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Malathion Induced Changes in the Serum Proteins and Hematological Parameters of an Indian Catfish *Heteropneustes fossilis* (Bloch)

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The indiscriminate use of pesticides in agricultural operations adversely affects the aquatic environment to a very great extent. This poses a great danger to freshwater organisms including fishes. Malathion (0,0-dimethyl phosphorothioate S- (1,2 dicarbenthoxyethyl), an organophosphorus insecticide acts as a neurotoxin due to its ability to block neurotransmission by inhibiting the enzyme, acetylcholinesterase (O'Brien 1960). In addition to other changes, hematological abnormalities (Mukhopadhyay and Dehadrai 1980; Mishra and Srivastava 1983) and changes in blood serum proteins (Richmonds 1989) have also been observed in fishes exposed to malathion. The purpose of this study was to study the changes in serum proteins and hematological parameters of Heteropneustes fossilis exposed to malathion.

MATERIALS AND METHODS

Live specimens of Heteropneustes fossilis (28.0-32.0 g) were procured from a swamp located in North Bihar, India. Fish were acclimated in the laboratory for 1 wk. Fish were fed with chopped goat liver. Bioassays to determine the 96-hr LC_{50} were conducted (in plexiglass aquaria) employing the technique as described by American Public Health Association (1981). Malathion containing 50% active ingredients (Northern Minerals Ltd., Haryana, India) was used in this study. The water used in the experiment had the following mean values for the water quality characteristics: temperature 28°C, pH 7.35, DO_2 7.5 mg/L, CO_2 4 mg/L, alkalinity 115 mg/L as $CaCo_3$, hardness 140 mg/L as $CaCo_3$. Groups of 10 fish (30 ± 1.0 g) were exposed to a sublethal concentration, 1.2 mg/L, nearly one-tenth of the 96-hr LC_{50} value (11.676 mg/L) for 24, 48 and 72-hr in plexiglass aquaria. Controls were treated under identical conditions with the same number of fish.

Fish were anesthetized with 100 mg/L tricaine methanesulfonate (MS-222). The caudal peduncle was cut off with a sharp razor blade and free flowing blood was collected for the hematological study. Hematological parameters were estimated by standard methods as described by Blaxhall and Daisley (1973). The RBC and WBC counts

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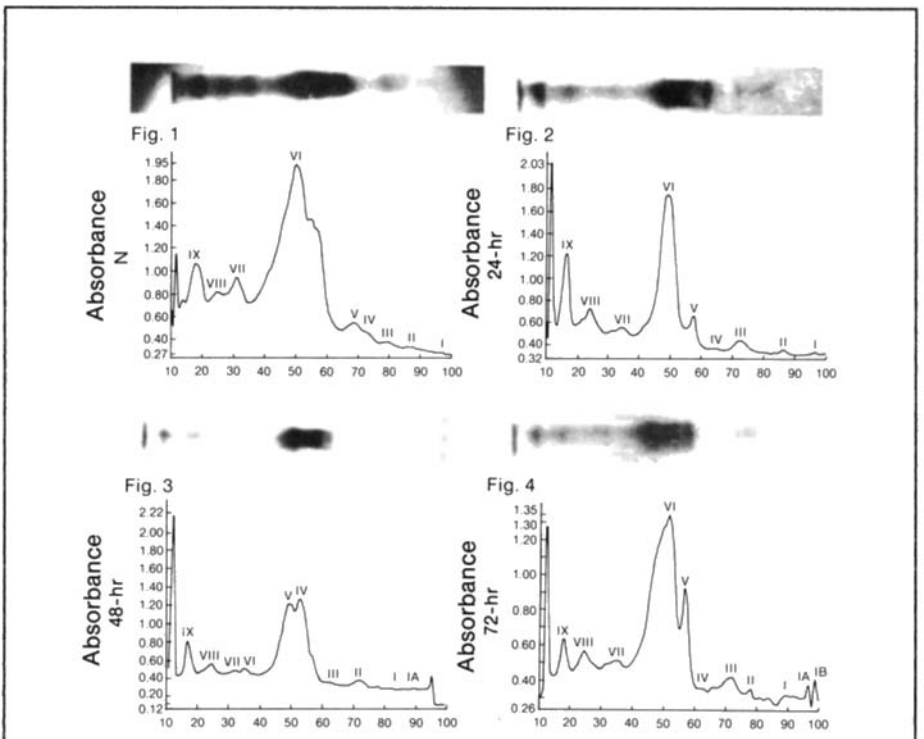
were made by Neubauer hemocytometer. Hemoglobin % determination was performed by Sahli's hemometer.

For the electrophoretic study the blood samples were allowed to stand at room temperature for 1 hr. The blood samples were centrifuged at 1000 x g for 15 min. The serum was removed and stored at 4^0C. It was used within 24-hr for electrophoretic assays. Serum proteins were analyzed by polyacrylamide gel electrophoresis (Clarke 1964). Twenty microliters of serum and a few drops of bromophenol blue were applied to each tube. The bromophenol blue served as the tracking dye. A constant current of 5 mA/tube was applied. Electrophoresis was terminated in 60 $\pm$ 5 min, when the tracking dye was about 1 cm from the bottom of the tube. After electrophoresis the gels were removed from the tubes. The gels were stained with 1% aqueous Coomasie Brilliant Blue and destained with 7% acetic acid until the bands displayed maximum staining effect with no background staining. Gels were scanned with LKB Ultrascan XL densitometer. Blood samples from 10 fish were analyzed for each time period.

RESULTS AND DISCUSSION

The electropheorogram of blood serum from control fish showed nine (I to IX) distinct fractions (Fig. 1). After 24-hr exposure, changes were observed in the relative position, height and area of some protein fractions (Table 1). A new fraction of protein (IA) was noticed after 48-hr exposure (Fig. 3). In addition to this fraction, another new fraction of protein (IB) was observed after 72-hr exposure (Fig. 4). The respective position, height and area of the fractions of proteins changed from 24 to 74-hr exposure. All the protein fractions seemed to slow down their mobility and shifted their relative position in a decreasing order as compared to control, except the VII one. Most of the protein fractions (II, IV, VI, VII and VIII) in 24-hr, VI, VII, VIII and IX in 48-hr and IV, VI, VII, VIII and IX in 72-hr were seen decreased in height. Some of the fractions III, V and IX in 24-hr, I to V in 48-hr and I to III and V in 72-hr increased in height as compared to control. Compared to control values, the area of all the protein fractions decreased except I and III in 24-hr, and I to V and IX in 48-hr exposures. After 72-hr, only three fractions (I to III) increased, whereas, six protein fractions (IV to IX) decreased in their area.

The slowest moving serum protein fraction from the blood of golden shiner, Notemigonus crysoleucas was identified as euglobulin by Summerfelt (1964). This author demonstrated antibody function in the slowest moving component of the serum. Menzel's (1970) electrophoretic study of the blood proteins of a fish, Notropis cyprinidae resulted in the identification of the slowest migrating fraction as a globulin fraction. Ney and Smith (1976) analyzed the serum proteins of bluegills using polyacrylamide gel electrophoresis, and identified the slowest moving fraction as a globulin fraction. According to Post (1966) who studied the serum proteins and antibody production in rainbow trout, Salmo gairdneri, specific humoral antibodies were found to be present in the



Figures 1-4. Electropheorograms of Sera
Fig 1 From control fish
Fig 2 After 24-hr exposure
Fig 3 After 48-hr exposure
Fig 4 After 72-hr exposure
I - IX = Peaks showing different serum proteins.

electrophoretically least mobile proteins. Based on the above findings, serum protein fraction I, in the present study was considered to include the globulin fraction. The appearance of two new serum protein fractions (IA and IB) of a very low mobility may be due to the immune response of the fish.

Formation of a new fraction of protein may be due to the breakdown of red blood cells or other cellular components. Findings of our SEM study on respiratory organs (unpublished data) reveal broken microridges, loss of cell boundaries in gill filaments, beaded appearance and fusion of secondary lamellar surface. Richmonds and Dutta (1989) observed necrosis in the gills of bluegills, Lepomis macrochirus exposed to malathion. The differences in position, height and area observed in the sera of exposed fish may be due to the possible changes in the amount of different proteins caused by the necrosis of the cellular components.

Previous studies on blood serum proteins have shown that under

Table 1. The position, Height and Area of different peaks of serum proteins from control and malathion exposed H. fossilis

Peak	Position (mm) (in mm from tracking dye)				Height (AU) (AU = absorbance units)				Area (AU x mm)			
	Control	24-hr	48-hr	72-hr	N	24-hr	48-hr	72-hr	N	24-hr	48-hr	72-hr
I	97.0	96.60	82.40	89.40	0.01	0.01	0.19	0.06	0.036	0.080	1.681	0.438
II	87.20	86.20	71.80	78.00	0.07	0.05	0.28	0.10	0.385	0.186	2.353	0.419
III	85.60	72.80	62.40	71.80	0.07	0.13	0.24	0.16	0.247	0.921	1.480	1.195
IV	79.20	64.80	52.80	62.20	0.12	0.06	1.16	0.10	0.665	0.194	6.636	0.397
V	68.60	57.60	49.40	56.80	0.28	0.35	1.10	0.67	2.412	1.237	6.071	2.278
VI	50.20	49.20	35.40	51.80	1.68	1.44	0.40	1.09	29.943	10.677	2.481	7.612
VII	31.20	34.80	32.20	35.40	0.67	0.24	0.38	0.25	4.105	1.754	1.856	2.360
VIII	25.20	24.40	24.60	25.00	0.55	0.41	0.45	0.30	3.047	2.708	2.756	2.203
IX	18.20	16.80	17.20	18.20	0.81	0.91	0.69	0.37	5.657	5.491	5.937	2.411

Table 2. Hematological changes in Heteropneustes fossilis on exposure to Malathion (mean values)

Blood parameters	Control	Treated 24-hr	48-hr	72-hr
Total RBC count ($\times 10^6/mm^3$)	4.05	3.01	2.05	2.10
Total WBC count ($\times 10^4/mm^3$)	1.57	4.52	4.06	2.40
Hemoglobin (g/dl)	14.40	13.40	12.20	11.40

conditions of stress (Bouck 1972) or heavy metal exposure (Qayyum and Gayazuddin 1978; Dutta et al. 1983; Rai 1987) the number of protein fractions either increased or decreased. Rai (1987) observed disappearance of some protein fractions and emergence of a new protein with a very low mobility in fish exposed to mercury. In the present study the protein fractions were not only affected, but also two new protein fractions of very low mobility appeared after exposure to malathion.

Noticeable differences were observed in the hematological parameters of exposed H. fossilis (Table 2). In control fish the total RBC count was 4.05 x $10^6/mm^3$. The total RBC count showed a decreasing trend with increasing exposure time. The reduction in RBC number may be due to microcytic or normocytic anemia (Tuschiya 1979). In 24-hr exposure the WBC count increased (4.52 x $10^4/mm^3$) as compared with control (1.57 x $10^4/mm^3$), whereas, in 48-hr exposure there was a slight decrease in WBC count compared to 24-hr exposure. This decreasing trend continued after 72-hr exposure (2.4 x $10^4/mm^3$). The hemoglobin percentage in control was 14.40% which decreased to 13.40%, 12.20% and 11.40% during 24, 48 and 72-hr exposures respectively. These findings in H. fossilis are in partial agreement with the results of other researchers (Dalela et al. 1981, Mishra and Srivastava 1983, El-Domiaty 1987, Gill et al. 1991.)

The increase in WBC count after 24-hr and 48-hr exposures may be attributed to the response of the fish to malathion exposure, where malathion may work as an antigen. A rise in the small lymphocyte count and a fall in the large lymphocyte count in fish exposed to both Cu and Pb have been observed by Gill et al. 1991. The reduction in the WBC count in 72-hr exposure compared to 24-hr and 48-hr exposures may be due to the malfunctioning of the hematopoietic system caused by malathion. Reduction in T-lymphocyte subpopulations following acute exposure to 4 ppm nitrogen dioxide was observed by Damji and Richters (1989). It has also been noted by Harvey et al. (1991) that deoxynivalenol (DON) induces changes in the hematopoietic system of chicks and alters immune responses. WBC are inextricably involved in the regulation of immunological function and a prolonged exposure of H. fossilis to malathion may inflict immunological deficiency.

The present study reveals that malathion, an organophosphorus pesticide, randomly used all over the world has profound effect on

serum proteins as well as other blood parameters.

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