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Effect of copper, lead and cadmium on some hematological parameters of *Heteropneustes fossilis* (Bloch)

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Abstract: **Pollutants are known to induce biochemical changes in fish before the more drastic cellular and systemic dysfunctions are observed. Therefore, monitoring can be effectively used to detect such an affliction at an early stage of exposure. The blood often shows pathological changes before the external signs of poisoning can be seen. Hence, it is inferred that hematological studies in fish could be utilized as tools for investigating changes caused by environmental pollutants. The present investigation was carried out to study the effect of copper, lead and cadmium on some hematological parameters like TEC, PCV, Hb and TLC in the air breathing freshwater fish *Heteropneustes fossilis* (Bloch). All the parameters showed a considerable decrease except TLC. The duration of exposure didn't show much variation. The sub-lethal compounds used were effective in the order Copper sulphate>Lead acetate>Cadmium chloride.**

Keywords: **Pollutant, Hematological, TEC, PCV, TLC.**

I. INTRODUCTION

Copper is an essential trace metal required in small concentration for several metabolic functions in fish. The incorporation of copper into a wide variety of enzymes that play an important role in physiological processes like cellular respiration, free radical defense, neurotransmission etc. Apart from the vital role in various enzymatic processes [1], [2], when present in excessive concentration, copper is known to exert adverse toxicological effects when present in high concentration in water [3]. It becomes extremely toxic when the internal concentration exceeds the capacity of physiological detoxification processes. Various researchers reported bioaccumulated copper concentration in the tissues of fishes [4], [5], [6].

Lead is a naturally occurring metal present in the earth's crust, rock, soil and water. Most water borne lead is derived from human activities such as mining, smelting, coal burning, cement manufacturing, use of gasoline, batteries and paint [7]. In fish, the toxicity of lead occurs through gills, which are the respiratory organs. Lead as a pollutant induces lipid peroxidation in tissues and causes an irreversible damage to the respiratory organs of fish.

Itai-itai disease was caused due to cadmium poisoning in Japan, in 1912. The cadmium poisoning caused softening of the bones and kidney failure. The disease is named for the severe pains caused in the joints and spine. The cadmium was released into rivers by mining companies in the mountains causing the disease. "Itai-itai" disease is known as one of the four big pollution diseases caused in Japan. Due to the cadmium poisoning, the fish in the river started to die, and the rice irrigated with river water did not grow well. The cadmium and other heavy metals accumulated in the sediments of the

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river. This water was then used to irrigate the rice fields which absorbed heavy metals, especially cadmium. The cadmium accumulated in people who consumed contaminated rice causing the fatal "itai-itai" disease.

The blood of fish is under constant influence of aquatic environment in comparison with terrestrial vertebrates. Since the fishes live in intimate contact with aquatic environment, they are susceptible to physical and chemical changes which are reflected in their blood components [8]. The blood often shows pathological changes before the external signs of poisoning are visible. Hence, it is inferred that hematological studies in fish could be utilized as tools for investigating changes caused by environmental pollutants.

II. MATERIAL AND METHODS

The fishes were caught gently with the help of a hand net from the experimental tank and immediately anaesthetized using 2% paraldehyde. The blood was collected in an EDTA coated vial and stored till analysis. Blood collection techniques were used as per method suggested by Blaxhall [8].

The pooled blood samples of *Heteropneustes fossilis* were taken from the heart with the help of a 24 number needle. The blood was drawn from the heart by piercing the needle through the body wall. The insertion was exactly in the middle line, 0.5-1.0 cm cranially from the posterior margin of the operculum and directed dorso-caudally at an angle of 45^0.

The quantitative changes in RBC, Hemoglobin, WBC and the Packed cell volume were recorded at a regular interval of 10, 20 and 30 days during exposure to sub lethal dose of heavy metals and their combination. The estimation of total erythrocytes and total leucocyte in the blood of fish was done with the help of Neubaeur german hemocytometer. The estimation of hemoglobin content was performed by employing Sahli's acid haematin method.

III. OBSERVATION AND RESULTS

In case of *Heteropneustes fossilis*, the hematological status under stress of copper sulphate after 10, 20 and 30 days are presented in Table 1. The observed and recorded changes in hematological parameters for lead acetate for 10, 20 and 30 days exposure are shown in Table 2, while for cadmium chloride, values are presented in Table 3.

TABLE 1: Hematological parameters of *Heteropneustes fossilis* (Bloch) exposed to sublethal concentration of copper sulphate

Exposure	Control	Duration of exposure in days		
		10	20	30
TECx10^6/mm^3	3.30±0.29	2.95±0.45	2.90±0.20*	2.86±0.15**
Hb%	12.82±0.24	11.39±0.60*	11.25±0.65*	11.17±0.47
TLCx10^3/mm^3	26.82±1.69*	32.58±1.73*	32.84±1.67*	33.12±1.58**
PCV (%)	39.22±0.69*	36.82±0.96*	36.42±0.86*	35.93±084**

TEC – Total Erythrocyte Count; Hb% - Haemoglobin Percentage; TLC – Total Leucocyte Count; PCV – Packed Cell Volume; C – Control; E – Experimental * P<0.05; ** P<0.01; NS: Non-significant

TABLE 2 : Hematological parameters of *Heteropneustes fossilis* (Bloch) exposed to sublethal concentration of lead acetate

Exposure	Control	Duration of exposure in days		
		10	20	30
TECx10^6/mm^3	3.28±0.21	2.95±0.24*	2.90±0.12*	2.88±0.12**
Hb%	12.83±0.48	11.51±0.60*	11.43±0.51**	11.33±0.46**
TLCx10^3/mm^3	26.73±1.62*	32.27±1.56*	32.46±1.64*	32.69±1.55**
PCV (%)	39.92±0.97	37.74±0.89	37.10±0.88*	36.56±0.95**

TEC – Total Erythrocyte Count; Hb% - Haemoglobin Percentage; TLC – Total Leucocyte Count; PCV – Packed Cell Volume; C – Control; E – Experimental * P<0.05; ** P<0.01; NS: Non-significant

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TABLE 3: Hematological parameters of *Heteropneustes fossilis* (Bloch) exposed to sublethal concentration of cadmium chloride

Exposure	Control	Duration of exposure in days		
		10	20	30
TECx10^6/mm^3	3.29±0.26	2.99±0.87	2.94±0.17*	2.87±0.11**
Hb%	12.63±0.41	11.43±0.37*	11.29±0.35*	11.24±0.28**
TLCx10^3/mm^3	25.72±1.77*	30.90±1.89*	31.07±0.93**	31.25±1.51**
PCV (%)	39.61±0.88	37.55±0.95	37.11±0.79*	36.67±0.77**

TEC – Total Erythrocyte Count; Hb% - Haemoglobin Percentage; TLC – Total Leucocyte Count; PCV – Packed Cell Volume; C – Control; E – Experimental * P<0.05; ** P<0.01; NS: Non-significant

TABLE 4: Percentage variations in hematological parameters of *Heteropneustes fossilis* exposed to copper, lead, cadmium.

Parameter	Duration of exposure	Copper sulphate	Lead acetate	Cadmium chloride
Total Erythrocyte Count (-)	10	10.48	10.06	9.18
	20	12.21	11.38	10.77
	30	13.36	12.23	12.78
Haemoglobin (-)	10	11.15	10.28	9.47
	20	12.24	10.94	10.61
	30	12.89	11.69	11.04
Total Leucocyte Count (+)	10	21.49	20.71	20.14
	20	22.68	21.45	20.81
	30	23.48	22.31	21.50
Packed Cell Volume (-)	10	6.12	5.46	5.21
	20	7.61	7.06	6.31
	30	8.39	8.41	7.42

IV. DISCUSSION

Environmental pollutants such as heavy metals have been recognized to interfere with several physiological and biochemical parameters and alter the capacity of fish to perform the vital body functions. When the toxicant concentration is higher in the water body, it results in the death of fish. Hence, the death of an organism was taken as the end point of toxicological studies formerly [9]. However, Adams (1990) reported that sublethal concentrations of toxicant also induce significant changes in the biological organization of fish.

Copper is one of the trace metals essential for the healthy life of many living organisms. It is a constituent of haemocyanin, a metalloprotein which helps in oxygen transport in aquatic animals. Its deficiency or excess also causes problems. Lead particles from the atmosphere are brought down to water bodies through rain and also as dry deposits [10]. Lead poisoning in fish results in haematological, neuronal, muscular and effect like coagulation of surface mucus [11], [12]. Cadmium has been recognized as one of the potent aquatic pollutants present in industrial effluents [13]. The main effect of increase in concentration of cadmium in aquatic ecosystems is the accumulation of cadmium in tissues and organs. It has been demonstrated that, cadmium causes pathological changes in liver, gills and skeletal systems [14]. In congruence with the earlier reports the present work revealed that the sublethal concentration of the copper, cadmium and lead has resulted in change of the normal hematological parameters of *Heteropneustes fossilis* (Bloch).

The blood composition of the fish could be used as one of the indices to indicate their health status. In fish, exposure to chemical pollutants induces either increase or decrease in hematological parameters. Blood tissue truly reflects the changes occurring in organisms. Therefore, detailed information is obtained on general metabolism and physiological status of fish of different age and habitat [15], [16], [17]. Hematological studies play an important role in monitoring fish health, due to pollution load, stress and disease. So, it is considered as an important tool to study the impacts of toxins. In

the present investigation, *Heteropneustes fossilis* (Bloch) exposed to the sublethal concentrations of copper, cadmium, lead showed considerable alteration in the level of different blood parameters due to the presence of heavy metals at sublethal levels.

The reduction of erythrocyte count produced erythropenia which is attributed to an increased fragility leading to shortening of the life span of the erythrocytes. The toxicants influence the malfunctioning of the haemopoietic system. So, the haemopoietic tissues fail to release the blood cells. In the present study, the decrease in the haemoglobin content and packed cell volume was noticed in the blood of *Heteropneustes fossilis* (Bloch) exposed to sublethal dose of heavy metals. The haemoglobin percentage came down by 10.83%, 9.99%, 9.26% in 30 days exposure to copper sulphate, lead acetate, cadmium chloride respectively.

Some researchers reported decrease in hemoglobin content in fish *Heteropneustes fossilis* due to exposure of paper mill effluents. The decrease in total erythrocyte count, hemoglobin percentage and packed cell volume (PCV) indicate the occurrence of anemia associated with erythropenia. Various workers [18], have also obtained similar results while investigating with different toxicants. The erythrocytes also show deformity and this may be the possible cause of decrease in hemoglobin percentage. Several researchers have reported reduction in RBC and hemoglobin in different fishes exposed to different toxicants, *Cyprinus carpio* exposed to cadmium [19], and *H. fossilis* exposed to dimecron [20].

Significant decrease in RBC count, hemoglobin percentage and haematocrit value reflects the anemic state of fish. Anemia associated with decreased RBC, hemoglobin percentage and hematocrit value has been recorded to occur in response to heavy metals [21], [22]. Web (1966) attributed the decrease in RBC count and hemoglobin concentration to haemolysis. The reduced RBC count and hemoglobin concentrations suggest that, an acute anemia was induced in the fishes under cadmium intoxication [23].

Hemoglobin is synthesized in the body of an organism from protein and iron. Liver is the storehouse of iron in the body. This might have led to the non-availability of sufficient iron in the body of the exposed fish leading to a decrease in hemoglobin percentage. A reduced hemoglobin percentage could have led to a decrease in oxygen carrying capacity of the blood.

The average leucocyte count recorded in the normal fish is about 38.80 x 10^8 /mm^3. However, the total leucocyte value of *Heteropneustes fossilis* after 30 days exposure to sublethal dose of copper sulphate was found to be 46.64 x 10^3/mm^3. These values clearly indicated a significant increase in the total leucocyte count. After treatment, as much as 25.16% of leucocytes showed their rise in *Heteropneustes fossilis*. The total leucocyte values in case of heavy metal lead acetate, cadmium chloride were increased by 21.46%, 20.91% over control. This increased leucocyte count is due to leucocytosis and this might be due to the condition of stress caused by heavy metals at sublethal concentration.

V. CONCLUSION

This research article has particularly focused on the effect of some heavy metals on fish haematological parameters. It was clearly revealed that heavy metals adversely affect the fish hematology. A significant decrease in Total Erythrocyte Count (-),Haemoglobin (-) and cked Cell Volume (-) was seen. However, increase in Total Leucocyte Count (+) was also observed. Hence, it can be concluded that hematological studies in fish could be utilized as tools for investigating changes caused by environmental pollutants and this work can be regarded as a mile stone in the environmental assessment of heavy metals.

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