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Haematotoxic Effects of Fertilizers, Diammonium Phosphate and Urea, on Teleost *Clarias batrachus*

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ABSTRACT

The effects of two commonly used fertilizers diammonium phosphate and urea, on haematological parameters (haemoglobin, red blood cell count, haematocrit. and total leucocyte count) of fresh water fish Clarias batrachus were studied. The toxic effect of diammonium phosphate was more pronounced than that of urea. The toxic effect of diammonium phosphate resulted in a sudden fall of haematological parameters-Hb. RBC count, HCt-at higher concentrations and at lower concentrations gradual decreases were seen over comparatively longer durations. In urea intoxication, slight decreases in the three parameters were seen at lower concentrations during shorter intervals, while at higher concentrations significant decreases during shorter intervals were observed. Total leucocyte count (TLC) increased during toxicity with both the fertilizers but higher elevations in TLC were produced by diammonium phosphate than by urea.

Keywords: Haematotoxic Effects, Diammonium Phosphate, Urea and Teleost Clarias batrachus.

INTRODUCTION

Under natural ecological conditions and in the experimentally created aquatic environment polluted with contaminants under controlled laboratory conditions, haematological parameters have been studied as indicators or monitors of prevailing aquatic pollution (Joshi, 1978; Verma et al., 1979; Sharma and Gupta. 1982; Singh, 1982; Gopal et al., 1982; Goel et al., 1982; Naqvi, 1983; Mishra and Srivastava, 1983; Goel and Maya, 1986). In this laboratory, the effects of commonly used fertilizers are being studied (Sethi et al., 1986, 1987). In this report, the effects of two commonly used fertilizers, diammonium phosphate (nitrogen 18%, phosphoric acid 46%) and urea (nitrogen 46.4%), on haematological parameters (hemoglobin, red blood cell count, hematocrit, and total leucocyte count) of the

fish *Clarias batrachus* exposed for 24 to 144 hr to six different concentrations were studied, and the results are presented.

Fertilizer	Exposur	Room	Aquari	рН	Hardness	Hardness	Alkalinity	Dissolved
concentra	e Time	temp	a temp		(total	(permane	(methylora	oxygen
tion (g/L)	(hr)	(⁰ C)	(⁰ C)		p.p.m)	nt p.p.m)	nge p.p.m)	(p.p.m)
0.00*	00*	30.5*	24.0*	7.05*	184*	184*	260*	8.00*
	144*	30.0*	23.6*	7.15*	163*	163*	290*	7.10*
0.78	00	30.5	24.8	7.10	180	180	450	7.90
	144	33.0	25.2	7.20	148	148	405	7.20
0.83	00	30.5	24.3	7.25	162	162	465	7.70
	120	29.8	25.1	7.45	150	150	430	7.30
0.89	00	30.5	24.0	7.27	154	154	480	7.90
	96	28.4	24.8	7.41	142	142	465	7.10
0.94	00	29.5	23.1	7.34	150	150	495	7.60
	72	29.7	24.5	7.47	140	140	455	6.90
0.98	00	30.5	23.0	7.39	144	144	515	7.80
	48	30.4	24.3	7.60	136	136	570	7.00
1.08	00	30.5	22.2	7.44	133	133	530	7.60
	24	30.2	23.1	7.57	112	112	585	6.80

Table 1. Water Characteristic after Disslolving Diammonium Phosphate Fertilizer at Different Intervals and Concentration.

* Control values

Table 2. Effect Fertilizers Diammonium Phosphate and Uria on Haemoglobin Levels ofFresh Water Teleost Fish Clarias Batruchus.

Fertilizer	1. Effect of Diammonium Phosphate Haemoglobin (g%) mean ± SD Exposure time								
concentration	(hr)								
(g/L)									
	24	48	72	96	120	144			
		Control:	8.50 ±0.52						
0.78	6.57±0.22	5.12±0.17	3.95±0.23	3.52±0.38	1.19±0.09	0.82±0.11			
0.83	5.87±0.49	4.05±0.60	3.42±0.33	1.95±0.12	1.08±0.06				
0.89	4.20±0.57	3.12±0.26	1.42±0.30	0.94±0.11					
0.94	2.57±0.22	2.62±0.22	1.04±0.09						
0.98	1.32±0.12	0.94±0.08							
1.08	0.95±0.05								
		II E	ffect of Urea						
	Control : 8.80±0.18								
7.60	6.45±0.36	6.00±0.21	5.07±0.17	4.57±0.22	3.10±0.21	2.90±0.08			
11.60	5.45±0.36	5.30±0.18	5.25±0.40	4.72±0.17	2.62±0.43				
16.35	3.72±0.17	3.55±0.31	2.17±0.45	1.82±0.17					
20.40	2.60±0.18	2.27±0.17	2.00±0.29						
24.80	2.50±0.29	1.95±0.12							
31.45	1.75±0.12 Ten observations were made in each case				se				

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MATERIAL AND METHODS

The fish were collected from the river Gomti of Lucknow with the help of local fishermen using hand nets. They were brought to the experimental laboratory in large plastic containers in natural water, avoiding injuries and stresses of all kinds. The fish were washed five times in tap water, then treated with 2.5% KMn0₄ to remove external infections. Normal, uninfected, and apparently healthy fish selected for the experiment were transferred to large glass aquaria and acclimated for 120 hr. Concentrations of DAP and urea lethal (90-100%) to C. batrachus at 24, 48, 72, 96, 120, and 144 hr have been recorded earlier (Singh, 1982; Naqvi, 1983).

Water characteristics were analysed throughout the study before and after dissolving an amount of fertilizer, using the standard procedure (APHA, 1976). Proper oxygen supply was maintained throughout the experiment. Fish were tested at measured intervals and exposures to particular concentrations. Blood was collected in vials by puncturing the caudal vein and was mixed with 1% EDTA (ethylene diamino tetra acitic acid) Haematological parameters-haemoglobin (g%). RBC count (million/cmm), HCt (%), and TLC (cells/cmm)-were analyzed using a semiautomatic blood cell counter (Boehringer-Mannheim Diagnostics, HC-555).

		releostrisi	i ciunus Dutit	iciius.					
Fertilizer	I. Effect of Diammonium Phosphate								
concentratio	RBC Count (million/cmm) mean ± SD								
n (g/L)	Exposure time (hr)								
	24	48	72	72 96		144			
		Control:	3.56±0.14						
0.78	1.05±0.55	1.25±0.02	1.08±0.06	0.89±0.12	0.84±0.09	0.62±0.06			
0.83	0.87±0.09 1.29±0.15		1.30±0.14	1.03±0.13	0.78±0.06				
0.89	1.14±0.11 1.16±0.08		0.95±0.04	0.69±0.02					
0.94	0.89±0.05	0.89±0.06	0.67±0.04						
0.98	0.91±0.03	0.67±0.12							
1.08	0.80±0.10								
II. Effect of Urea									
		Control :	3.43±0.06						
7.60	3.28±0.30	2.95±0.05	2.76±0.05	2.66±0.11	2.30±0.11	2.13±0.11			
11.60	2.94±0.07	2.80±0.05	2.72±0.06	2.38±0.03	2.18±0.09				
16.35	2.73±0.07	2.66±0.12	2.24±0.11	2.17±0.13					
20.40	2.57±0.10	2.58±0.16	2.13±0.06						
24.80	2.63±0.17	2.27±0.11							
31.45	2.25±0.11	Ten observations were made in each case							

Table 3. Effect of Fertilizers Diammonium Phosphate and Urea on RBC of Fresh Water
Teleost Fish Clarias Batrachus.

OBSERVATIONS AND RESULTS

(A) DAP on Haematological Parameters

1. **Water characteristics:** pH increased slightly at the termination of the experiment (Table-1), while other water characteristics-hardness, temperature, dissolved oxygen, etc. analyzed before and after dissolving different amounts of fertilized was almost the same.

2. **Hemoglobin:** Hemoglobin content decreased at all concentrations and expo-torts. However, at higher concentrations (0.94, 0.98, 1.08 g/L) a sudden fall in hemoglobin was observed, while at lower concentrations (0.78, 0.83 g/L) hemoglobin decreased gradually and was minimum at highest exposures (Table-2), which proved lethal to the fish.

Table 4. Effect of Fertilizers Diammonium Phosphate and Urea on Hematocrit of Fresh
Water Teleost Fish Clarias Batrachus.

Fertilizer	I. Effect of Diammonium Phosphate								
concentration	Hematocrit (%) mean ± SD								
(g/L)	Exposure time (hr)								
	24	48	72	96	120	144			
		Control:	22.35±1.08						
0.78	2.42±0.33	2.47±0.12	3.20±0.21	4.22±0.25	3.87±0.29	2.80±0.25			
0.83	2.72±0.17	3.35±0.26	4.40±0.40	4.12±0.49	2.55±0.31				
0.89	2.40±0.31	2.30±0.18	2.50±0.29	2.45±0.34					
0.94	2.65±0.68	2.57±0.17	2.47±0.17						
0.98	2.55±0.26	2.62±0.26							
1.08	3.20±1.00								
II. Effect of Urea									
	Control : 24.45±0.62								
7.60	23.32±1.26	22.82±0.38	18.3±2.05	17.15±2.17	15.27±1.26	10.82±0.07			
11.60	21.17±0.74	18.62±1.08	17.87±0.84	14.35±0.64	10.42±0.65				
16.35	15.52±0.60	15.05±0.88	13.95±0.58	9.95±0.36					
20.40	1285±1.06	11.80±1.25	9.00±0.35						
24.80	10.67±0.38	8.85±0.31							
31.45	9.40±0.42	9.40±0.42 Ten observations were made in each case							

3. RBC Count: At all concentrations, the RBC count was lowest at the termination of the experiment. Smaller decreases were seen for initial exposures at lower concentrations, and at higher concentrations the cell count decreased suddenly (Table-3).

4. Haematocrit: The haematocrit content, during shorter intervals and at lower concentrations was almost unaffected, but decreased over longer durations (Table-4).

5. TLC: At all concentrations, TLC increased gradually with exposure time (Table-5) and was maximum at the end, which proved lethal to experimental fish.

(B) Effect of Urea on Haematological Parameters

1. Water characteristics: Variable changes in water characteristics were observed (Table-6) after dissolving the fertilizers, pH of the medium changed slightly while the other characteristics remained almost unaffected.

2. Hb, **RBC Count and HCt:** These three parameters decreased at all concentrations and exposures (Tables-2, 3 and 4). Maximum lowering of 80.11% for Hb content, 37.90% for RBC count and 15.60% for HCt were recorded.

3. TLC: During urea intoxication, TLC increased but the increase was not pronounced as in DAP toxicity.

DISCUSSION

The significant alterations in haematological parameters due to toxicity by various pollutants have been observed in fish (Van Vuren and Hattingh, 1978; Srivastava and Agarwal, 1981; Raizada and Gupta, 1982). Phosphorus is one of the important constituents of the animal body and is present in soils as tri, di and monophosphate. Phosphates are transported to and from water as adsorbed materials on soil particles and as part of microrganisms. Solid residues, water effluents, and air emissions from phosphate fertilizer-producing industries cause the pollution of water, air, and land. In our experiment diammonium phosphate toxicity caused a sharp fall in RBC count, haemoglobin content and hematocrit. A decrease in RBC number and hemoglobin was observed in the fish *Trichogaster fasciatus* after exposure to the fungicide RH-216 (Raizada and Gupta, 1982)

Fertilizer concentrati	I. Effect of Diammonium Phosphate TLC (cells/cm2) mean \pm SD Exposure time (hr)							
on (g/L)								
	24	48	72	96	120	144		
		Control:	22850±1195					
0.78	46625 ±	56475±	54000±2535	47100±	54725±3387	50475±		
	1206	3920		1471		1828		
0.83	37725±	43775±	46225±1209	49625±	55500±2434			
	3287	1873		1941				
0.89	40450±	55550 ±	66575±1150	6457± 5994				
	580	2458						
0.94	45825±	57950±	63875 ± 2808					
	2321	2471						
0.98	60600±	68000±						
	2034	2160						
1.08	74000±							
	2449							
		1	II. Effect of Urea			•		
		Control :	20175±1027					
7.60	250751±	31425±	26500 ± 3415	29525	24650 ± 911	23400±		
	6393	1709		±1014		1329		
11.60	26925±	24000±	22750 ± 1424	23150 ±	23425 ± 613			
	4832	4832		1554				
16.35	30825±	30875±	33850 ± 1975	33700 ±				
	2392	1150		1061				
20.40	36125±	37225±	38825 ± 3009					
	1314	1541						
24.80	40200±	42825±						
	535	1685						
31.45	38750±		Ten observatior	ns were made in	n each case			
	3500							

 Table 5. Effect on Fertilizers Diammonium Phosphate and Urea on TLC of Fresh Water

 Teleost Fish Clarias Batrachus.

Fertilizer	Expos	Room	Aquari	рΗ	Hardn	Hardness	Alkalinity	Alkalinity	Dissolved	
concentr	ure	temp	a temp		ess	(permane	(phenopht	(methyl	oxygen	
ation	Time	(⁰ C)	(⁰ C)		(total	nt p.p.m)	haline	orange	(p.p.m)	
(g/L)	(hr)				p.p.m)		p.p.m)	p.p.m)		
0.00*	00*	35.5*	28.0*	7.05*	242*	242*	15.0*	240.0*	9.10*	
	144*	35.0*	27.6*	7.65*	252*	252*	10.0*	255.0*	8.80*	
7.60	00	35.5	27.8	7.95	239	239	18.01	301.0	9.00	
	144	35.0	28.2	8.23	226	226	51.5	408.0	8.15	
11.60	00	35.5	27.3	8.05	236	236	24.0	312.0	9.15	
	120	34.8	28.1	9.00	224	224	144.0	392.0	8.45	
16.35	00	35.5	27.0	8.10	230	230	32.0	351.5	9.10	
	96	34.4	27.8	8.52	219	219	122.0	418.0	8.60	
20.40	00	34.5	26.1	8.12	226	226	78.0	368.0	9.20	
	72	34.6	27.5	8.38	207	207	39.0	421.5	8.65	
24.80	00	35.5	26.0	8.16	216	216	95.5	392.0	9.05	
	48	35.2	27.3	8.80	192	192	58.0	422.5	8.70	
31.45	00	35.5	24.2	8.22	207	207	122.0	412.5	8.90	
	24	35.0	25.7	8.90	185	185	70.0	446.5	8.20	

Table 6. Water Characteristic after Dissolving Urea Fertilizer at Different Intervals and Concentration.

* Control Values

with CCl₄, injections (Sharma and Gupta, 1982), while in *H. fossilis* 7.6 ppm of malathion in the medium resulted in a fall in haemoglobin content from 12.35 to 11.42 g% (Mishra and Srivastava, 1983).

CONCLUSION

These data lead to the conclusion that lowering of RBC count and haemoglobin levels and leucocytosis in the blood of fresh water fish *C. batrachus* and *H. fossilis* are common effects of the pollutants diammonium phosphate and urea fertilizers in the aquatic environment.

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