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Toxicity of heavy metal copper with reference to haematological alterations in a live cat fish species *Heteropneustes fossilis* (Bloch)

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Abstract

The present study deals with the effect of Copper on hematological parameters of a live cat fish species *Heteropneustes fossilis* (Bloch) under optimal laboratory and physicochemical condition. Most of the physicochemical parameters like DO, FCO₂, p^H, TA and TH of the experimental aquariums are observed to be maintained at the productive range. During the experiment, LC₅₀ was determined which is 4.5 mg.l⁻¹ (CuSO₄ as Cu) and thereafter fishes were treated with sub lethal LC₅₀ concentration (1/5th of LC₅₀ concentration i.e. 0.9 mg.l⁻¹ CuSO₄ as Cu) and examined the haematological alteration after a treatment period of 24 hrs, 48 hrs, 72 hrs and 96 hrs interval. The result reveals that there is decrease in RBC and Hb% during the investigation period. Moreover significant increase of WBC number has been observed with increasing time during the investigation period.

Keywords: Copper sulphate, Haematological alteration, *Heteropneustes fossilis*, toxicity.

1. Introduction

Insects now a day's pollution has become a serious hazard which can change the normal characteristics of the environment affecting the whole biota globally. There are several pollutants which can affect the environment, among which heavy metals are regarded as one of the most potent toxicant which can alter normal physiology of organisms inhabiting in the polluted area. Aquatic bodies are also being constantly polluted by these toxicants which can cause deleterious impact such as reduction of RBC as well as Hb% content and alteration of WBC value of fish species.

Among all pollutants, heavy metal pollution is considered as the important issue as these pollutants are highly soluble and persistence with biomagnifications in water which cause serious threat to aquatic ecosystem (Shukla and Tripathi, 2012) [15]. Heavy metals contamination of aquatic ecosystem was initiated not only by natural processes but also by anthropogenic activity (Kumar, 1989; Seth, 2000; Kar *et al.*, 2008; Begum *et al.*, 2009) [10, 14, 9, 2]. Copper is a heavy metal which is essential for normal life activities (Winz *et al.*, 2002) [17]; but in excess it becomes a toxicant and impose deleterious effects on living organisms (Shukla and Tripathi, 2012) [15].

2. Materials and Methods**2.1. Model organism**

The study has been carried out on *Heteropneustes fossilis* which is well known as stinging cat fish with accessory respiratory organs and is native of Assam. It is commonly found in freshwater ecosystem such as ponds, wetland etc.

2.2. Procedure

Around 100 live, healthy and disease free fishes irrespective of sexes (weight range 10-30g and length range 13-15 cm) were collected from Gorjan Beel (Wetland). The fishes were washed with 0.2% KMnO₄ to avoid any dermal infection. The fishes were kept in aquarium for acclimatization in laboratory condition for 10 days. During this period fishes were fed with commercial food. After acclimatization the fishes were divided into several groups (6-7), of which one serves as control which is kept in aerated water and the other groups are treated with different concentration of CuSO₄ as Cu up to 96 hrs of exposure to determine LC₅₀ value. After the determination of LC₅₀ value, the fishes were kept in sub lethal concentration (1/5th of

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LC₅₀ value). Blood samples were collected from the anesthetized fishes by puncturing the Heart at 24hrs intervals for hematological studies. From the collected samples TLC and Hb% was studied by Neubauer's chamber (Daice and Lewis, 1977) [4] and Sahli's method respectively. The physicochemical parameters of water are analyzed as per A.P.H.A., 1988 [1] and NEERI, 1989 [12].

Table 1: Different concentration of copper sulphate as Copper with its mortality rate

Concentration of CuSO ₄ as Copper (mg.l ⁻¹)	Number exposed	Number survived	Number respond
00	10	10	0
3.5	10	8	2
4	10	6	4
4.5	10	5	5
5	10	4	6

3.2. Behavioral changes

With the exposure of CuSO₄ as Cu near to LC₅₀, the model fish species exhibits restlessness, sometimes loss of balance along with occasional jerky movements.

3.3 Hematological parameter

The present investigation reveals that there is a significant alteration of hematological parameters such as RBC count, Hb% and total WBC count due to exposure of sub lethal concentration of CuSO₄ as Cu. It has been found that the

3. Result

3.1. Determination of LC₅₀ value

The LC₅₀ is the concentration at which 50% death of fishes occur for the exposure of 96 hours. After the exposure of CuSO₄ as Copper for 96hrs the LC₅₀ value has been found 4.5mg/l (Table-1).

increase of exposure of time leads to the subsequent decreases of RBC count. Similarly the value of Hb% also exhibits decreasing trend with exposure of time interval. Moreover, the value of total WBC count is found almost reverse to that of RBC count. At 24hrs exposure of CuSO₄ as Cu, the value of WBC count has been observed to increase drastically with an exceptional and interesting decrease of WBC count at 48 hrs interval of exposure of Copper. However, the value is again increases after the exposure of 72hr and 96 hr exposure of CuSO₄ as Cu (Table-2).

Table 2: Effect of sub-lethal concentration of Copper sulphate as Copper on hematological parameters of *Heteropneustes fossilis* at different hours of exposure.

	Control	CuSO ₄ as Copper Exposure			
		24 hrs exposure	48 hrs exposure	72 hrs exposure	96 hrs exposure
Hb percentage(g/100ml)	5.1±0.14	3.85±0.21	3.65±0.070	2.93±0.28	1.8±0.14
Total RBC count(10 ⁶ mm ³)	395±12.02	274±12.73	272±4.95	271±31.82	202±65.76
Total WBC count(10 ³ mm ³)	130±8.48	220±33.94	296±89.80	214±6.36	300±68.58

3.4. The physicochemical parameters of the entire experimental aquariums were maintained at the range as shown in Table-3. The most important physicochemical

parameters like DO, FCO₂ and p^H are found at normal range in all experimental aquariums. The recorded value of TA and TH are maintained above 60 mg.l⁻¹ and 55 mg.l⁻¹ respectively.

Table 3: Physicochemical parameters of water in experimental aquariums

Parameters	Average±SD		
	LC ₅₀ Experimental Aquarium	Control Aquarium	CuSO ₄ as Copper treatment Aquarium
DO (mg.l ⁻¹)	7.2±0.22	6.84±0.56	6.82±0.53
Free CO ₂ (mg.l ⁻¹)	7.7±3.17	7.54±2.16	9.5±2.74
p ^H	6.72±0.29	6.99±0.26	6.64±0.31
Total Hardness (mg.l ⁻¹)	58.6±3.91	57.0±3.0	57.2±4.66
Total Alkalinity (mg.l ⁻¹)	62.4±5.55	60.2±4.49	61.2±6.02

4. Discussion

The present investigation indicates that there is an alteration of physiological and behavioral pattern due to exposure of sub-lethal concentration of CuSO₄ as Cu. Losses of balance, restlessness, jerky movement are the some behavioral changes found during the experimental period. Similar result was also observed by Chandra, 2008 in fishes exposed to various pesticides. The decrease of RBC and Hb% content is significantly noticed due to exposure of sub lethal concentration of CuSO₄ as Copper which is corroborate with the findings of Goel and Sharma, 1987 [8] Dutta *et al.*, 2015 [6]. Ramesh and Saravanan, (2008) [13] also observed similar observations during the acute treatment of the fish *Cyprinus carpio* with chlorpyrifos.

White blood cells provide defense against any foreign

material that entering in the body by increasing their number. In the present investigation increase in numbers of WBC is observed which is assumed to be the result of stimulation of immune system by heavy metal Copper. Gill and Pant, 1985 also reported the stimulation of the immune system causes an increase in lymphocyte by an injury or tissue damage.

There is no negative impact of physicochemical parameters on the model fish as the most of the physicochemical parameters of the experimental aquarium like DO, FCO₂ and p^H are maintaining at the productive range. Dutta *et al.*, 2015 [6] also opined that the effect of lower DO and higher FCO₂ concentration is minimum for live fish species in this type of experiment. Moreover, high concentration of FCO₂ is not always toxic (Das and Das, 1994) [5] under optimal DO level. Natural water with 40.0 mg.l⁻¹ or more total alkalinity (TA) is

considered more productive than the water of lower alkalinity (Moyle, 1946) ^[11] which is evident in the present study. The TH of water of the experimental aquariums represents hard water since its lower limit is $> 50 \text{ mg.l}^{-1}$ (Swingle, 1967) ^[16].

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