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Toxic Effects of Heavy Metals (Cu, Ni, Fe, Co, Mn, Cr, Zn) to the Haematology of *Mastacembelus armatus* Thriving in Harduaganj Reservoir, Aligarh, India

Mehjbeen Javed [α] & Nazura Usmani [σ]

Abstract - Investigations were made on the blood of *Mastacembelus armatus* exposed to heavy metals (Cu, Ni, Fe, Co, Mn, Cr and Zn). The study reveals the contamination of Harduaganj Reservoir with heavy metals. The heavy metal content in reservoir water were in the order of Fe > Cu > Zn > Mn > Ni > Co > Cr. The values for the heavy metals such as Fe (8.71 mgL^{-1}), Ni (0.12 mgL^{-1}), and Mn (0.21 mgL^{-1}) were higher than the limits suggested by UNEPGEMS. Whereas the concentration of heavy metals Cu, Cr and Zn were within the limits proposed. *Mastacembelus armatus* thriving in the reservoir water exhibited the influence of contamination.The exposed fish showed the significant decrease in total RBC count (2.16 $x10^6 mm^{-3}$), significant increase in total WBC count (3.84 x 10^3 mm^3) and insignificant decrease in Hb (9.48 gdL^{-1}) and when compared to control.

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I. Introduction

Aquatic pollution from sources like effluents from industries, power plants untreated domestic and sewage waste etc. have adverse effects on aquatic ecosystem. Due to which the animals thriving in these water bodies exposed to unnaturally high levels of contaminants. Along with other animals fishes are the animals that cannot escape from the detrimental effects of these contaminants. The heavy metals are of particular concern due to their persistence and undegradable nature. The metal contamination in aquatic ecosystem is considered to be unsafe not only for fishes but, also for human beings because they consume fishes whuch are best sources of proteins and essential amino acids . Fish blood is known to exhibit pathological changes before the onset of any external symptoms of toxicity and it truly reflects the physical and chemical changes occurring due to heavy metal accumulation in body of fish. Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management and disease investigations (Mulcahy 1975; Bansal et al. 1980). It is a pathophysiological indicator of the whole body function and therefore blood parameters are important in diagnosing the structural and functional status of fish exposed to a toxicant. The accumulation of heavy metals in a fish depends mainly on the concentration of the metal in the water and exposure period. Fishes exposed to heavy metal pollutants can induce either increases or decreases in haematological levels. A number of haematological indices such as haemoglobin (Hb), haematocrit (Hct), red blood cells (RBCs), white blood cells (WBCs) and so on, have been used as an indicator of metal pollution in the aquatic environment. Furthermore, it should be noted that haematological indices are of different sensitivity to various environmental factors and chemicals (Vosyliene, 1999b). Previous haematological studies of pollutants brought to the knowledge that erythrocytes are the major and reliable indicators of various sources of stress (O' neal and Weirich, 2001). Hematological abnormalities have also been studied in various toxicants exposed fish: *Channa punctatus* to lead (Hymavathi and Rao 2000); *C. punctatus* to cadmium (Karuppasamy et al. 2005); and *Labeo rohita* to synthetic detergents (Chellan et al.1999).

Harduaganj Thermal Power station (HTPS) / Kasimpur power station (Fig. 1) is located at Harduaganj (27.218° N and 79.378° E), district Aligarh, India. The waste water containing heavy metals from the power plant reaches the nearby Harduaganj reservoir. This reservoir has water filled area of 13.5 ha. This power plant have a total capacity of power generating of 700 MW, uses sulphur rich bituminous coal as fuel at the rate of 11,65,069 tonnes/ annum. The pollutants,being released in the man made Harduaganj reservoir through condensation process using certain gases, fly ash and traces of heavy metals. Hence, it is obvious that this may also exert tremendous impact on the aquatic ecosystem. Fishes, specially are being the potential indicators of pollution, and clearly indicate the pollution status of the reservoir.

Thus haematological studies are useful not only in assessing the health of fish subjected to changing

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environmental conditions but, also for the deteriorating water quality. Keeping this in view, a study was conducted to assess the changes in fish blood exposed to excess of potentially toxic heavy metals in the wastewater and to provide the fish as a bioindicator.

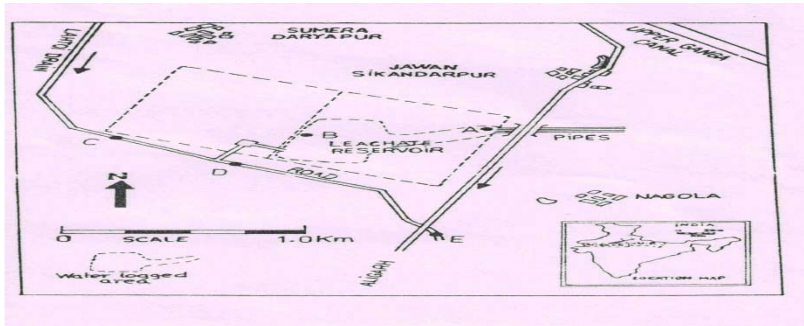

Figure : 1 Map showing the Harduaganj Reservoir, Aligarh, India.

II. Materials and Methods

Water was collected at the time of fish collection into the pre-cleaned and acidified glass bottles. The bottles were immediately brought to the laboratory and acidified with concentrated HNO_3 to pH less than 2.0. On spot fixation of water was done to measure the dissolved oxygen (D.O). Total solids (T.S), total dissolved solids (T.D.S) and total suspended solids (T.S.S) using standard techniques (APHA 2005).The temperature and pH were recorded at the site using laboratory thermometer (Deluxe, 6) and pH strips (S.D Fine chemicals, 0 - 0.1).

The analysis of water for the presence of heavy metals (Cu, Ni, Fe, Co, Mn, Cr and Zn) was performed according to APHA (2005). Blanks were prepared along with each set of the sample. Standard solutions for heavy metals were prepared using standard techniques (APHA 2005).

The water sample was analyzed for heavy metals by Atomic Absorption Spectrometer (Perkin Elmer, AA 800, multiple cathode lamps) with specific cathode lamps for each metal and Nitrous oxide-Acetylene was used as flame. The following analytical conditions of the instrument were used for atomic absorption of these metals Table 1.

Table : 1 Instrumental analytical conditions of heavy metal analyses.

Heavy Metals	Wavelength (nm)	Slitwidth (nm)	Optimm working range(μgml^{-1})	Sensitivity (μgml^{-1})	Lamp current (mA)
Cu	327.4	0.2	2.5- 10	0.050	3.0
Ni	341.5	0.2	6-25	0.12	3.5
Fe	372.0	0.2	20-80	0.45	7.0
Co	346.6	0.2	90-450	2.3	6.0
Mn	403.1	0.2	7-27	0.15	5.0
Cr	425.4	0.2	7-40	0.17	6.0
Zn	213.9	0.2	0.4-1.5	66	5.0

Fish *Mastacembelus armatus* is a predominant species of the reservoir, (stock 5, n= 5, length 14.20±1.5 cm, weight 35±0.42g,), were collected from the fishermen working beside the Reservoir. Procured fishes were washed with double distilled water and wiped dry with clean muslin cloth. Blood was collected from heart puncture of fish by heparinised syringe in vials containing heparin anticoagulant, which was used to estimate the haematological parameters. The vials and fishes were kept in separate ice boxes and brought to the laboratory for further analysis. Control fishes (Stock 5, n=5, length=14.99±1.43cm, weight= 36.12±0.98g) were procured from market and reared in laboratory. These fishes were kept in chlorine free tap

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water which was changed every morning and maintaining ideal water quality conditions.

The RBC counts were made by Neubaur Haemocytometer. Blood was diluted 1:200 with Hayem's solution. Counting was done under the binocular microscope in the five smaller squares i.e. in the 1st, 5th, 13th, 21st and 25th. The RBC's on the lower and right sides of a square were added in the total, while those on the upper and left sides were rejected. Total numbers were reported as10^6 mm^{-3} (Wintrobe, 1967).

The WBC counts were made by Neubauer Haemocytometer. Blood was diluted 1:20 with Turk's diluting fluid and placed in haemocytometer. Four large (1 sq mm) corner squares of the haemocytometer were counted under the binocular microscope. The total number of WBC was calculated in $mm^3 \times 10^3$ (Wintrobe, 1967).

Haemoglobin (Hb) was determined with haemoglobin test kit (DIAGNOVA, Ranbaxy, India) using the cyanmethaemoglobin method.

Experiments were conducted in triplicates. Values are presented as Means ± SEM. Values of RBC, WBC and Hb of fish blood were compared statistically with control by using student's t-test (2- tailed) with the help of SPSS 17. The level of significance was established at $P < 0.01$.

Table : 2 Water quality parameters of Harduaganj Reservoir water.

Water quality parameters	Values
Temperature	27.6±0..36°C
pH	6.9±0.29
Dissolved oxygen (D.O)	6.9±0.0 mgL^{-1}
Total solids (TS)	652±0.70 mgL^{-1}
Total Dissolved solids (TDS)	407±0.06 mgL^{-1}
Total Suspended solids (TSS)	245±0.50 mgL^{-1}
Cu	0.86± 0.06 mgL^{-1}
Ni	0.12± 0.01mgL^{-1}
Fe	8.71± 1.66 mgL^{-1}
Co	0.11± 0.01 mgL^{-1}
Mn	0.21± 0.05 mgL^{-1}
Cr	0.10± 0.01 mgL^{-1}
Zn	0.30± 0.02 mgL^{-1}

Values are Mean ± SEM, (n= 3).

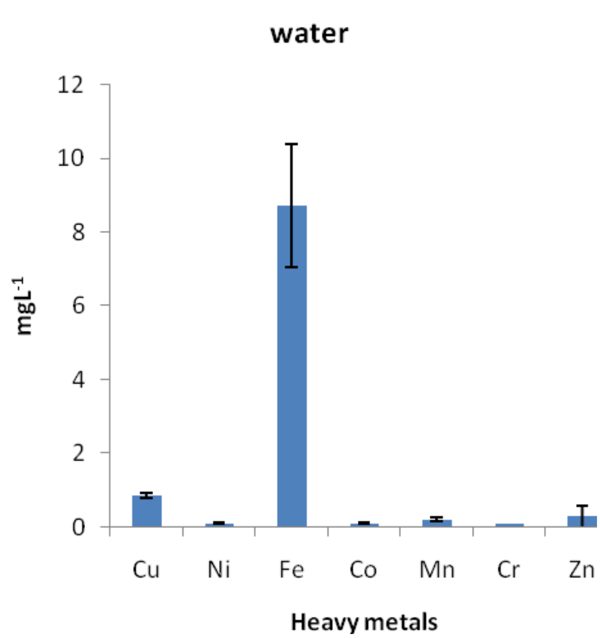

Figure : 2 Heavy metal concentration in water of Harduaganj Reservoir.

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Table : 3 Summary of water quality guidelines and standards by International Organization or country.

Param eter↓	WHO (guidelines) mgL^{-1}	E U (standards) mgL^{-1}	Canada (guidelines) mgL^{-1}	Australia (guidelines) mgL^{-1}	N Z (guidelines) mgL^{-1}	Japan (standards) mgL^{-1}	USA (standards) mgL^{-1}	Present Study mgL^{-1}
Copper	2	2	2	1	2	1	1.3	0.86
Nickel	0.02	0.02	0.02	0.02	0.02			0.12*
Iron		0.2	0.2	0.3	0.01	0.3	0.3	8.71*
Cobalt								0.11
Manga -nese	0.5	0.05	0.05	0.5	0.5	0.05	0.05	0.21
Chrom-ium	0.05	0.05	0.05		0.05	0.05	0.1	0.1
Zinc	3			3		1	5	0.3

Adapted for Water Quality for Ecosystem and Human Health, 2006 (prepared and published by the United Nations Environment Programme.
Global Environment Monitoring System (GEMS)/ Water Programme).
Blank cells indicate that no, citable information was available.
*Indicates these values are far exceeding the recommended limit.

Table : 4 Total count of RBC's, WBC's, and Haemoglobin in the control and exposed *Mastacembelus armatus.*

Variable	Control (Mean±SEM)	Exposed (Mean±SEM)
No. of RBC ($10^6 mm^{-3}$)	4.31±0.34*	2.16±0.08*
No. of WBC ($10^3 mm^{-3}$)	2.55±0.16*	3.84±0.18*
Haemoglobin (g/dL)	9.70±0.23	9.48±0.52

Values are given as Mean ± SEM, (n = 5), *Significantly different at (P < 0.01

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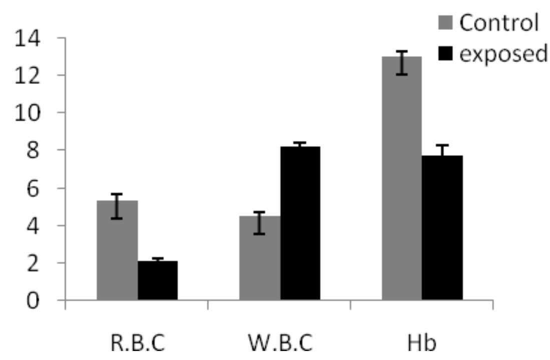

Figure : 3 Graphical representation of total Count of RBCs, WBCs and Hb in the Control and exposed *Mastacembelus armatus.*

III. Results and Discussion

Table 2 and figure 2 shows the mean values of physicochemical parameters and concentration of heavy metals (mg L^{-1}) in water. The temperature, pH, dissolved oxygen and total, suspended and dissolved solids are upto the ideal water quality guidelines (Vijay kumar, 1999). Therefore these are no more responsible for stress which the *Mastacembelus armatus* was undergoing. The heavy metal content in reservoir water were in the order of Fe (8.71) > Cu (0.86) > Zn (0.30) > Mn (0.21) > Ni (0.12) > Co (0.11) > Cr (0.10). The heavy metal content in reservoir water was compared with the quality guidelines and standards of United Nations Environment Programme Global Environment Monitoring System (UNEPGEMS, 2006) (Table 3). The Fe and Ni were found to be exceeding than the recommended limits. The results of hematological studies of *Mastacembelus armatus* are summarized in Table 4 and Figure 3.

Water quality parameters are one of the major factors responsible for individual variation in fish hematology. Since hematological parameters are necessary for clinical diagnosis of a disease and pathological conditions in human, these criteria should receive enough attention in assessing the health of the fish with regard to aquatic pollution and has been accepted by many workers such as McCarthy et al. (1973) and Christensen et al. (1978). While evaluating the total effect of heavy metals on the hematological indices of *Mastacembelus armatus*, a synergetic effect of these metals was found on the erythrocyte count, concentration of hemoglobin, and the present leucocytes. Blood forms a unique compartment between external and internal environments and any agent including toxic substances that causes stress and can alter blood composition either directly or indirectly by altering osmotic and ion regulation.

In the present study, the erythrocyte count of healthy controls showed a mean value of 4.31 x 10^6 mm^{-3} and Hb 9.70 gdL^{-1}. The fish *Mastacembelus armatus* procured from Harduaganj Reservoir showed mean values of RBC 2.16 x 10^6 mm^{-3} and Hb 9.48 gdL^{-1}. The values mentioned above showed a significant ($P < 0.01$) decrease when compared to the control. The reported value of Hb is insignificant. This study on hematological changes in fish serves as an effective tool in the diagnosis of the extent of environmental pollution and also the abiotic fish diseases. Hypoxia, anemia, and hyperthermia are related stresses causing an osmotic imbalance and decreased capacity of the RBC to carry sufficient oxygen unless otherwise compensated by erythropoiesis or suitable physiological adjustments. Lowering of TEC count coupled with low Hb content here may be due to destructive action of pollutants on erythrocytes and as a result of which the viability of the cells may be affected as was also reported by Karuppasamy (2000). This supports the findings of present study that is lowering in RBC count and Hb content. Multiple form of hemoglobin allows fish to adjust more efficiently to physiological stress such as varying water temperature and oxygen concentration (Hochachka and Somero 1973). Hemolysis occurs in response to toxicity that leads to alteration in the selective permeability of the membrane (Das et al. 1987). All these reports are in agreement with the present study of reduction in TEC count and Hb content of fish from polluted lakes due to the inhibition of aerobic glycolysis curtailing synthesis of iron and hemoglobin via the lowered energy status in fish (Joshi et al. 2002). It has been suggested that heavy metal exposure decreases the TEC count, Hb content due to impaired intestinal absorption of iron.

The results of the total count of white blood cells revealed that the blood of the control fish showed a mean value of 2.55 x 10^3 mm^3. Exposed Mastacembelus armatus showed the mean value of WBC as 3.84 x 10^3 mm^3. The values mentioned above showed a significant increase when compared to the control ($P < 0.01$).

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Increase in TLC in the present study was a result of direct stimulation for its defense from diseases due to the presence of polluted substances. Progressive increased levels of TLC have been reported in *C. punctatus* exposed to lead (Hymavathi and Rao 2000) and *Clarias batrachus* exposed to mercuric chloride (Joshi et al. 2002). Leukocytosis is directly proportional to severity of stress condition in maturing fish and is a result of direct stimulation of immunological defense due to the presence of pollutants (heavy metals) in this reservoir. The observations made in present study for WBC are also in good agreement with those of Karuppasamy et al. (2005) and Hardikar and Gokhale (2000).

Hence, the present investigation results confirm that stress due to various heavy metals present in the reservoir does create hematological disturbances, erythrocyte destruction (hemolysis), and leukocytosis in fish population, affecting the immune system and making the fish vulnerable to diseases.

IV. Acknowledgements

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