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Studies on the effects of hemotoxic assessment on fishes

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Abstract
In the present findings in which the Urea toxicity is more pronounced than Diammonium Phosphate on the fishes followed by Calcium ammonium niterate and Muriate of potash. The toxic effect of urea resulted in sudden fall of hematological parameters-Hb, RBC count, HCt at higher concentration and at lower concentration gradual decreases, in *Clarias batrachus*, maximum lowering of 80.11% for Hb content, 37.90% for RBC count, and 15.60% for HCt is recorded. The effect of CAN is not so pronounced as in DAP and Urea. In *Clarias batrachus*, maximum decrease of 24.05%, 26.68% and 34.91% below control is observed in Hb. RBC count and TLC respectively. In *Clarias batrachus*, at lower concentrations (5.60, 6.15, 6.70 g/l) and shorter exposures, the three parameters studied-Hb, RBC count and HCt remained almost unaffected giving an idea that fishes tolerate these concentrations of fertilizer muriate of potash.

Keywords: hemotoxic and fishes

Introduction
In present study is an endeavor to assess the effects of most commonly used two chemical fertilizers viz. urea on the haematological parameters of two very important and medicinally valued fish species in the region of North Bihar. The presence of fertilizers in rivers and ponds through drainage system and runoff waterways cause serious ecological disturbances, adversely affecting aquatic flora and fauna specially the fishes [1-7]. The aquatic animals maintain their internal body chemistry by a variety of regulatory adjustments. In the aquatic environments, when the levels of pollutants exceed the capacity of regulatory adjustments, it leads to haematotoxic changes [8-15]. The lethal concentrations, for 90 to 100%, fishes were worked out on two fresh water teleost fishes, *Clarias batrachus* and *Anabas Testudineus* 24, 44, 72, 96, 120 and 144 hours exposures to four commonly used fertilizers-Diammonium Phosphate, Urea, Calcium Ammonium Nitrate and Muriate of Potash. The details of concentrations are given in Table-1.

Material and Methods
The fishes were hand netted from river Kamla, Darbhanga with the help of local fishermen. They were brought to laboratory in large plastic containers in natural water avoiding injuries and stresses as far as possible; washed four times in tap water, and treated with 2.5% KMnO4, to remove external infections. Uninfected, normal and healthy fishes, selected for the experiments, were transferred to large glass aquaria, and acclimated for 72 hours. The lethal concentrations of fertilizer Diammonium phosphate for 24, 48, 72, 96, 120 and 144 hours were recorded earlier [16, 17].
The characteristics of water were analysed at the beginning and at the termination of each experiment, using the standard procedures [6]. Throughout the experiment, proper oxygen supply was maintained through an electric aerator. Fishes were taken out at 24, 48, 72, 90, 120 and 144 hours of intervals and exposures to particular concentrations. Blood was collected by puncturing the caudal vein in vials and mixed with 1% EDTA (Ethylene Diamino-Tetra Acetic Acid). Haematological parameters Haemoglobin, RBC Count, Hct and TLC were analysed by using semi-automatic blood cell counter-Boehringer Mannheim Diagnostics, HC-555.

Results and Discussion

1. Effect of diammonium phosphate fertilizer on teleost fishes *clarias batrachus* and *anabas testudineus*

The toxic effect of Diammonium Phosphate was more pronounced than that of urea in both the fishes. The toxic effect resulted in a sudden fall of hematological parameters- Hb, RBC count, HCt at higher concentration, and at lower concentrations gradual decreases were seen over comparatively longer durations.

In H. fossilis, the maximum decreases of 50.79%. 52.75% and 82.16% from the Controls were seen in Hb, RBC count and HCt respectively. Leucocytosis was evident at all concentrations and exposures.

2. Effect of urea fertilizer on haematological parameters of teleost fishes, *clarias batrachus* and *anabas testudineus*

Hematological parameters- Hb, RBC count and HCt decreased during urea intoxication in both fishes. In *Clarias batrachus*, maximum lowerings of 80.11% for Hb content, 37.90% for RBC count, and 15.60% for HCt were recorded. TLC increased at all concentrations and exposure of urea. In *Anabas Testudineus*, maximum lowering of 69.62%, in RBC count, 82.74% in Hb content and 83.62% in HCt were recorded.

3. Effect of-calcium ammonium nitrate fertilizer on teleost fishes, *clarias batrachus* and *anabas testudineus*

Fertilizer CAN decreased Hb, RBC count and HCt in both the fishes at all concentrations and exposures, The effect of CAN was not so pronounced as in DAP and Urea. In *Clarias batrachus*, maximum decrease of 24.05%, 26.68% and 34.91% below control were observed in Hb. RBC count and TLC respectively. TLC, at higher concentrations and shorter exposures increased gradually and reached the peak value when the fshes died.

4. Effect of muriate of potash fertilizer on teleost fishes, *clarias batrachus* and *anabas testudineus*

In *Clarias batrachus*, at lower concentrations (5.60, 6.15, 6.70 *g*/*l*) and shorter exposures, the three parameters studied-Hb, RBC count and HCt remained almost unaffected giving an idea that fishes tolerated these concentrations. However, due to apparent failure in adjustment of body chemistry, the fishes died in prolonged exposures i.e. 96 to 144 hours when the Hb levels had fallen from the controls. At higher concentrations (7.10, 7.80. 8.75 *g*/*l*) and shorter exposures (24 to 72 hours), maximum lowering of 25.75% in Hb, 31.25% in RBC count and 36.10% in HCt were recorded. TLC at lower concentrations (5.60, 6.15, 6.70 *g*/*l*) and shorter exposures, were similar to controls, while it increased gradually with progressing concentrations in longer time intervals giving an idea of fishes being infected and unable to adjust the toxic effect of fertilizer. TLC was highest 43.77% above the controls, at lowest concentration of 5.60 *g*/*l* in 144 hours after which the fertilizer proved lethal to fishes.

All facts furnish ample support to our findings regarding a decline in Hb content, RBC count and HCt values on exposures of the fishes to fertilizers polluted aquatic environment, but whether the exact cause of these anemic symptoms is the impairment of haemoglobin synthesis due to interference by urea with the absorption of iron at the intestinal mucosa, or it is due to an. impairment of the concerned enzyme systems at the corpuscular level or it is due to increased destruction of erythrocytes caused by a misbalanced cell plasma relationship, or it is due to a combination of one or more of these factors can only be settled by detailed biochemical investigations. The results hive opened a wide scope for further experimentation and work in relation to these findings is in progress in my laboratory. Leucocytosis in response to environmental pollution in aquatic animals has been reported by a number of workers. Leucocytosis occurs as an immunological response to internal toxins as well as environmental pollution. It is certainly a pathological state and a diagnostic symptoms of morbidity. The total leucocyte count in case of fish like that in other vertebrate does reflect the state of internal as well as external environment, significant toxicity was observed due to agricultural fertilizers in fishes [16-17].

Conclusion

Our findings lead to the inference that the pollution of' aquatic environment with fertilizers DAP, Urea. CAN and MP even at low concentrations causes anaemia in the fresh water fishes *Clarias batrachus* and *Anabas Testudineus* and that with a rise in concentration of the pollutant, both the symptoms increase in intensity.

Table 1:
90-100% lethal concentrations (g/l) of different fertilizers to fresh water teleost fishes

Name of the fertilizers	*Clarias batrachus* Exposure Time in Hours						*Anabas testudineus* Exposure Time in Hours					
	24	48	72	92	120	144	24	48	72	92	120	144
Diammonium Phosphate	1.10	1.01	0.97	0.92	0.86	0.81	3.00	2.70	2.15	1.85	1.50	1.15
Urea	31.30	24.7	20.30	16.25	11.80	7.50	36.6	31.9	27.70	21.35	12.15	8.50
Calcium Ammonium Nitrate	20.80	17.4	15.70	13.50	11.30	9.20	8.90	7.70	6.30	5.65	4.20	3.75
Muriate of Potash	8.75	7.80	7.10	6.70	6.15	5.60	6.50	6.10	5.80	5.55	5.30	4.50

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