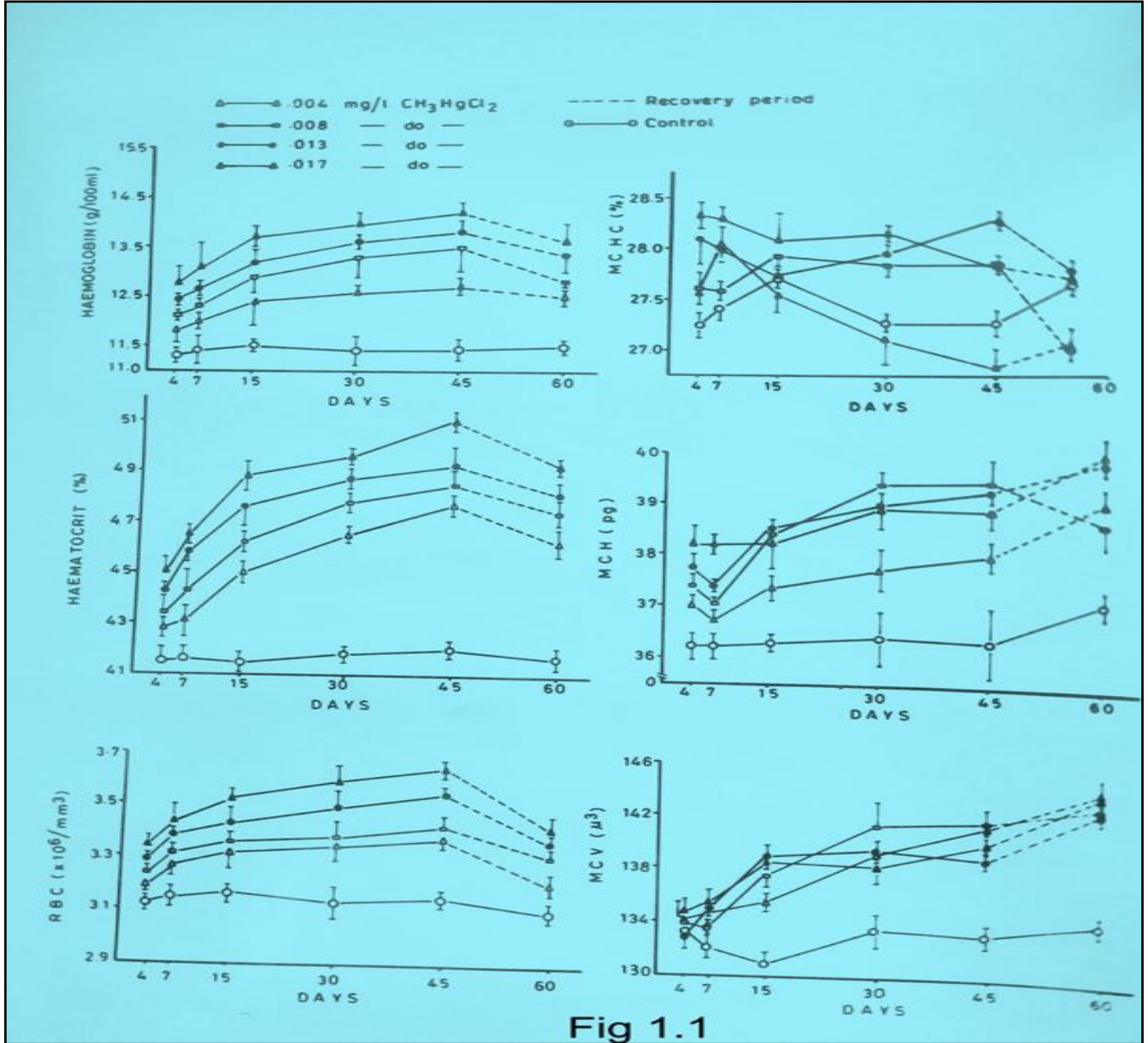


Fig 1.1

Table showing % of MCV, MCH, MCHC:

Days	Exposure Level ppm	Percentage increase (+) or decrease (-)		
		MCV	MCH	MCHC
15	0.004	+3.52	+2.95	+0.90



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		1	2	
	0.008	+5.03	+5.84	+0.75
		1	2	
	0.013	+6.0	+6.09	+0.43
		1	2	
	0.017	+5.82	+5.37	+1.65
45		1	1	
	0.004	+6.50	+4.50	-1.57
		1	3	
	0.008	+6.16	+8.61	+2.09
		1	3	
	0.013	+4.23	+8.03	+3.99
		1	2	
	0.017	+5.07	+7.07	+2.12
60 (R)		2	2	
	0.004	+7.37	+4.20	-2.02
		2		
	0.008	+6.50	+3.50	-2.00
		1	3	
	0.013	+3.49	+7.63	+0.58
		2		
	0.017	+7.31	+7.90	+0.26

1=P<0.05, 2=P<0.01, 3=P<0.001, (R) Recovery

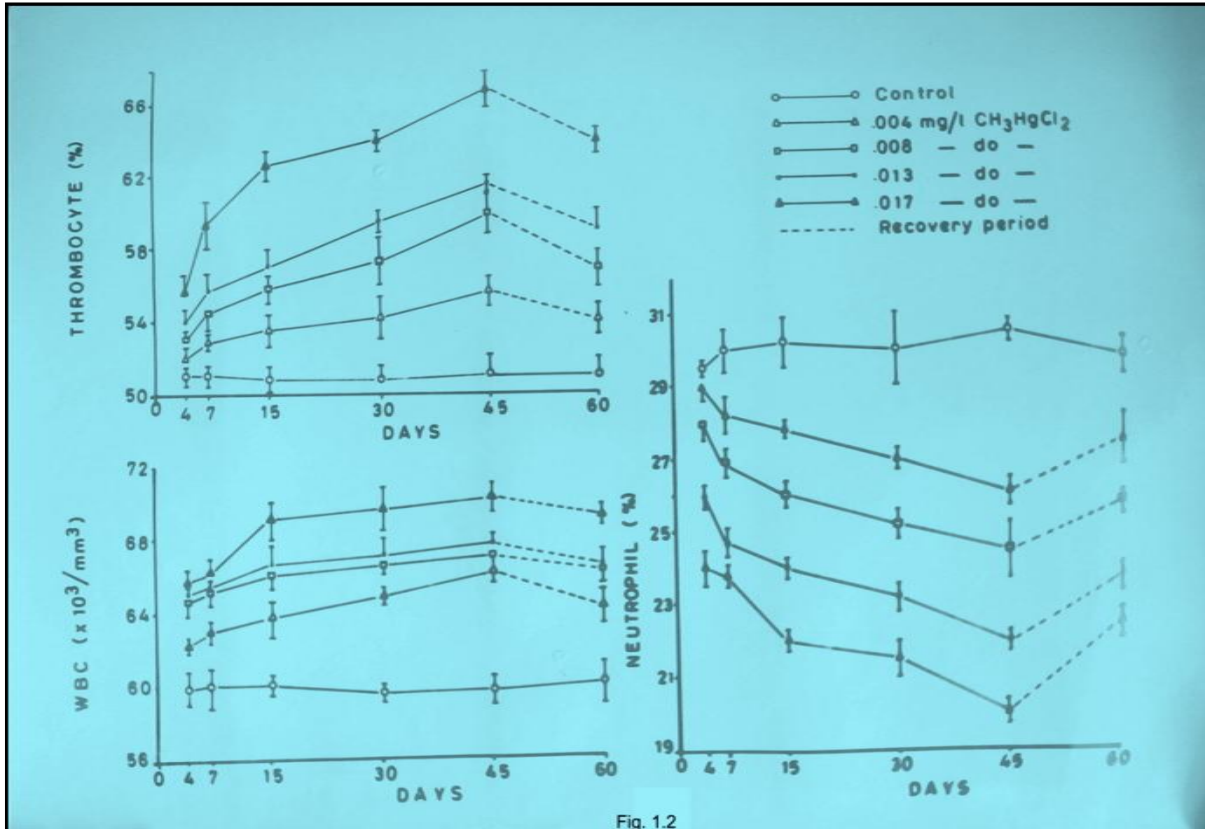


Fig. 1.2

It will be seen that organic mercury pollution results in macrocytic hyperchromic Polycythemia of RBC and that the response is dependent on the exposure level with respect to time and concentration. Being higher at higher levels of exposures and lower at lower levels, as observed in graph (Fig1.1) so far as Mean Corpuscles Haemoglobin Concentration is concerned there is a relative decreases at the lower level tested particularly in G2 as compared to control and this decrease is statically significant indicating thereby that increase in blood cells volume was greater than a corresponding increase in haemoglobin concentrations.

C.Total and differential leucocyte count:

The changes observed in WBC, thrombocytes and neutrophil values have been presented graphically in Fig (1.2). It will be seen that mercury pollution results in leucocytosis mainly contributed by thrombocytosis although there was significant neutrophilic leucopenia. In the other cell types apparently there was no change in population relative to total leucocytic count.

Key data of significances is tabulated below:

Days	Exposure Level (ppm)	Percentage in increase (+) or decrease (-)		
		WBC	t	n
15	0.004	1 +6.17	+5.31	1 -7.90
	0.008	2 +10.00	1 + 9.84	2 -13.91
	0.013	2 +11.00	1 +12.20	2 -20.53
	0.017	2 +15.02	2 +23.22	3 -27.15
45	0.004	2 +10.36	1 +9.02	1 -14.43
	0.008	2 +12.04	2 +17.45	2 -19.67
	0.013	3 +13.42	2 +20.59	3 -27.87
	0.017	3 +17.39	3 +30.98	3 -34.42
60(R)	0.004	1 +7.00	1 +6.09	1 -7.72
	0.008	2 +10.33	2 +11.59	2 -13.42
	0.013	2 +10.67	2 +15.91	2 -20.13
	0.017	3 +15.33	3 +25.54	3 -24.50

1=P<0.05, 2=P<0.01, 3=P<0.001, (R) Recovery, t=thrombocytes , n= neutrophils.

IV.DISCUSSION

Channa punctatus is the hardiest of fishes but still the effect of methyl mercury chloride is observed on peripheral blood. The study of total blood cell count of fishes have importance in diagnosing the growth of fishes and its effect on human health also(Idriss et al ;2015).The acute chronic stress in fishes (Barcelles et al;2004)were similar to present conditions and haematological abnormalities have been recorded (Panigrahi et al;1987,Karuppasamy et al ; 20005,Oliveira et al;2016) more or less similar to present study were recorded.

It is seen that the response to methyl mercuric chloride after 45 days exposure to 0.017 ppm haemoglobin level increased by 24%. The haematological parameters are sensitive indices of the pollution stress. This investigation is further strengthened by comparative data of significance from total and differential studies of leucocytic population. Increase in RBC is indicative of stress resulting in a higher metabolic rate .Leucocytosis , mainly contributed due to thrombocytosis and characteristic neutropenia were even more significant as indices of methyl mercury pollution. When fishes were restored to control water ,then recovery was faster.



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