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Anemia in the Freshwater Teleost, *Heteropneustes fossilis*, under the Stress of Environmental Pollution

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Gorakhpur is an agricultural zone (approximate area, 60 km^2) with a tropical climate (temperatures: average, 24°C; range, 18-40°C). It boasts of a large freshwater body, viz., Ramgarh Lake (location: 26°42'-26°46'N, 83°23'-83°25'E; maximum area, approximately 15 km^2). The lake is a major source of fish and shellfish for this region. It is, however, heavily polluted. It receives the sewage refuse of the major part of Gorakhpur city through an approximately 6 km long and mostly open sewer, as well as the agricultural run-off from the large number of agricultural fields surrounding the lake on all sides (Narain and Srivastava 1979; Srivastava and Narain 1985). Toxicity of lake water on account of organic enrichment through sewage and contamination by agrochemicals has reduced the fishery catch of the lake to less than half during the past years. An evaluation of the toxicity of these pollutants is, hence, essential. It has already been established (Narain and Srivastava 1979; Narain and Nath 1982; Srivastava and Narain 1982, 1985) that these contaminants produce hematohistological, leucocytic and hemostatic anomalies, and affect the blood chemistry, in the freshwater catfish, Heteropneustes fossilis, which is a very common food-fish of this region. The deleterious effects on the number, volume, size, hemoglobin content and sedimentation rate of the erythrocytes of the catfish are described here.

MATERIALS AND METHODS

The experimental design was the same as that used in the earlier toxicological studies on Heteropneustes fossilis (Narain and Srivastava 1979; Narain and Nath 1982; Srivastava and Narain, 1982, 1985). Fishes, collected from unpolluted habitats, were stored in tap water (Table 1) at room temperature. Healthy individuals (17.4 ± 0.5 g body-weight, 15.4 ± 0.9 cm fork-length) were exposed to sewage and its chemical factors, the

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fertilizers urea and potash, the chlorinated insecticides BHC and Stardrin 20 Shaw Wallace (active ingredient, 20% endrin), and the organophosphorus insecticides Nuvacron 40 Ciba (active ingredient, monochrotophos) and Dimecron 100 Ciba (active ingredient, phosphamidon).

Sewage was collected just before its entry to the lake and diluted to 25% with tap water. It was analyzed monthly; the yearly averages are given in Table 1. The chief chemical factors of the sewage (Sewage Factors), i.e., N, NH_3-H, PO_4, SO_4, HCO_3 and Ca, were reproduced individually by adding KNO_3, NH_4Cl, $Na_2HPO_4.12\ H_2O$, Na_2SO_4, Na_2HCO_3 and $CaCl_2$ to tap water so as to produce the listed values respectively. Urea, potash, endrin, BHC, Nuvacron and Dimecron were used in concentrations of 2 g/L, 2 g/L, 0.02 mg/L, 0.2 mg/L, 12 mg/L and 20 mg/L respectively. The concentrations of pollutants used were those which permitted 50-60% of these fishes to survive for 40 days in the polluted media as compared to control.

Every experimental set (containing medium polluted with sewage or agrochemicals), and its accompanying control set (containing tap water), comprised four cylindrical glass aquaria (diameter, 24 cm), each having 12 fishes in 12 L of the medium which was changed daily. Ten to twelve fishes from these aquaria were used for blood sampling after 10, 20, 30 and 40 days. The first half of the experiments (up to 20 days) is called 'acute' and the second half (20-40 days) 'chronic' exposure. The room temperature during storage and experiments was 18.6°C $\pm$ 0.15 SE.

The pH of the media used was as follows: control, 7.3 $\pm$ 0.0001 SE; sewage, 8.0 $\pm$ 0.04 SE; N, 7.65 $\pm$ 0.005 SE; NH_3-H, 7.64 $\pm$ 0.003 SE; PO_4, 7.5 $\pm$ 0.005 SE; SO_4, 7.71 $\pm$ 0.004 SE; HCO_3, 7.76 $\pm$ 0.006 SE; Ca, 7.83 $\pm$ 0.005 SE; urea, 8.54 $\pm$ 0.023 SE; potash, 6.84 $\pm$ 0.008 SE; endrin, 7.81 $\pm$ 0.003 SE; BHC, 7.84 $\pm$ 0.003 SE; Nuvacron, 7.36 $\pm$ 0.009 SE; Dimecron, 6.12 $\pm$ 0.080 SE.

Table 1. Chemical characteristics of sewage and control medium (tap water).

Characteristic	Sewage*	Tap water*
Dissolved oxygen	.....	7.200 $\pm$ 0.3600
Free carbon dioxide	19.50 $\pm$ 3.590	90.000 $\pm$ 3.3600
Total nitrogen (N)	0.30 $\pm$ 0.014	0.002 $\pm$ 0.0001
Ammonia nitrogen (NH_3-H)	1.75 $\pm$ 0.080	0.002 $\pm$ 0.0001
Phosphate (PO_4)	43.59 $\pm$ 7.091	0.010 $\pm$ 0.0008
Sulphate (SO_4)	0.18 $\pm$ 0.030	0.004 $\pm$ 0.0006
Total alkalinity (HCO_3)	0.45 $\pm$ 0.091	0.120 $\pm$ 0.0010
Calcium (Ca)	0.11 $\pm$ 0.003	0.060 $\pm$ 0.0020

*Values represent yearly means (in parts per thousand $\pm$ standard error.

Blood was collected from the caudal artery of both the experimental and control fishes to determine RBC count, whole blood hemoglobin (Hb), hematocrit (PCV) and erythrocyte sedimentation rate (ESR); mean corpuscular hemoglobin concentration (MCHC) was calculated from these. To determine changes in RBC size, the criteria of Soivio et al. (1974) were used; increased PCV with decreased MCHC, and vice versa, were taken as indicative, respectively, of RBC swelling and shrinkage.

Only those experimental values which differed significantly ($P<0.05$) from corresponding control values were taken as representative of change under stress.

RESULTS AND DISCUSSION

The normal hematological values are recorded in Table 2, and the stress-mediated changes in these values are summarized in Tables 3 and 4.

Sewage lowered the RBC count temporarily. NH_3-H lowered and SO_4 raised it at chronic stage. Other factors were ineffective. Fertilizers lowered the count at chronic stage. Insecticides lowered the count, Nuvacron at acute and others at chronic stage.

Sewage lowered hemoglobin level temporarily. Ca had no effect, PO_4 and SO_4 were predominantly elevators (effect of SO_4 was better sustained), while N, NH_3-H and HCO_3 were elevators at acute and depressors at chronic stage. PO_4 was effective only at chronic stage. Urea lowered Hb level at chronic stage. Potash had a mixed but predominantly depressant action. Endrin lowered Hb level at acute stage. Other insecticides were elevatory at acute and depressant at chronic stage.

Sewage generally increased hematocrit. The effect of the sewage factors on PCV was predominantly elevatory. Response of PCV to fertilizers was mixed, though reduction seemed to predominate. Potash was effective only at chronic stage. Chlorinated insecticides tended to increase PCV, being more effective at acute stage. Organophosphorus insecticides reduced it, their effect being better sustained.

Table 2. Normal hematological values of _H. fossilis_.

Characteristic	Mean	Range	S.E.
RBC count ($\times 10^6$ = No./mm^3)	2.33	1.71- 3.05	0.06
Hb (g/100 ml)	9.79	7.60-12.80	0.29
PCV (%)	29.13	20.00-33.50	0.74
MCHC (%)	34.18	24.92-57.00	1.50
ESR (mm per hr)	1.94	1.50- 2.00	0.06

Reduction of erythrocyte sedimentation rate was the predominant effect of sewage. N, SO_4 and HCO_3 increased ESR at chronic stage. NH_3-H, PO_4 and Ca had a predominantly lowering effect, that of PO_4 being the best sustained. Urea increased ESR at acute stage and reduced it at chronic stage. Potash lowered it at both stages. The insecticides increased ESR, Dimecron at acute and others at chronic stage. The effect of chlorinated insecticides was better sustained.

Reduction of mean corpuscular hemoglobin concentration was the predominant effect of sewage. All the sewage factors, except PO_4, usually reduced MCHC. Effect of fertilizers appeared early, tended to wear off ultimately, was not very sharply defined, and seemed to

Table 3. Hematological changes in *H. fossilis* exposed to sewage and sewage factors.

Pollutant	Days of exposure	Percent change				
		RBC	Hb	PCV	ESR	MCHC
Sewage	10	-34	-21	+36	0	-42
	20	0	-25	-12	-25	-15
	30	0	0	+60	-33	0
	40	0	-20	+38	0	-42
N	10	0	+22	0	0	0
	20	0	0	+23	0	-14
	30	0	0	0	+25	0
	40	0	-31	-12	+25	-27
NH_3-H	10	0	+38	0	-25	0
	20	0	0	+11	0	-14
	30	0	0	+20	-25	-26
	40	-20	-31	0	0	-41
PO_4	10	0	0	0	-25	0
	20	0	0	- 6	-25	0
	30	0	+51	+13	-25	0
	40	0	0	+31	-38	0
SO_4	10	0	0	+47	0	-44
	20	0	- 8	-20	0	+16
	30	0	+44	+52	+25	0
	40	+15	+16	+44	+25	-32
HCO_3	10	0	+23	+41	0	0
	20	0	-18	-17	0	0
	30	0	0	0	0	-21
	40	0	-34	+56	+13	-63
Ca	10	0	0	+25	-25	-33
	20	0	0	-40	-25	+45
	30	0	0	+22	-12	-23
	40	0	0	0	-12	-37

Values represent statistically significant ($P<0.05$), and 0 denotes insignificant ($P>0.05$) or no change, from the corresponding controls.

be predominantly an increase of MCHC. The chlorinated insecticides were predominantly depressive, and the organophosphorus insecticides elevatory, for MCHC. The effect of organophosphorus insecticides was much better pronounced and defined. BHC was effective only at very chronic stage.

Regarding the contribution of individual sewage factors in the total action of sewage on the stressed fishes, it appears that: (a) NH_3-H was chiefly responsible for the RBC loss produced initially, and SO_4 for the recovery achieved thereafter; (b) N, NH_3-H and HCO_3 were responsible for the Hb loss produced initially, and PO_4 and SO_4 for the recovery achieved thereafter (the role of NH_3-H was major during Hb loss because it also lowered RBC count, and that of SO_4 major during recovery because it had the best sustained effect on Hb level and also raised RBC count); (c) a cumulative effect of all factors was responsible for the increase of PCV and lowering of

Table 4. Hematological changes in H. fossilis exposed to agrochemicals.

Pollutant	Days of exposure	Percent change				
		RBC	Hb	PCV	ESR	MCHC
Urea	10	0	0	-21	0	+56
	20	0	0	+21	+50	-18
	30	0	0	-24	-50	+58
	40	-15	-23	-27	-50	0
Potash	10	0	-21	0	0	-15
	20	0	0	0	-50	0
	30	0	+29	+21	-50	+30
	40	-19	-31	-42	-25	0
BHC	10	0	0	0	0	0
	20	0	+29	+29	0	0
	30	0	0	0	+75	0
	40	-20	-37	0	+50	-43
Endrin	10	0	-12	+43	0	-22
	20	0	0	+29	0	-22
	30	-14	0	0	+25	+21
Nuvacron	10	-10	+22	0	0	+56
	20	0	+19	-25	0	+60
	30	0	0	-46	+50	+141
	40	0	-22	-27	0	0
Dimecron	10	0	0	0	0	+67
	20	0	+20	-57	+100	+189
	30	0	0	-45	0	0
	40	-21	-20	-27	0	+24

Values represent statistically significant ($P<0.05$), and 0 denotes insignificant ($P>0.05$) or no change, from the corresponding controls.

MCHC; and (d) NH_3-H, PO_4 and Ca (specially PO_4) were mainly responsible for the lowering of ESR.

An anemic condition is generally indicated by the tendency of lowered RBC count and Hb content seen in Heteropneustes fossilis exposed to environmental pollution.

Inhibited production and increased destruction of erythrocytes are the generally recognized (McLeay 1973) causes of low RBC count, as recorded in stressed Heteropneustes fossilis and other fishes like Channa punctatus exposed to organophosphorus (Lone and Javaid 1976). In Heteropneustes fossilis exposed to sewage, urea, and Nuvacron, RBC destruction must be contributing largely to RBC loss because erythrolysis is seen to be marked in these fishes (Narain and Srivastava 1979). In Heteropneustes fossilis exposed to sewage, noticeable pooling of blood is observed in the gills (unpublished work) and could also be contributing to the erythrocytopenia of these fishes. Aggregation of RBCs in gills is known to cause reduction in the number of circulating RBCs of stressed fishes like Carassius auratus subjected to hypoxia (Westfall 1943). Low Hb level, as recorded in stressed Heteropneustes fossilis and other fishes like Channa punctatus exposed to organophosphorus (Lone and Javaid 1976) and Oncorhynchus kisutch exposed to chlorinated wastewater (Buckley 1977), would be an expected consequence of RBC loss. However, inadequate hemoglobinization of RBCs can also be a cause. This is particularly indicated in Heteropneustes fossilis individuals exposed to sewage and potash because hemoglobin-deficient RBCs are seen to appear in circulation of these individuals (Narain and Srivastava 1979). Production of hemoglobin deficiency in the erythrocytes of Heteropneustes fossilis individuals exposed to sewage has also been confirmed by electron microscopy (Narain and Nath 1982).

The RBC loss incurred by Heteropneustes fossilis exposed to sewage and Nuvacron seems to trigger a compensatory reaction whereby erythropoiesis increases and ultimately restores the RBC number. Further evidence of this is provided by the appearance of immature RBCs in the blood of Heteropneustes fossilis individuals exposed to sewage (Narain and Srivastava 1979). Hyperplasia (increased erythropoiesis) with shift to the left (ripening of immature stages) has also been reported in other stressed fishes like Oncorhynchus kisutch exposed to kraft pulp mill effluent (McLeay 1973) and chlorinated wastewater (Buckley 1977). Some amount of blood regeneration also seems to occur in Heteropneustes fossilis exposed to chlorinated insecticides since immature red cells are seen to appear in the blood of these fishes (Narain and Srivastava 1979); but the compensation appears inadequate since the RBC count remains subnormal.

Macrocytosis seemingly accompanies the anemia of *Heteropneustes fossilis* exposed to sewage and chlorinated insecticides. The general tendency of increase in the PCV and decrease in the MCHC of these fishes points at RBC swelling which must be persistent because PCV does not decrease even when RBC count falls. Swelling of RBCs has often been related to hypoxia (Soivio et al. 1974). This could also be the case in *Heteropneustes fossilis* exposed to sewage because the polluted environment of the fish contained practically no dissolved oxygen; but the tolerance and response of this species to anoxia must be known before making a definite conclusion. On the other hand, microcytosis seems to occur in *Heteropneustes fossilis* made anemic by fertilizers and organophosphorus insecticides. The tendency of PCV to decrease and MCHC to increase suggests RBC shrinkage in these fishes. The tendency is much better marked and sustained in *Heteropneustes fossilis* individuals exposed to the organophosphorus insecticides, indicating a greater and more persistent shrinkage in these individuals. Decreased PCV is also recorded in other stressed fishes like *Oncorhynchus kisutch* exposed to kraft pulp mill effluent (McLeay 1973) and chlorinated wastewater (Buckley 1977). However, during acute exposure of *Heteropneustes fossilis* to organophosphorus insecticides, although RBCs shrink and either decrease or remain unaltered in number yet the Hb level is raised. This is intriguing. Pathological release of cellular hemoglobin into plasma may be a reason. In man (Eastham 1977), plasma hemoglobin increases when heme from lysed or damaged RBCs is released under pathological conditions. In that case, the erythrolysis observed in *Heteropneustes fossilis* exposed to Nuvacron (Narain and Srivastava 1979) may be a contributing factor in these fishes.

So, while the category of anemia induced in *Heteropneustes fossilis* depends on the type of stress causing it, one or more of the medically known (Britton 1969;Eastham 1977) causes of anemia (blood loss, dyshemopoiesis, hemolysis, hemoglobin loss) are exhibited by most of the stressed individuals at one time or another.

Further, in view of the tendency of reduction in hemoglobin, the anemic *Heteropneustes fossilis* may also be expected to suffer from iron deficiency. This would be more likely in individuals exposed to fertilizers and sewage because they show low ESR, which is one of the known clinical signs of iron deficiency anemia (Britton 1969). On the other hand, the high ESR of *Heteropneustes fossilis* exposed to insecticides could be related to RBC loss; such a correlation does exist in man (Britton 1969).

The present observations strengthen our recommendation that erythrocyte characteristics can provide an effective baseline for evaluating pollution-related damage to fish and hence may be used for diagnosis in the field of pollution-related fish pathology as routinely as in clinical medicine.

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