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Haematological Response of Freshwater Fish Puntius Sophore (HAM.) to Copper Exposure

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Abstract- Sublethal concentrations of copper ($CuSO_4.5H_2O$) administered to freshwater fish, *Puntius sophore* for a period of 30 days brought about significant haematological alterations. RBC/TEC count, Haemoglobin, Haematocrit and MCHC content progressively decreased while WBC/TLC count, MCV and MCH increased. LC_{50} of $CuSO_4$ for *P.sophore* was estimated as 1.6 mg/l. Alterations in haematological parameters were dose and duration dependent. The highest concentration of copper (0-8 mg/l) proved lethal and resulted in mass mortality of the fish on 15^{th} day of the experiment.

Index Terms- *Puntius sophore*, haematological response

I. Introduction

Aquatic pollution undoubtedly has direct effects on fish health and survival. Heavy metals are regarded as serious pollutants of the aquatic environment because of their persistence and tendency to be concentrated in aquatic organisms (Veena et al, 1997). Most heavy metals released into the environment find their way into the aquatic phase as a direct input by various anthropogenic processes, atmospheric deposition and erosion due to rainwater (Kalay and Canli, 2000). Copper is an essential heavy metal. It plays an important role in various biological processes including oxidative phosphorylation, gene regulation and free radical homeostasis as essential cofactor. However, when its concentration exceedes metabolic requirements, it becomes harmful and play a major role among pollutants (Singer et al, 2005). Copper sulphate ($CuSO_4$) has been widely used to control algae and pathogens in fish culture ponds, increasing copper concentrations in water. Copper sulphate though important as essential nutrient but becomes highly toxic to fish if its concentration required to control algae or pathogen agents is not below the threshold of fish. In light of this, present study was conducted to investigate the haematological changes in the fish *P.sophore* on exposure to copper treatment.

II. Materials and Methods

The test fish *Puntius sophore* (length 7-9 cm and weight 9-10 gms) were collected with the help of cast net from Ghomanasa stream located 20 km north-west of Jammu. Fish were acclimatized to laboratory conditions for a fortnight. Copper was given in the form of copper sulphate ($CuSO_4.5H_2O$). Experiments employing different doses of sublethal concentration of copper were carried out in 30 litre capacity tubs. Three sublethal concentrations of copper; viz, 0.16 mg/l (10% of LC_{50} value), 0.4 mg/l (25% of LC_{50} value) and 0.8 mg/l (50% of LC_{50} value) were employed for experimental purpose. From the acclimatized group, 150 fish were selected and distributed into four groups; viz, Control group (normal water), Group II (0.4 mg/l Cu) and Group III (0.8 mg/l Cu). Haematological parameters were analyzed after an interval of every 5 day for 30 day experimental duration. Blood was collected with the help of insulin syringe rinsed with an anticoagulant (Heparin). Total erythrocyte count (TEC) and Total leucocyte count (TLC) was made by using an improved Neubauer haemocytometer (Shah & Altindag 2004). Haemoglobin value was estimated by Sahli's haematin method. Haematocrit was estimated by Wintrobe tube method. MCV (fl), MCH (pg) and MCHC (g/l) were calculated as:

$$MCV = \frac{PCV \times 10/RBC}{\text{RBC Count}}$$

$$MCH = \frac{\text{Hb in g/l of blood}}{\text{RBC in million/mm}^3}$$

$$MCHC = \frac{\text{Haemoglobin in g/100ml} \times 100}{\text{Vol. of packed RBC's in 100 ml}}$$

The data obtained from the experiment were subjected to statistical analysis.

III. Results and Discussion

Erythrocytes : Compared to control group, significant decline has been observed in TEC, Hb and PCV values in all the metal treated fishes. The maximum decline in these values has been found in Group III fishes (highest concentration employed) (tab. 1a, fig. 1). One way ANOVA results reveal that the changes in TEC were significant ($p<1$, about 0.99) at all intervals but highly significant ($p=1$) only after ten days of metal exposure in all the treated groups. The reconnaissance of the data on percental decline in TEC further enlights that the maximum decline was observed during first five days 0-5 days (about 24%, 30% and 35% in Group I, II and III resp.). This may apparently be due to the fact that upon exposure to copper the fish comes under stress and immediate response is a drastic decline in TEC. Reduction in TEC s observed in *P.sophore* according to present investigator can be very safely attributed to 1) erythrocyte cell lysis besides 2) inhibition of denovo formation of erythrocytes in haemopoietic tissues. Distorted shape of RBC's (fig. 2) as observed in smear preparation very clearly discern the qualitative effect on formed elements of blood. Similar results were also

reported by Katalay and Parlak (2004) while studying effect of pollution on *Gobius niger*.

On day 15/16 of the experiment, mass mortality occurred in Group III fishes. It simply indicates that 0.8 mg/l of copper concentration is highly fatal and appears to have caused an irreparable damage by tune of as much as 53.8% decline in TEC compared to other groups where decline is to the tune of 47.8% in Gp.I and 50.4% in Gp.II. it may be very safely inferred that such high percentage of decline in population of RBC simply cannot meet the O_2 requirement of fish creating hypoxic conditions and hence result in death of fish.

In the surviving Gp. I and II fishes, during 15 and 25 day interval (tab. 1a and fig.1) declining trend though was maintained, the magnitude of decline gets lowered which according to present author may simply be an adaptation (compensatory mechanism) on part of fish to meet its O_2 demand under stressful conditions of the metal toxicity. Similar viewpoint was also given by McDonald and Wood (1993), Handy et al, (1999) and Das et al, (2006). From 25th day onwards very interestingly, fishes of both the groups observe rise in TEC percental decline. This second bout of decline is indicative that the fish like other organisms also have tolerance limit to cope up the stress of metal toxicity.

The study of the data on TEC and Hb however reveal that the fall in TEC and Hb was not totally parallel. While TEC dropped by 48% in Gp. I, 50% in Gp. II and 54% in Gp. III, Hb values fall by 31%, 35% and 27% only in Gp. I, Gp. II and Gp. III respectively at the end of the experiment. This means that during the experimental duration greater number of erythroblasts may possibly have been released in circulation. The greater amount of Hb, therefore, appears to have been incorporated either in the erythrocytes or erythroblasts. This is evident from the increase in MCH values of the metal exposed fish when compared to control (tab.3a, fig. 3). The rise observed in MCH makes it clear that to compensate the increasing O2 demand, there occurred an increased incorporation of Hb content per cell.

MCHC values after observing a slight initial rise in all the treated groups (tab. 4a, fig. 4) exhibit an overall decline. This decline in MCHC is the reflection of concomitant fall of Hb observed observed throughout the experiment. Such decline clearly is the indication of anaemia in the metal exposed fish. Also that the increase in MCHC value is more prominent in Gp.III fishes at the end of 10 days which may be an outcome of the increased lysis of RBC's and gradual fall in Hb. The Hb released from lytic RBC's may have contributed to the rise in MCHC observed in the present study. Immature erytrocytes (though not functional RBC) which start forming later when haemopoetic machinery get stimulated, atleast contribute to Hb and hence rise in MCHC is understandable here. No further data on MCHC of this group could be further obtained because these fish, which have received highest concentration of copper observed mass mortality.

Overall decline observed in PCV values was highest in Gp. III and lowest in Gp. I fishes. Thus, the decline observed in PCV follows the same trend as is shown by TEC in terms of dose dependency. Taking RBC as the essential basic component from which Hb and PCV (observed values) and calculated values (MCV, MCH and MCHC) are determined, the alterations in these values is simply the multiple reflection of RBC destruction as a result of metal toxicity to *P.sophore*. As a consequence of this, not only normal physiology of the fish gets disturbed but they also exhibit hypochromic microcytic anaemia. The anaemic fish being very less energetic may fall easy prey to secondary infections.

IV. Leucocytes

WBC'c or leucocytes are the cells of immune system defending the body against both infectious diseases and foreign materials. Tab. 5a and fig.5 clearly shows an increase in TLC values in all the metal treated groups as compared to control group. The maximum percental rise was observed in Gp. III fishes (49.8%). One way Anova results reveal that the changes in TLC was significant ($p<1$, about 0.99) at all intervals but highly significant only after 10 days ($p=1$) of metal exposure. Similar increase in TLC has also been reported earlier by Garg et al (1989), Singh (1995), Das & Mukherjee (2000) and Tyagi & Srivastava (2005) following treatment with various xenobiotics. The indepth study of the data (tab.5a, fig. 5) clearly reveals that the increment was highest in 0-5 days which present author feels may be a protective response on the part of the fish to combat stress caused by metal toxicity. The pathway of TLC increase appears to be lymphopoiesis (Gupta, 2008) because the lymphocytes which range from 50-60% in control group undergoes an appreciable increase in the metal treated groups (75-80%). In this context, the observations of Meenakala (1978) who stated that the stimulated lymphopoiesis and/or enhanced release of lymphocytes from lymphomyeloid tissues support the presently held viewpoint of lymphocytes to be the chief contributor for the increase in TLC. Mahajan & Juneja (1979) and Agrawal & Srivastava (1980) also advocated the increase in TLC to be the increased lymphocytes only.

From the day 5-10, although a rise in TLC was maintained in all the treated groups,5 but the magnitude of rise begins to fall to the tune of 20% in gp. I, 28% in gp. II and 29% in gp. III compared to 25%, 30% and 40% in gp. I, II and III respectively during first five days. The rise in TLC subsequently becomes lower and lower with the increasing time duration. Present author feels that during this time period, the internal defense mechanism of the fish may have become operational to fight the infection/ foreign invasion by the addition of more lymphocytes in the circulation. Thus, increase in TLC observed presently seems to increase the immunity of the fish against stress caused by metal toxicity.

V. Conclusion

From the overview of results, it can be concluded that exposure of fish *P.sophore* to metal toxicity (copper presently) even at low concentrations cause marked changes in haematological parameters. Such changes generally go unnoticed in the natural environment and their impacts on human beings are often overlooked. It is therefore recommended, that effluents containing heavy metals should only be disposed after their proper treatment. Further, there should be strict and regular monitoring of these toxicants in the water bodies to check possible environmental hazards.

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Table 1: Alterations in TEC (x10^6 cells/mm^3) of *Puntius sophore* exposed to different sublethal concentrations (10%, 25% and 50% of LC_{50} value i. e. 0.16, 0.4 and 0.8 mg/l) of Copper at 5 day interval.

Status and Exposure Period	Sublethal Copper concentrations employed		
	10%	25%	50%
Control	2.34±0.06	2.34±0.06	2.34±0.06
1 day	2.05±0.09	1.91±0.15	1.84±0.04
5 days	1.78±0.08	1.64±0.02	1.53±0.03
10 days	1.62±0.05	1.48±0.07	1.2±0.07
15 days	1.49±0.31	1.39±0.11	1.08±0.13
20 days	1.42±0.18	1.38±0.08	-
25 days	1.35±0.04	1.37±0.12	-
30 days	1.22±0.06	1.16±0.04	-

Table 2: Alterations in Haemoglobin of *P. sophore* exposed to different sublethal concentrations (10%, 25% and 50% of LC_{50} value i.e. 0.16, 0.4 and 0.8 mg/l) of Copper at 5 day interval.

Status and Exposure Period	Sublethal Copper concentrations employed		
	10%	25%	50%
Control	5.2±0.05	5.2±0.05	5.2±0.05
1 day	5.1±0.03	5.0±0.06	5.0±0.06
5 days	4.8±0.09	4.7±0.07	4.6±0.02
10 days	4.5±0.04	4.6±0.02	4.3±0.04
15 days	4.3±0.06	4.2±0.07	3.8±0.05
20 days	4.0±0.12	3.8±0.08	-
25 days	3.8±0.18	3.7±0.03	-
30 days	3.6±0.03	3.4±0.05	-

Table 3: Alterations in PCV (Packed Cell Volume) of *P. sophore* exposed to different sublethal concentrations (10%, 25% and 50% of LC_{50} value i. e. 0.16, 0.4 and 0.8 mg/l) of Copper at 5 day interval.

Status and Exposure Period	Sublethal Copper concentrations employed		
	10%	25%	50%
Control	24.6±2.42	24.6±2.42	24.6±2.42
10 days	21.2±2.11	20.4±1.26	17.5±2.06
30 days	18.4±1.87	17.8±2.56	-

Table 4: Alterations in MCH (Mean Corpuscular Haemoglobin) of *P. sophore* exposed to different sublethal concentrations (10%, 25% and 50% of LC_{50} value i. e. 0.16, 0.4 and 0.8 mg/l) of Copper at 5 day interval.

Status and Exposure Period	Sublethal Copper concentrations employed		
	10%	25%	50%
Control	2.22	2.22	2.22
1 day	2.48	2.61	2.71
5 days	2.69	2.80	3.0
10 days	2.77	3.10	3.58

15 days	**2.88**	**3.02**	**3.50**
20 days	**2.80**	**2.75**	**-**
25 days	**2.81**	**2.70**	**-**
30 days	**2.95**	**2.93**	**-**

Table 5: Alterations in MCHC (Mean Corpuscular Haemoglobin Concentration) of *P. sophore* exposed to different sublethal concentrations (10%, 25% and 50% of LC_{50} value i. e. 0.16, 0.4 and 0.8 mg/l) of Copper at 5 day interval.

Status and Exposure Period	Sublethal Copper concentrations employed		
	10%	25%	50%
Control	**21.13**	**21.13**	**21.13**
10 days	**21.22**	**22.54**	**24.57**
30 days	**19.56**	**19.10**	**-**

Table 6: Alterations in MCV (Mean Corpuscular Volume) of *P. sophore* exposed to different sublethal concentrations (10%, 25% and 50% of LC_{50} value i. e. 0.16, 0.4 and 0.8 mg/l) of Copper at 5 day interval.

Status and Exposure Period	Sublethal Copper concentrations employed		
	10%	25%	50%
Control	**44.9**	**44.9**	**44.9**
10 days	**80.78**	**93.13**	**121.52**
30 days	**123.6**	**132.2**	**-**

Table 7: Alterations in TLC ($x10^3$cells/mm^3) of *P. sophore* exposed to different sublethal concentrations (10%, 25% and 50% of LC_{50} value i. e. 0.16, 0.4 and 0.8 mg/l) of Copper at 5 day interval.

Status and Exposure Period	Sublethal Copper concentrations employed		
	10%	25%	50%
Control	**15.63±0.32**	**15.63±0.32**	**15.63±0.32**
1 day	**18.71±1.20**	**19.85±0.82**	**19.46±0.76**
5 days	**19.5±0.61**	**20.25±2.47**	**21.75±1.80**
10 days	**23.45±0.84**	**25.98±1.52**	**28.0±2.34**

15 days	**25.33±0.38**	**27.15±0.61**	**31.15±0.90**
20 days	**26.49±0.31**	**28.2±1.65**	**-**
25 days	**27.63±0.91**	**29.78±2.32**	**-**
30 days	**28.50±0.51**	**30.9±1.82**	**-**