



## Impact of heavy metals on hematological profile in fish from the Bhaghar Lake, India: A preliminary study

### Abstract

Hematological parameters have been recognized as valuable tools for monitoring fish health induced by any changes in the quality of waters and other related environmental factors. In present study, assessment of heavy metals concentration in fresh water Bhaghar Lake (located at Tahsil-Ramnagar, District- Barabanki, State-Uttar Pradesh, India) concerning their effects on hematological parameters in fish *Channa punctatus* from it were carried out. The water and sediments of the lake were analyzed for zinc (Zn), copper (Cu), and lead (Pb) concentrations. At the same time, blood parameters of the exposed fish were also examined for any hematological modifications. The results revealed that concentrations of the metals differs significantly ( $p < 0.05$ ) between the lake water and sediments. The concentration (mg/l) of the metals were in the order of  $Cu > Zn$ , whereas Pb was not detected in any sample. Blood parameters such as blood cell count (red and white blood cells), hemoglobin, hematocrit, mean cell hemoglobin concentration, mean cell volume, and mean cell hemoglobin were noted to be altered from their normal value in fish ( $p < 0.05$ ). In conclusion this study indicated that Cu and Zn polluted the lake water and posed their harmful impact on fish health. Further this is the first report on distribution of heavy metals and proximate composition of important edible fish from this fresh water lake in India.

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### Keywords

Bhaghar Lake, Hematological parameters, *Channa punctatus*, heavy metals

### Introduction

Over the last few years, rapid industrial progression have taken place the intensity of Cu and Zn in the stream and reservoir has risen, affecting fisheries and natural resources are depleted. There have been heavy metals being while they have great biological hazards; they have serious environmental threats, Relevance as a micronutrient. Sewage and manufacturing effluents are significant sources of Cu and Zn in aquatic environments. Many employees have already confirmed Cu and Zn toxicity to fish. (Eriksen *et al.*, 2001; Wepener *et al.*, 2001; Paquin *et al.*, 2002; Singh, *et al.*, 2008; Lodhi *et al.*, 2006; Ketpadung and Tangkrockolan, 2006). Copper sulphate and Zinc sulphate is commonly used as an algacide to regulate phytoplankton in fish ponds and lakes, and as well as herbicide, employed in aquatic weed management Since 1882 (Effler *et al.*, 1980; Carbonell and Tarazona, 1993). Fish species are the essential and effective copper and Zn pollution biomarker of Aquatic Bodies (Taylor *et al.*, 2000; Lodhi *et al.*, 2006). The metallic ion found in the water enters the body of the fish and receives collected in different tissues, such as the liver and kidneys (Al-Mohanna, 1994). The blood criteria used were As a responsive stress indicator for fish subjected to various water sources, Pollutants and toxins, like pesticides, metals, chemical compounds, factory

effluents, etc. When copper and Zn were treated on *Channa punctatus*, it was found with acute anemia, along with a decrease in RBC count, Hb percent and PCV percent in the fish (Singh, 1995). The metal that reaches the fish system is removed slowly (Newman and Mitz, 1988; James and Sampath, 1996; James *et al.*, 1996;) that's why blood factors get influenced on account of heavy metal poisoning. The water of Bhaghar Lake is mainly use for fish production and irrigation. It is also subjected to other activities like cloth washing, human, cattle bathing etc. Unnaturally, the lake receives the large quantity of untreated discharges from near-by villages and agro-chemical (fertilizers etc) from agriculture. The heavy metals are the constituents of pesticides, which leach out with rainwater and irrigation in Bhaghar lake (aquatic ecosystem). In lake these metals are considered as most important toxic agent imposing their harmful impacts on aquatic flora and fauna. Since they are present throughout the lake and detected in critically high amount. Heavy metals such as Cadmium (Cd), Mercury (Hg), Copper (Cu), Lead (Pb) and Zinc (Zn) are the most important toxicants which are reported to affect aquatic environment of the fresh water fishes. Most of the metals are being accumulated in the tissues and cause the organ poisoning of fish. The heavy metals become toxic when they are not metabolized by body and accumulate

in the soft tissues. Heavy metal Copper is an essential trace nutrient, which is discharged in fresh water environment in large concentration as an industrial effluence and evenly affects the fresh water fauna specially fish. The elevated level of Cu may become acutely or chronically toxic to aquatic lives (Kumar et al., 2018). The current study is carried out to assess heavy metals present in water lake Bhaghar in Tehsil Ramnagar, Barabanki (U.P.) and variations in different hematological variables of freshwater fish resides in it.

### Materials and Methods

**Sample collection:** Water samples were collected from three different places of water lake Bhaghar in Tehsil Ramnagar, Barabanki (U.P.). Samples were employed for further analysis of the concentration of heavy metals.

**Heavy metal detection:** The collected samples were sent to Biotech park, Lucknow, India. Cu, Zn and Pb were detected in collected samples through the ISO 3025 (P 42):1992 (RA2014), ISO 3025(P 49): 1994 (RA 2014), ISO (P 47): 1994(RA 2014) respectively in Biotech park.

**Fish collection and Weight and length measurement:** Fish (*Channa punctatus*) was caught from lake Bhaghar in Tehsil Ramnagar, Barabanki (U.P.) on 15 day, 30 day and 45 days from work start. Fish were anesthetized and their body weight (g) was measured after their body surface was dried with a Kimwipe. Fish length (cm) was measured from the anterior most point of the mouth, to the posterior most point of the caudal fin. Body - mass index (BMI) was expressed as body weight (g) divided by the square of the body length (cm) (Zang et al., 2013).

**Blood collection:** Insulin syringe with a 22 gauge needle was used to withdraw blood from fish. The needle was inserted, right behind the anal fin on underside of the fish. Needle was pushed toward the vertebral column until it reached the base (Duman et al., 2019). Heparine sodium (1%) was used as an anticoagulant (Svobodova et al., 2008).

**Analysis of blood Parameters:** The collected blood samples were immediately subjected to hematological analysis. The blood was diluted with appropriated diluting fluids for RBCs and WBCs counts were determined using improved Neubauer hemocytometer and calculated (Blaxhall and Daisley, 1973). Replicated counts were made for each blood samples. Sahli's hemoglobinometer was used to estimate hemoglobin (HB) percentage (HB%). Hematocrit (HCT) was determined using micro hematocrit capillaries filled with blood and centrifuged at 8,700×g for 5 min and expressed as percentage of total blood volume (Wintrobe, 1974). Mean corpuscular volume (MCV), mean cell hemoglobin (MCH) and mean cell hemoglobin concentration (MCHC) were calculated from the average values of HB% (Dacie and Lewis, 1984). For the determination of erythrocyte sedimentation rate (ESR), the blood was mixed well and 200 mm was drawn into a Westergren tube. The tubes were placed vertically and were left undisturbed for 60 min; after that, the level of the column of sediment was noted as ESR. Biochemical estimation of blood glucose, blood protein, blood cholesterol and blood urea were determined by following standard methods (Folin and Wu, 1920; Annino, 1976; Henry, 1968; Fawcette

and Scott, 1960). The Acid phosphatase (ACP), Alkaline phosphate (ALP), Serum glutamate acetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT) were estimated by the method of King (1965). The data were analysed statistically and presented in standard manner. Method described by Jendrassik and Grof (1938) was used for the determination bilirubin in serum.

### Results and Discussion

In the present study, significant alterations in hematological parameters of Indian freshwater fish, *Channa punctatus*. The alterations observed in various blood parameters like Hb%, RBCs and PCV % decreased significantly after in comparison with control (table 2). On the contrary, WBC counts, ESR, CT and MCV values were found significantly increased after 15, 30 and 45 days of exposure, as compared to control. After an initial increase over and above control in MCH for 15 and 30 days, it was found significantly decreased after 45 days of exposure.

Although the MCHC and MCH values were found significantly elevated during the exposure of 15 and 30 days. The increase in MCH was also significant after 15 and 30 days exposures. Both values decreased significantly after 45 days of exposure in comparison with control. The lymphocytes and eosinophils registered an increasing trend over and above the control. The percentage of neutrophils, monocytes and basophils were found decreased as exposure periods increased. Maximum decrease in neutrophils, monocytes and basophils was recorded after 45 days of exposure.

The blood parameters have been used as a sensitive indicator of stress in fish exposed to different water pollutants and toxicants, such as heavy metals, biocides, pesticides, industrial effluents, etc. The exposure of *Channa punctatus* to sub-lethal concentration of copper significantly decreased Hb%, RBCs count and PCV% values leading to anaemia. The anaemia might have led to a fall in the red blood cell count, hemoglobin concentration, and haematocrit volume. Anaemia, under copper induced stress, may also be due to blood cell injury and disrupted hemoglobin synthesis (Mckim et al., 1970; Gross et al., 1975). The anaemic condition in fish results from an unusually low number of red blood cells or too little hemoglobin in the red blood cells. Similar results with significant reduction of RBC and Hb% content in fishes exposed to different heavy metals have been reported previously by Goel et al. (1985) and Goel and Sharma (1987). According to Pamila et al. (1991), the reduction in hemoglobin content in fish exposed to toxicant could also be due to the inhibitory effect of the toxic substance on the enzyme system responsible for synthesis of hemoglobin. Devi and Nandan et al. (1975) suggested that heavy metal exposure also decreased the RBCs, Hb% and PCV% due to impaired intestinal absorption of iron. Anaemia is an early

**Table-1:** Summarization of heavy metals concentration in water

Heavy metal	Test Method Used	Result (mg/L)
Copper as (Cu)	IS 3025 (P 42): 1992 (RA 2014)	0.340
Zinc as (Zn)	IS 3025 (P 49): 1994 (RA 2014)	0.236
Lead as (Pb)	IS 3025 (P 47): 1994 (RA 2014)	Not Detected

**Table-2:** Effect of heavy metals on hematological parameters of *Channa punctatus*

Parameters	Control	Days of exposure period		
		Collected on 15day	collected on 30 day	Collected on 45 day
Hb gm (%)	9.64 ± 0.16	10.06 ± 0.13*	8.80 ± 0.10*	6.60 ± 0.11*
RBC(x10 <sup>6</sup> /mm <sup>3</sup> )	1.96 ± 0.05	2.46 ± 0.05*	2.08 ± 0.90*	1.84 ± 0.02*
WBC(x10 <sup>3</sup> /m <sup>3</sup> )	59.00 ± 1.03	81.30 ± 1.94*	91.88 ± 1.34*	92.48 ± 0.91*
ESR ( mm / hr)	3.90 ± 0.45	8.00 ± 0.46*	8.56 ± 0.32*	12.56 ± 0.66*
PCV (%)	31.00 ± 1.00	27.7 ± 0.32*	24.66 ± 1.19*	23.33 ± 0.33*
CT (Second)	26.60 ± 1.45	30.7 ± 1.74*	38.00 ± 2.07*	52.00 ± 1.15*
MCV (cμ)	107.11 ± 2.00	112.31 ± 3.10 <sup>NS</sup>	118.16 ± 0.70*	126.75 ± 2.38*
MCHC (%)	32.87 ± 0.61	36.95 ± 1.60 <sup>NS</sup>	32.08 ± 1.02*	28.28 ± 0.64*
MCH (μg %)	36.67 ± 0.05	40.10 ± 0.64*	43.28 ± 1.22*	35.84 ± 0.17 <sup>NS</sup>
Bilirubin(mg/ml)	0.68 ± 0.32	0.48 ± 0.32	0.50 ± 0.28	0.51 ± 0.23
Serum glucose (mg/ml)	17.02 ± 0.05	17.18 ± 0.18**	17.78 ± 0.22**	17.98 ± 0.20**
Serum protein (g/dl)	3.98 ± 0.51	3.78 ± 0.48	3.65 ± 0.50	2.75 ± 0.38*
Triglyceride(mg/dl)	49.80 ± 0.31	53.62 ± 0.33	52.32 ± 0.3	56.28 ± 0.32
Cholesterol (mg/dl)	72.20 ± 0.22	78.38 ± 0.33	78.65 ± 0.53	80.32 ± 0.18*
Acid phosphatase (U/L)	1.34 ± 0.78	1.36 ± 0.72	1.38 ± 0.48	1.42 ± 0.48
Alkaline phosphatases(U/L)	3.28 ± 0.56	3.30 ± 0.45	3.34 ± 0.26	4.02 ± 0.26
SGOT(U/L)	65.05 ± 0.08	66.10 ± 0.20	66.70 ± 0.32	69.42 ± 0.43
SGPT(U/L)	32.35 ± 0.41	32.83 ± 0.32	33.05 ± 0.18	72.18 ± 0.18

Values are mean ± SEM, n=3, \* = significant at p < 0.05 level, Hb% = Hemoglobin, RBC = Red blood cells, WBC = White blood cells, ESR = Erythrocyte sedimentation rate, PCV% = Packed cell volume, CT = Clotting time, MCV = Mean Corpuscular Volume, MCHC = Mean corpuscular Hemoglobin concentration, MCH = Mean corpuscular Hemoglobin

manifestation of acute and chronic intoxication of heavy metals. Significance of these changes may be understood in terms of reduced oxygen consumption in fish resulting in death due to heavy metal pollution (Christensen *et al.*, 1972).

High white blood cell counts indicate damage due to infection of body tissues, severe physical stress, and as well leukemia. In most cases, abnormal red cell morphology is noted. White blood cell counts were found increased following copper exposure as shown in table 2. Similar findings were also documented significantly higher in fish exposed to increased copper concentration (Devi, 1975). Mishra and Srivastava (1980) also reported an increase in leucocytes count when they exposed fishes to heavy metals. Some of the most common causes of heavy metal toxicity are inflammatory lesions associated with tissue damage, anaemia and neoplasia. Further, an increase in fibrinogen or serum globulins or a decrease in serum albumin, may also cause an increase in the ESR. Increase in ESR and MCV values and total leucocytes count suggested that the anaemia was of macrocytic type (Sampath *et al.*, 1998).

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