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0014-4754/85/070952-04$1.50 + 0.20/0

Catfish blood chemistry under environmental stress

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Summary. Blood chemistry of *Heteropneustes fossilis* exposed to sewage, fertilizers and inseticides showed signs of anemia, dehydration and disturbance in the pituitary-interrenal endocrine axis and the excretory function of gills. Hepatic and renal tissue damage was also indicated.

Key words. Heteropneustes fossilis; environmental stress; blood chemistry; sewage; fertilizer; insecticides; pollutants.

The chief body of fresh water in Gorakhpur is Ramgarh Lake, which lies at 26°42′–26°46′N and 83°23′–83°25′E and occupies approximately 15 km^2 (fig). It serves as a major source of fish and shellfish for this region which possesses a tropical climate with temperatures of 24 (18–40) °C and occupies an approximate area of 60 km^2 (fig.). The lake is heavily polluted; untreated sewage refuse from most of the city, including some of the most thickly populated areas, is discharged into the lake through a mostly open sewer covering a distance of approximately 6 km along its course. Moreover, Gorakhpur is primarily an agricultural zone; Ramgarh Lake is surrounded by agricultural fields, and even the areas inundated by it are used for cultivation when they are not immersed (fig.), so fertilizers and insecticides from surrounding agricultural fields also drain off into the lake. Organic enrichment through sewage, and contamination by agrochemicals, have progressively added to the toxicity of the water of the lake, which is evident from the fact that the fishery catch of the lake has dwindled by more than half during the past decade; whereas fish and shellfish from the lake used to be transported to other parts of the country, they hardly suffice for local requirements now. A toxicity assessment of the pollutants is therefore necessary. The pollutants have been found to have noticeable effects on the hematohistological, leucocytic and hemostatic features of feshwater fish and shellfish[1–4]. The effects of these pollutants on some chemical parameters of the blood of a common Indian freshwater catfish, *Heteropneustes fossilis*, are described here.

Material and methods. The experimental design was the same as that used earlier[1,2]. Fish were exposed up to 40 days to the following pollutants at the highest concentrations which permitted survival of 50% of the fish population for 30–40 days:

A) Sewage. Sewage was collected just before its entry to the lake and used in a concentration of 25%.

B) Sewage factors. Some selected chemical constituents of sewage (sewage factors) were determined. The yearly average values (in ppt) were found to be as follows:

Total nitrogen (N), 0.3; ammonia nitrogen (NH_3-H), 1.8; phosphate (PO_4), 43.6; sulphate (SO_4), 0.2; total alkalinity (HCO_3), 0.5; calcium (Ca), 0.1. These values were respectively 150, 850, 44, 45, 4 and 2 times higher than those in control.

The sewage factors were individually reproduced by adding KNO_3, NH_4Cl, $Na_2HPO_4 \cdot 12\,H_2O$, Na_2SO_4, $NaHCO_3$ and $CaCl_2$ to unpolluted water so as to produce the values listed above.

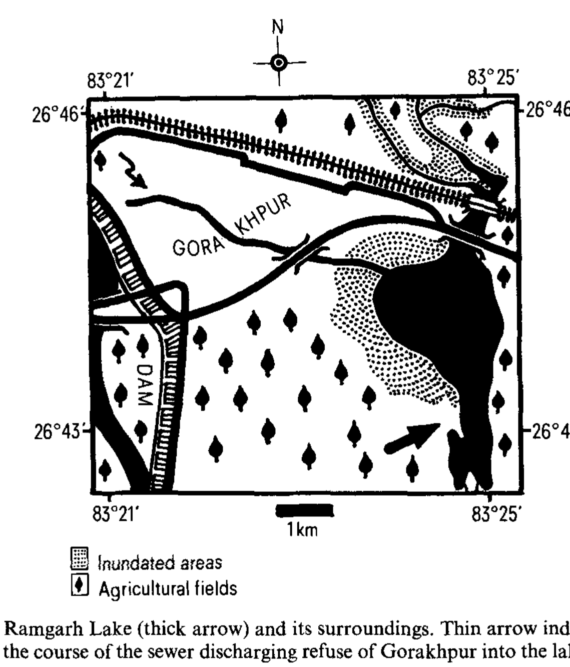
C) Fertilizers. Urea and potash (potassium oxide), 2 g/l each.

D) Insecticides. Chlorinated: BHC, 0.2 mg/l; endrin, 0.02 mg/l. Organophosphorus: Nuvacron (monochrotophos), 2 mg/l; Dimecron (phosphamidon), 20 mg/l.

Blood samples were collected every 10 days from both the treated and untreated fish. The samples were used for determining glucose level in whole blood, cholesterol level in serum, total protein level in serum, urea level in whole blood, and acid and alkaline phosphatase activity in serum. For every determination, 20–24 fish were used.

Experimental values differing significantly ($p < 0.05$) from corresponding controls have been considered as representative of change under stress, the rest being deemed normal.

Results. The normal values of the specified selected chemical parameters of the blood of *H. fossilis* are as follows:



Ramgarh Lake (thick arrow) and its surroundings. Thin arrow indicates the course of the sewer discharging refuse of Gorakhpur into the lake.

Blood glucose, 84.2 mg%; serum cholesterol, 253.9 mg%; total serum protein, 8.0 g%; blood urea, 7.3 mg%; serum acid phosphatase activity, 1.7 mg phenol/h/100 ml; serum alkaline phosphatase activity, 1.7 mg phenol/h/100 ml.

The changes produced in these values on account of pollutional stress are summarized in the table. Most of the tested pollutants raise the level of blood glucose, serum cholesterol and blood urea and the activities of serum acid and alkaline phosphatases at one or more exposure times. However, sewage appears to be predominantly depressant for blood glucose level, fertilizers for serum cholesterol level and Dimecron for serum alkaline phosphatase activity. Regarding the effect on total serum protein level, fertilizers and organophosphorus insecticides seem to be predominantly elevatory and sewage and chlorinated insecticides predominantly depressant. The rise in blood urea level and serum phosphatase activity was found to be, for the most part, quite pronounced.

As for the contribution of individual sewage factors to the total action of sewage on the stressed fish, it appears that: a) SO_4 and HCO_3 are chiefly responsible for raising serum cholesterol level;

Changes in some chemical parameters of the blood of *H.fossilis* subjected to the stress of environmental pollution

Pollutant	Days of exposure	Per unit change Blood glucose	Serum cholesterol	Total serum protein	Blood urea	Serum acid phosphatase	Serum alkaline phosphatase
Sewage	10	0	0	0	0	− 0.8	+ 0.9
	20	− 0.5	+ 1.7	− 0.1	+ 0.5	0	+ 1.1
	30	− 0.6	0	0	+ 0.4	0	+ 1.3
	40	0	+ 1.2	0	+ 0.5	+ 2.0	0
N	10	+ 1.4	0	− 0.6	+ 0.6	0	+ 18.5
	20	+ 0.7	0	0	0	0	+ 9.1
	30	0	0	0	0	0	0
	40	0	+ 0.2	0	+ 1.5	+ 17.9	0
NH_3-H	10	+ 4.1	0	0	+ 5.1	0	+ 7.0
	20	+ 1.8	0	+ 0.9	+ 1.7	+ 1.0	0
	30	0	0	0	0	0	0
	40	0	0	0	+ 27.2	0	0
PO_4	10	0	0	− 0.2	+ 6.2	0	+ 4.2
	20	+ 3.4	0	0	0	0	0
	30	0	0	− 0.2	0	0	0
	40	+ 1.8	+ 0.3	0	0	+ 3.1	0
SO_4	10	+ 0.6	+ 0.5	0	+ 3.0	0	+ 7.5
	20	+ 1.0	+ 0.7	0	0	+ 4.2	0
	30	0	+ 0.8	0	0	0	0
	40	0	+ 1.7	+ 1.0	+ 5.7	0	0
HCO_3	10	+ 2.5	+ 0.7	+ 0.6	+ 3.3	0	+ 4.7
	20	+ 3.2	+ 1.1	+ 0.6	+ 6.7	+ 15.0	0
	30	+ 2.1	+ 0.8	0	+ 4.3	0	0
	40	+ 3.3	+ 1.7	0	+ 31.1	0	0
Ca	10	+ 4.2	0	0	0	0	+ 5.4
	20	+ 3.0	0	0	0	0	0
	30	0	0	− 0.2	0	0	0
	40	0	+ 0.8	0	+ 5.0	+ 2.3	0
Urea	10	0	0	+ 0.1	+ 5.3	− 0.9	0
	20	0	0	0	+ 5.5	0	+ 8.5
	30	+ 0.8	− 0.3	+ 0.1	+ 10.7	0	0
	40	+ 0.9	− 0.3	+ 0.1	+ 36.4	+ 3.0	+ 4.1
Potash	10	0	− 0.2	+ 0.1	0	− 0.9	0
	20	0	− 0.3	0	0	0	0
	30	+ 0.8	− 0.3	+ 0.1	0	0	+ 6.1
	40	0	− 0.2	0	+ 6.0	+ 2.8	+ 6.2
BHC	10	0	+ 1.4	0	0	0	0
	20	+ 0.4	+ 1.7	0	0	+ 7.2	+ 46.9
	30	0	+ 0.7	− 0.2	+ 9.2	+ 41.4	+ 10.9
	40	0	0	− 0.2	+ 45.8	+ 56.4	+ 18.3
Endrin	10	0	+ 0.6	0	0	0	+ 6.2
	20	+ 1.0	+ 1.0	− 0.1	0	+ 6.1	0
	30	+ 1.6	+ 0.7	0	+ 2.1	+ 14.8	+ 10.1
	40	+ 2.0	+ 0.7	0	+ 2.3	+ 18.1	+ 14.5
Nuvacron	10	+ 0.5	0	+ 0.3	0	− 0.7	+ 4.1
	20	+ 0.5	0	0	+ 0.7	0	0
	30	+ 0.9	0	0	+ 1.3	0	0
	40	0	+ 0.3	0	+ 5.8	+ 2.7	0
Dimecron	10	0	0	+ 0.3	0	− 0.9	− 0.7
	20	+ 0.7	0	0	0	0	− 0.5
	30	0	0	0	0	0	− 0.4
	40	0	+ 0.3	0	+ 9.5	+ 2.7	0

NB. 20–24 fish sampled for each determination. Values represent statistically significant ($p < 0.05$) changes from the corresponding controls. 0 denotes insignificant changes ($p > 0.05$) or no change from the corresponding controls.

b) N, PO_4 and Ca contribute mainly to the lowering of total serum protein level; and c) a cumulative effect of all factors raises blood urea level and serum phosphatase activity.

Discussion. Stressed *H.fossilis* generally show hyperglycemia and hypercholesteremia. In higher animals, hyperglycemia forms a part of the physiological response to stress and is caused by stress-induced increase in ACTH-liberation and glucocorticoid secretion[5]. This appears to be generally true for fish also[6]; stressful environmental changes have been found to disturb the pituitary-interrenal endocrine axis, releasing ACTH from the pituitary and stress hormones from the interrenal tissue, and causing the blood glucose level to rise as a consequence. The interrelationship between hyperglycemia and damage to insulin cells is well known in higher animals. Hyperglycemia also appears to be closely associated with hypercholesteremia in these animals. Cholesterol is a precursor for the synthesis of many corticosteroid hormones and undergoes ACTH-affected dehydroxylation during the process[5]. Hence, a lipid mobilization, reflected by raised serum cholesterol level, could be expected during stress-induced increase in corticosteroids. Mobilization of fat and a rise in the lipid content of blood also occurs when gluconeogenesis increases during diabetes[7]. So, if any endocrine stimulation and/or insulin cell damage is caused in stressed *H.fossilis* then it would be more likely in individuals exposed to endrin because they show the greatest sustained rise in both blood glucose and serum cholesterol levels.

Conversely, *H.fossilis* exposed to sewage show hypoglycemia and those exposed to fertilizers develope hypocholesteremia. In mammals[7], hypoglycemia is caused by damage to glucagon cells or hypopituitarism. Damage to glucagon-secreting cells has been observed in stressed fish like *Channa punctatus* treated with cobalt[8]. Involvement of hypopituitarism in the production of hypoglycemia would appear more likely if a direct relationship between blood glucose and blood cortisol, as suggested to exist in the case of *Esox lucius* treated with mercury[9], occurred generally in fish. As regards hypocholesteremia, it is clinically known to signify anemia[7]. This could also be the case in *H.fossilis* exposed to fertilizers. Hematohistological[1] and other hematological (unpublished work) including ultrastructural[4] studies have established the production of anemic conditions in these stressed fish; the fish exhibit all the medically known causes of anemia (i.e. blood loss, dyshemopoiesis, hemolysis and hemoglobin loss), those exposed to sewage and chlorinated insecticides developing macrocytosis and those exposed to fertilizers and organophosphorus insecticides developing microcytosis.

Hyperglycemia and hypercholesteremia are also medically known to be associated with hepatic and renal disorders[7].

H.fossilis exposed to fertilizers and organophosphorus insecticides show hyperproteinemia. Hemoconcentration by dehydration is an important cause of hyperproteinemia in man, and loss of sodium from body fluids is a medically well known cause for dehydration[7]. In the case of fish[6], sodium is reabsorbed from the urine by renal tubules, and sodium is taken up from the medium by the gills. Hence, a lowering of sodium level in the body fluids, leading to dehydration conditions, could be expected in the event of dysfunction of or damage to kidney and gills.

Conversely, *H.fossilis* exposed to sewage and chlorinated insecticides show hypoproteinemia. Medically[7], this condition is known to occur during kidney disorders like nephrosis. Changes in serum protein level are also medically known to be associated with liver pathology[7].

Blood urea of stressed *H.fossilis* is increased considerably. This response could represent a physiological adjustment for excretory convenience. Fish excrete ammonia, exchanging NH_4^+ with Na^+ across the gills[6]. However, gills are extremely vulnerable to environmental toxicity[6], and the Na-uptake by gills is also seen to be reduced in many stressed fish[10]. In such an event, the NH_4^+-Na^+ exchange would be inhibited and NH_4^+ would tend to accumulate. Conversion of ammonia to less toxic urea would, hence, be needed under these circumstances. A shift from ammonotelic to ureotelic would also facilitate conservation of water which would be needed during the stress-induced dehydration conditions mentioned above. Such a shift has previously been reported in other stressed fish needing water conservation, e.g. mudskippers removed from water[6].

The serum phosphatase activity of stressed *H.fossilis* also generally rises considerably. Clinically[7,11], serum alkaline phosphatase activity rises mainly during bone disease and also during obstruction of enzyme excretion by liver while both acid and alkaline phosphatase activities of serum increase when carcinoma extends to bone and metastatizes therein. So, the marked rise in the activity of both the enzymes in stressed *H.fossilis* could be meaningful. Moreover, in many cases of human leukemia[12] and multiple myeloma[7,11], the serum protein level also rises. Hence, the hyperproteinemia occurring alongside the rise in serum phosphatase activity in *H.fossilis* exposed to fertilizers and organophosphorus insecticides could be further meaningful. It might also be mentioned here that some similarity has been observed in the leucocytic picture of human leukemia and that of *H.fossilis* exposed to organophosphorus insecticides[1].

As mentioned above, many of the presently observed hematological changes also appear to be indicative of hepatic, renal and branchial damage in the stressed fish. Such damage has been histologically confirmed in the case of *H.fossilis* exposed to sewage. These stressed fish show extensive injury to hepatocytes, nephric capsules, renal tubules[13,14], gill epithelium and pilaster cells (unpublished work). The observed injury includes: disarray of hepatic muralia; vacuolization and loss of staining of the cytoplasm of hepatocytes; nuclear shrinkage in hepatocytes; thickening of the walls of bile ducts and blood vessels in the liver; desquamation of epithelium and degeneration of cytoplasm in the kidney tubule cells; accumulation of debris in the lumen of renal tubules; glomerular shrinkage; formation of fibrous and hyaline casts within the renal tissue; hypertrophy and hyperplasia of the branchial epithelium; edema in the subepithelial tissue of gills; blood pooling in the branchial tissue; and collapse of the pilaster system of the gills.

* Financial grant from U.P. State Counc. Sci. Technol., India, is acknowledged.

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