

## Toxic Impacts of Urea on the Hematological Parameters of Air Breathing Fish *Heteropneustes fossilis* (Bloch)

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**Abstract:** Urea is a common fertilizer widely used in agriculture in India. The present work was designed to study the effect of sub-lethal concentration of urea on some haematological parameters of *Heteropneustes fossilis* (Bloch). Comparison of control and treated fish showed change in blood parameters due to toxic effects of this fertilizer. Initial increase in both studied total count (TC) of RBCs and haemoglobin concentration and then gradual decrease of these two parameters with the increase of doses, indicating slow recovery from adverse effects of urea in this fish. Differential leukocyte count indicated significant response due to toxic effect of this fertilizer. Though the count of lymphocytes, heterophils and eosinophils decreased but gradual increase of basophils, neutrophils and thrombocytes was taken place with increasing dose of urea. Elevation in the number of these cells revealed recovery and exhibited resistance of this fish to environmental xenobiotic compound like urea.

**Key words:** Urea • Hematological Parameters • *Heteropneustes fossilis*

### INTRODUCTION

Urea [ $\text{CO}(\text{NH}_2)_2$ ] is one of the widely used fertilizer in India. Due to indiscriminate use in agriculture field these fertilizer is ultimately washed out and is continuously adding to the water bodies affecting the aquatic flora and fauna life. Urea when mixed with water body, it was found to diminish fish production and also cause mortality [1]. Urea has also negative correlation between fish production and level of organic nitrogen [2]. In some cases urea was reported to cause pernicious physiological changes in fish [3]. *Channa punctatus* when exposed to sub-lethal concentration of malathion revealed a declining trend of RBCs, Haemoglobin and increasing trend of WBCs. Experiment was also done with the effect of plant seeds extracts on air breathing fish [4]. Whereas, the growth performance of major carp was better in urea treated pond in-comparison of untreated fish as observed by Abbas [5]. Nanda *et al.* [6] has been reported prevalence of hypoxic stress and tissue damage in *Heteropneustes fossilis* due to sub-lethal toxicity of rotenone. In most cases release of tissue specific enzyme into the circulation and changes in the haematological profile was observed when fishes are exposed to toxic

environment [7]. Sampath *et al.* [8] reported that fish blood response long before any type of outward manifestation of a disease. After entreating the environment pesticides can be circulated into different ecosystem through air, water, soil, different food chain and other agents [9, 10]. Ansari and Waleema [11] reported that nutrient fertilizers and chemical fertilizers were the main cause of pollution of aquatic water bodies. Study was also conducted on fresh water fish to observe the response of plant nutrient which was considered as chemical pollutant and wide histopathological changes in liver, ovary as well as testis were observed [12]. Ibrahim [13] has observed that fishes were suffering from several histopathological problems due to changes in water quality of the river Nile. When fishes are exposed to chemical pollutant, there are either increases or decreases in haematological level and these changes depend on fish species, age and other characteristic of the fish. The present work deals with the effect of urea which is a widely used fertilizer in West Bengal, India on certain blood parameters of *Heteropneustes fossilis* (Bloch) as it is a common fresh water air breathing fish of West Bengal, India.

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## MATERIALS AND METHODS

**Fertilizer Used:** Urea [ $\text{CO}(\text{NH}_2)_2$ ] was selected and purchased from the market to use during this experiment. This fertilizer was utilized at different sub-lethal doses.

**Experimental Design:** Live and healthy fish *Heteropneustes fossilis* (Bloch) were collected from the local market, Salt Lake, Kolkata, West Bengal. Fish showing injuries and emaciation were discarded. *Heteropneustes fossilis* having a mean weight of  $49.64 \pm 0.27$  g and length of  $21.04 \pm 0.52$  cm, breadth  $3.88 \pm 0.76$  cm were selected for experiment.

The fish were reared in a glass aquaria (60 x 48 x 48)  $\text{cm}^3$  containing dechlorinated tap water for seven days in laboratory to the sub-lethal toxicity study. Fish were fed with protein rich fish foods regularly, purchased from market during acclimatization. Separate aquaria were set up for each concentration. Five fish were released into each tank in 25L of dechlorinated tap water. Water was changed in every 24 hrs and un-digested food was siphoned off. Water temperature was maintained at  $29^\circ\text{C}$  during the whole experimental period. Physicochemical condition of the aquaria water was as follows: dissolved oxygen  $12.02 \text{ mg L}^{-1}$ , free  $\text{CO}_2$   $62.64 \text{ mg L}^{-1}$ , nitrite  $0.5 \text{ mg L}^{-1}$ , nitrate  $5 \text{ mg L}^{-1}$ , ammonium  $0.5 \text{ mg L}^{-1}$ , pH 7 (approx.).

Control fishes were kept in tap water without any treatment. Fishes were exposed for 7 days and 14 days in different dosage of urea such as  $0.0078 \text{ g L}^{-1}$ ,  $0.0156 \text{ g L}^{-1}$ ,  $0.03125 \text{ g L}^{-1}$ ,  $0.0625 \text{ g L}^{-1}$  and  $0.125 \text{ g L}^{-1}$ .  $\text{LC}_{50}$  was measured  $1.3 \text{ mg L}^{-1}$  of water for urea during this experiment following the standard method [14].

Blood samples were collected from the sampled fish by tail ablation and the blood samples were used to analyse different parameters. viz. Red blood cells (RBCs) total count, Differential leukocyte count and Haemoglobin percentage.

**Erythrocyte Count:** Total count of RBCs was done with the help of the improved Neubauer Haemocytometer slide and studied under light microscope (Olympus CH<sub>2</sub>Oi). The erythrocyte count in fish blood was determined by using 0.85% NaCl diluting fluid. The dilution fluid is 1 part blood: 200 parts diluting fluid. The counting was done in 5 of 25 small square of haemocytometer slide: 4 small squares at four different corners and a central small square [15, 16].

**Calculation:** The number of RBCs/ $\text{mm}^3$  of blood = the total number of cells counted X dilution X 4000 / the number of small squares in which counting has been done.

**Differential Count of Leukocyte:** Blood film was prepared with the Leishman's stain following the standard method used in human blood film preparation. The counting was done in narrow longitudinal strips of the blood film starting from one end of the film to the other end, avoiding lateral edges. While counting the number, different types of leukocytes were observed. The counting was replicated three times.

**Haemoglobin Percentage:** The Haemoglobin percentage was estimated by Sahil's Haemoglobinometer. The blood was blown out from the haemoglobin pipette into the haemoglobin tube containing N/10 HCl. The contents of the haemoglobin tube was stirred with glass stirrer and allowed to stand for 10 to 20 minutes. Then N/10 HCl drop by drop was added to the haemoglobin tube while stirring with the glass rod till the colour in the haemoglobin tube match exactly with that of the standard brown plates. Dilution of blood was read on the haemoglobin tube in terms g/100ml. The data were subjected to statistical analyses and r and t values were calculated [17].

## RESULTS AND DISCUSSION

The effect of urea on *Heteropneustes fossilis* was studied in the present experiment. The fish were exposed for 7 days and 14 days to different concentrations of urea such as  $0.0078 \text{ g L}^{-1}$ ,  $0.0156 \text{ g L}^{-1}$ ,  $0.03125 \text{ g L}^{-1}$ ,  $0.0625 \text{ g L}^{-1}$  and  $0.125 \text{ g L}^{-1}$ .

The study revealed that RBCs count in control was  $2.26 \pm 0.23 \times 10^6 \text{ mm}^{-3}$  of blood. When the fish were treated with  $0.078 \text{ g L}^{-1}$  of urea, the count of RBCs comprised  $2.13 \pm 0.18$  and  $2.04 \pm 0.17$  of blood after 7 days and 14 days of treatment respectively (Table 1). Whereas the count of RBCs were  $3.02 \pm 0.20$  and  $2.51 \pm 0.13$ ,  $3.05 \pm 0.14$  and  $4.02 \pm 0.17$ ,  $2.70 \pm 0.52$  and  $1.92 \pm 0.24$ ,  $2.72 \pm 0.21$  and  $2.41 \pm 0.12$  of blood when the fish were treated with  $0.156 \text{ g L}^{-1}$ ,  $0.03125 \text{ g L}^{-1}$ ,  $0.0625 \text{ g L}^{-1}$  and  $0.125 \text{ g L}^{-1}$  of urea respectively. Thus the result showed decreasing trend in  $0.078 \text{ g L}^{-1}$  and increasing trend in comparison with the normal RBCs count (Fig.1). The value of haemoglobin percentage in control fish was  $9.4 \pm 0.61 \text{ g/100ml}$  of blood (Table 1). The experiment showed that the haemoglobin

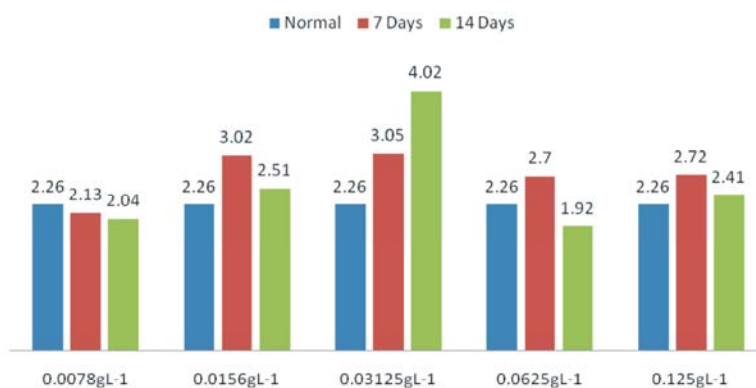
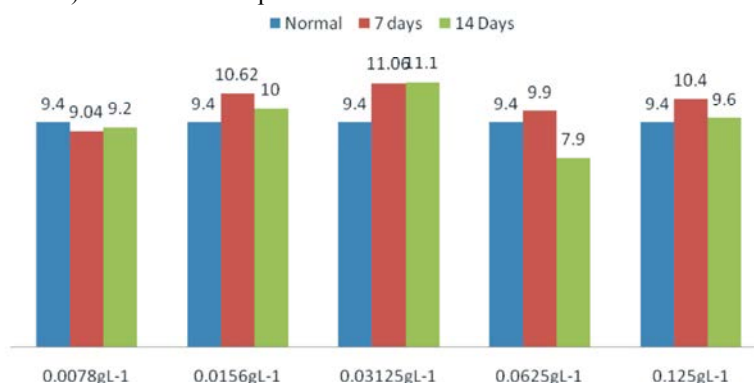
Fig. 1: RBCs count (10<sup>6</sup> mm<sup>-3</sup>) of *H. Fossilis* exposed to different doses of urea.Fig. 2: Haemoglobin percentage (g/100 ml) of *H. fossilis* exposed to different doses of urea.

Table 1: Total count of RBCs and haemoglobin percentage in control (single control was maintained throughout the experiment) and urea treated fish after 7 and 14 days of exposure to various concentrations

Parameter	Doses of Urea						
	Control	EP	0.0078 g <sup>-1</sup>	0.0156 g.L <sup>-1</sup>	0.0312 g.L <sup>-1</sup>	0.0625 g.L <sup>-1</sup>	0.125 g.L <sup>-1</sup>
Hb (g/100ml)	9.40±0.61	7days	9.04±0.50	10.62±0.12	11.06±0.43	9.90±0.25	10.40±0.43
			r=0.23 t=1.01	r=0.25 t=0.45	r=0.91 t=3.85*	r=0.16 t=0.28	r=0.54 t=1.12
		14days	9.20±0.42	10±0.25	11.10±0.38	7.90±0.19	9.60±0.36
			r=0.06 t=0.11	r=0.16 t=0.28	r=0.07 t=0.13	r=0.56 t=1.18	r=0.50 t=1.002
TC of RBCs (10 <sup>6</sup> mm <sup>-3</sup> )	2.26±0.23	7days	2.13±0.18	3.02±0.20	3.05±0.14	2.70±0.62	2.72±0.21
			r=0.57 t=1.13	r=0.36 t=0.64	r=0.65 t=0.94	r=0.45 t=0.89	r=0.06 t=0.10
		14days	2.04±0.17	2.51±0.13	4.02±0.17	1.92±0.24	2.41±0.12
			r=0.54 t=1.12	r=0.35 t=0.65	r=0.51 t=1.03	r=0.88 t=3.16*	r=0.57 t=1.11

EP= Exposure Period; TC= total count; \* Experimental value is significantly different from control with statistical significant (p<0.05); each value is mean of five observations±Standard Deviation.

percentage was 9.04±0.50 and 9.2±0.42 g/100 ml of blood after 7 and 14 days respectively, in 0.078 g L<sup>-1</sup> of sub-lethal concentration of urea. When fishes were treated with (0.0156 g L<sup>-1</sup>, 0.03125 g L<sup>-1</sup>, 0.0625 g L<sup>-1</sup> and 0.125 g L<sup>-1</sup>) higher concentration of toxicant the haemoglobin percentage constituted 10.62±0.12 and 10±0.25, 11.06±0.43

and 11.1±0.38, 9.9±0.25 and 7.9±0.19, 10.4±0.43 and 9.6±0.36 g/100ml of blood after 7 days and 14 days of exposure respectively (Fig. 2).

The percentage of lymphocytes in control fish was 32%. When the fish was treated with 0.0078 g L<sup>-1</sup> of urea, lymphocyte percentage lowered to 30% and 31%, where

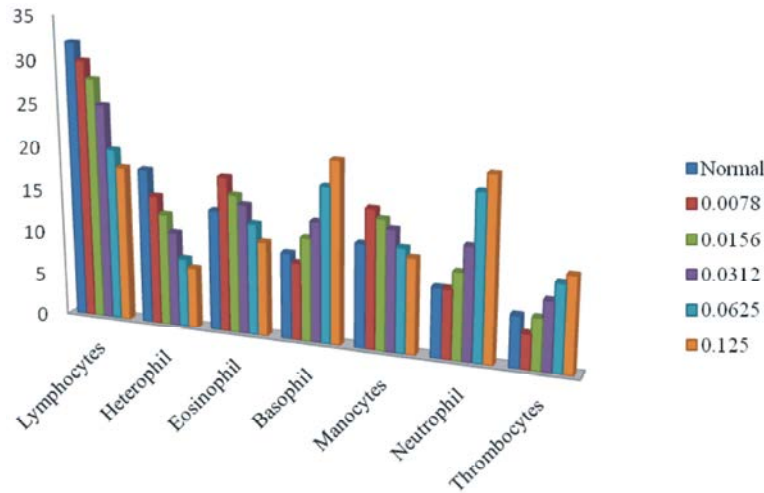


Fig. 3: Differential count of WBCs of *H. fossilis* exposed to normal & 7 days of urea.

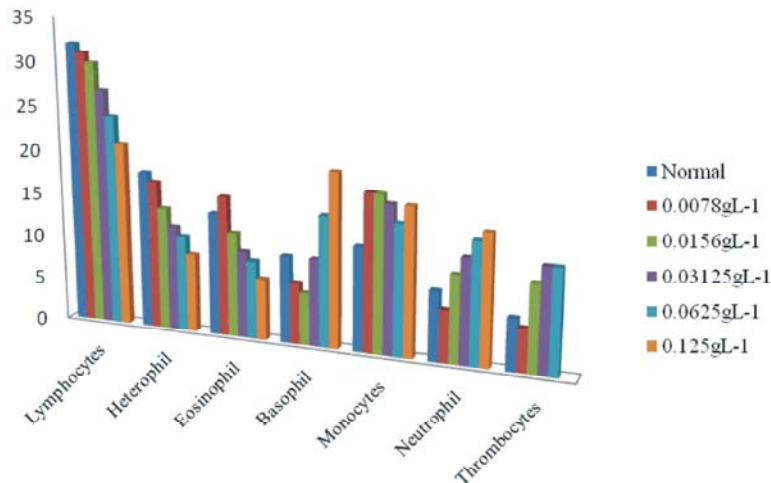


Fig. 4: Differential count of WBCs of *H. fossilis* exposed to normal & 14 days of urea.

as treatment with higher concentrations of urea induced decrease in lymphocyte percentage (Table 2). So the result clearly shows that percentage lymphocyte decreased with elevation of urea concentration.

The heterophils percentage of control fish was 18% where as the fish treated with 0.0078 g L<sup>-1</sup> of urea, the heterophils percentage was 15% and 17% during 7 and 14 days of EP respectively (Figs. 3 & 4). So, the study also showed that heterophils percentage was decreasing with increased doses.

The differential count also showed that eosinophils were 14% in control fish and the observation showed an increasing trend at 0.0078 g L<sup>-1</sup> then the number decreased with the increasing doses at 7 days EP. But percentage came down gradually at after 14 days EP. The basophils percentage in control fish was 10%, when the fishes were treated with 0.0078 g L<sup>-1</sup> of urea, the

basophils were 9% and 7% but this cell showed an increasing trend with the high doses for both 7 and 14 day of EP in comparison to control fish.

The number of monocytes in control fish was 12% which increases gradually and then maintain almost normal level when fishes were treated with 0.0625 g L<sup>-1</sup> and 0.125 g L<sup>-1</sup> EP for 7day respectively. Whereas, the number of monocytes showed an increasing trend with increased doses after 14 day of EP.

The percentage of neutrophils and thrombocytes in control was 8% and 6% respectively. The results clearly show that percentage of these two leukocytes increased with elevation of urea concentration.

In the present study urea treated *Heteropneustes fossilis* exhibited distinguishable response of haematological variables. It was suggested that haematological parameter reflects the ecological

Table 2: Differential count in control (single control was maintained throughout the experiment) and treated fish after 7 and 14 days of exposure to various concentrations of urea

			Doses of Urea				
			0.0078 gL <sup>-1</sup>	0.0156 gL <sup>-1</sup>	0.0312 gL <sup>-1</sup>	0.0625 gL <sup>-1</sup>	0.125 gL <sup>-1</sup>
Leucocytes	Control	EP					
Lymphocyte	32	7 days	30	28	25	20	18
		14 days	31	30	27	24	21
Heterophil	18	7 days	15	13	11	8	7
		14 days	17	14	12	11	9
Eosinophil	14	7 days	18	16	15	13	11
		14 days	16	12	10	9	7
Basophil	10	7 days	9	12	14	18	21
		14 days	7	6	10	15	20
Monocytes	12	7 days	16	15	14	12	11
		14 days	18	18	17	15	17
Neutrophil	8	7 days	8	10	13	19	21
		14 days	6	10	12	14	15
Thrombocyte	6	7 days	4	6	8	10	11
		14 days	5	10	12	12	11

EP= Exposure period

conditions of the habitat of the fish [18]. In sedentary and benthic species values are found to less in comparison to active predacious and pelagic species [19].

The sub-lethal concentration of urea resulted in the initial increase of RBCs count as well as Hb, at the exposure of both 7 and 14 days. This is in conformity with the observation of Sasikala *et al.* [20] who observed significant changes in haematological parameter in *Channa striata*. Initial increase in both studied parameters and then gradual decrease with the increase of doses, indicating slow recovery from adverse condition in the fishes. Roy and Nath [21] reported almost similar observation in case of Thiamethoxam treated *Oreochromis niloticus*. Then a gradual decrease in total count of RBCs and haemoglobin percentage indicates anaemia that could be due to break down and destruction of RBCs triggered by influx of urea into erythrocytes as in case of phenol-dosed fishes [22]. Patnaik and Patra [23] also indicated symptoms of anaemia due to reduction of number of erythrocyte and amount of haemoglobin in carbaryl induced *Clarias batrachus*. Similar phenomena were also reported in tobacco leaves dust treated African cat fish [24].

Haematological parameters have been considered as indicator of stress, induced by pesticides and variation in RBCs count and haemoglobin concentration was due to deleterious effect of pollutant on the erythropoietic tissue of *Mystus vittatus* [25]. Goger and Sawant [26] suggested that differential count (DC) of leukocytes is a reliable haematological index to study the change in environmental conditions.

In differential leukocytes count (DLC) number of besophils, monocytes, neutrophils and thombocytes were found to increase with increasing dose during 7 days and 14 days period. Such increase may be due to stress, because these cells play an important role in the defence mechanism of the body. More over the gradual increase with count indicated the recovery behaviour from this fertilizer stress.

Such high counts of these leukocytes indicated damage, due to infection of body tissues, severe physical stress and as well leukaemia. Similar finding was also documented significantly higher leukocytes in fish exposed to copper [27]. It was reviewed that though urea is a perturbing solute which inhibit enzyme activity and stability but in some species its acts as a major osmotic and some cellular salt like KCl and NaCl plays a crucial role in counter acting the effect of urea [28]. Eosinophil in 0.0078 g.L<sup>-1</sup> dose increase in both 7 and 14 days of exposure, then gradually decrease with increasing the doses on the other hand lymphocytes and heterophils (HET) decreased gradually with the increase of doses in the same day of exposure. In a similar observation Vasait and Patil [29] found decreasing lymphocyte count in *Nemacheilus botia* fish treated with organophosphorous insecticide.

HET is a highly phagocytic cell which can prevent antimicrobial activity. In the present study, the number of heterophils decreased with increasing doses indicating the negative effect of urea on these leukocytes, though it was reported that HET increased with the increase of stress condition [30, 31].

Urea is a common organic fertilizer used throughout India in agriculture. The study indicated that urea have deleterious effect on haematological parameters of *Heteropneustes fossilis*, i.e. the fertilizer have acute and long term side effects in aquatic animals like fish and may ultimately reach the upper level of food chain including human.

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