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HAEMATOLOGICAL STUDIES OF FRESH WATER FISH, *LABEO ROHITA* (HAM.) EXPOSED TO HEAVY METALS

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Abstract: The main aim of the present study is to know the effect of heavy metals in Tawa reservoir on *Labeo rohita* and their seasonal variations. During the study, *Labeo rohita* were exposed to pre monsoon, monsoon and post monsoon seasons at different sites in Tawa reservoir. The red blood cell (RBC) count decreases when compared to the control 3.42±0.47 x 10^6/mm^3, pre monsoon (2.93±0.49 x 10^6/mm^3), monsoon (2.81±0.47 x 10^6/mm^3) and post monsoon (2.90±0.50 x 10^6/mm^3) periods. The Heamoglobin percentage also decreases when compared with the controlled fish (9.16±0.75%), pre monsoon (8.73±0.14%) , monsoon (7.43±0.35%) and post monsoon seasons (8.71±0.16%). The number of white blood cells (WBC) increases more during monsoon period (5.80±0.32 x10^3/mm^3) as compared to control (4.63±0.37 x10^3/mm^3), pre monsoon (5.03±0.54 x10^3/mm^3) and post monsoon seasons (5.47±0.54x10^3/mm^3).The Haematocrit value decreases from control group of fishes to postmonsoon ones. The results are statistically significant at $p< 0.005$ level.
Keywords: *Labeo rohita*, Heavy metals, Monsoon, RBC, WBC, Hb.

Introduction

Heavy metals are one of the major pollutants, which are present in small quantities in the earth's crust. Heavy metals constitute an important class of toxic substances, which are encountered in numerous occupational and environmental circumstances. The heavy metals are present at <0.1% level in earth's crust. But anthropogenic, geochemical factors are releasing a large number of toxic heavy metals into the aquatic ecosystem (1). The heavy metals produce their toxicity by forming complexes or legends with organic compounds. These modified molecules lose their ability to function properly and thus result in malfunction or death of affected cells. When heavy metals bind to these groups they may inactivate important enzyme system or affect protein structure of an animal. Heavy metals are major pollutants of aquatic environment because of their environmental persistence and ability to be accumulated by aquatic organisms (2).

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Heavy metals when reach the aquatic bodies deteriorate the life Sustaining quality of water and cause damage to both flora and fauna (3). The problem of heavy metal pollution increases many folds due to their long half life period and properties of non-biodegradability, bioaccumulation and biomagnifications (4).

Blood parameters are considered pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants. A number of haematological indices such as haematocrit (Hct.), Hb, RBCs and WBCs, are used to assess the functional status of the oxygen carrying capacity of the bloodstream and have been used as indicator of metal pollution in the aquatic environment (5).The present study was undertaken to analyze the impact of sub lethal concentrations of heavy metals on haematological parameters of *Labeo rohita*.

Material and Methods

The test fish *labeo rohita* of almost same size (30 to 40cm) were caught alive in healthy conditions from Tawa reservoir Distt. Hoshangabad (M.P.) during pre monsoon, monsoon and post monsoon seasons, also some fishes were purchased from the members of Tawa corporate society. The fishes were transported from the reservoir in oxygenated bags to the Laboratory and immediately some control fishes transferred into the glass aquaria of 50 liters capacity containing well aerated, unchlorinated ground water for 15 days acclimatization. The other fishes with the active movement were only used for the experimentation. The fishes were screened for any physical damage, disease and mortality. The immobilized, injured, abnormal and dead fishes were discarded immediately. The live specimen of adult fresh fish *labeo rohita* having body weight 38-40grams were dissected immediately for biochemical and histological studies.

However the control fishes were feed every day twice with wheat flour pellets, boiled egg protein and grounded dried shrimps purchased from local fish market. Before stocking, the aquaria were washed with 0.1% $KMnO_4$ to free the walls from any possible fungal infections if any and acclimatization was judged satisfactory when the incidence of fish monolith was less than 10% of total fish during one week prior to the commencement of the experiment. The fishes were also treated with 0.1% $KMnO_4$ solutions to check any possible bacterial infections. The media in aquaria for control fish was renewed on alternate days to prevent accumulations of metabolites. Apart from one control aquaria and other experimental

fishes caught during pre monsoon, monsoon and post monsoon seasons were maintained for acute toxicity test, while the concentrations in the aquaria with one control fish group were maintained and the other groups of fishes was taken direct for experimentation to analyze the heavy metal concentrations in the different organs of the fish *L. rohita*.

Also the individual fish in the control aquaria were considered dead when they failed to respond to touch stimulus. After the death of each fish, its body was removed immediately from the container along with the aquaria water allotted for each fish. The aquaria water containing the compounds was renewed after each 24^{th} hour keeping the concentration constant throughout the experiment. After 15 day acclimatization of control fish, survived individuals in the container were counted. Then the control fish were dissected in the laboratory. Determination of heavy metal concentration values in adult *labeo rohita* had been done. Percentage of mortality in static bio assay was converted into probit mortality.

Results and Discussion

Blood is a pathophysiological reflector of whole body and therefore blood parameters are important in diagnosing the functional status of the animal exposed to toxicants. Hematological analysis therefore can serve as a rapid and economical method for assessing the metal toxicity on fishes. Anemia is one of the most sensitive pathological situations developed as a result of metals poisoning. A value of different hematological parameters of control and experimental fishes during pre monsoon, monsoon and post-monsoon seasons is compared in Table 1.

In present Observation the heavy metal concentrations in *Labeo rohita* during monsoon season, resulted into significant reduction in RBC count and Hb%, While increase in WBC count as compared to control were observed. The values of reduced RBC's were more marked in monsoon season exposure ($2.81\pm0.47x10^{6}/\mu l$) than control fish ($3.42\pm0.47x10^{6}/\mu l$) and pre monsoon ($2.93\pm0.49x10^{6}/\mu l$). The Hb% also depleted more significantly in monsoon exposure ($7.43\pm0.35\%$), a clear indication of comparatively more toxic nature of monsoon season because of high concentration of heavy metals than pre monsoon ($8.73\pm0.14\%$) and postmonsoon period ($8.71\pm0.16\%$). It is inferred that the decrease in Hb% in the present study may be due to anemia caused by heavy metal mixtures during pre monsoon, monsoon and post monsoon seasons. It may be due to the decrease rate

of production of erythrocytes or increased loss of these cells or impaired erythropoietin due to direct effect of heavy metals in the reservoir on haematopoietic centers.

However the value of WBC increases more in *Labeo rohita* during monsoon period (5.80±0.32x10³/µl) as compared to control fish (4.63±0.37x10³/µl), pre monsoon (5.03±0.54x10³/µl) and post monsoon fish (5.47±0.54x10³/µl). Hematocrit(PCV%) value of control fish were observed to be (28.06±0.84%), during pre monsoon the value decreases(26.33±1.06%) and during monsoon season exposure the Hematocrit value decreases more significantly (23.86±0.84%).Similarly the values of MCHC of control fish were to be (31.11±2.13%), but during pre monsoon the value were observed to be (30.80±2.05%) and during monsoon season the value will decreases to be (29.78±1.71%) , while during post monsoon exposure the value of MCHC will be (30.26±1.49%).However the value of MCH in control fish were to be (27.13±1.73%) and during pre monsoon period the value to be(28.01±2.36%).The MCV value of control fish were to be(84.8±12.58%) and during post monsoon seasons the value were to be(85.26±9.30%) as depicted in Table 1 . Similar findings were reported by (6-8).

Heavy metals have a great ability to accumulate in kidney, gill, liver and other tissue (9). Kidney and liver is the major target organs of heavy metal poisoning, as these are the hemopoietic organs (10). After seasonal changes heavy metals showed significant decrease of the erythrocyte count and Hb%. In most vertebrates including fishes, erythropoietin activity is regulated by the erythropoietin hormone produced in kidney. Erythropoietin promotes erythropoiesis by inducing hemopoietic stem cells to differentiate into erythroblasts, which forms RBCs. Erythropoietin also activates pyridoxal phosphate in developing maturing RBCs, including hemoglobin synthesis (6). Hypoxia constitutes the fundamental stimulus for erythropoisesis with the kidney as probable sensing organ for low blood oxygen tension. A structurally and normally functioning kidney is essential for erythropoietin production. Kidney damage usually cause a decrease in erythropoietin level, which in turns decreases RBCs production and hemoglobin synthesis even under hypo toxic condition (6). The decrease in hemoglobin on exposure of mercury chloride was due to decrease of RBCs number, which in turn might be due to sodium arsenate effect on erythropoietic organs primarily like kidney and spleen which lead into inhibited erythropoiesis and thus inadequate hemoglobin synthesis a similar view with Srivastava (11). Anemia can also be caused by other number of pathological conditions such as lysis of RBC, erythropenia, increased ESR or hemodilution. It has been reported leucopenia caused by

toxicant was among the relevant reasons of fish death. The above discussion led us to conclude that pre monsoon, monsoon and postmonsoon alters the normal hematological value. Such response would be result to toxicity of chemical substance which induces tissue damage. Tawari also observed the similar results in all the Hematological parameters WBC, PCV, Hb% and platelets reveled significant difference between control and poisoned fishes on exposure of $CdSO_4$ and $PbNo_3$ (8).

The erythrocyte counts decreased more in high concentration that is during monsoon period in *Labeo rohita* in Tawa reservoir as compared with control group. The values of reduced RBC's were more marked in monsoon season exposure than control fish and pre monsoon fish. The Hb% also depleted more significantly in monsoon exposure, a clear indication of comparatively more toxic nature of monsoon season because of high concentration of heavy metals than pre monsoon and postmonsoon period. Although in present study value of RBC, Hb% decreases with increase in concentrations of heavy metals. However other authors have determined that acute 48 hour exposure of catfish *Clarias garepinus* from Claridae family to copper concentration induce significant decrease in the count of erythrocyte as well as hemoglobin concentration. Although after 48 hours exposure these two indices slightly increase. Hemolysis and anemia were determined in catfish (*Clarias laser*) after 96 hour exposure to 32.0 mg/L of copper (12). The lethargic condition of fish in late, period of $CuSO_4$ exposure corroborates the findings of (13). The presence of immature erythrocyte and broken fragment of RBCs corroborates the findings (14). During present investigation the number of erythrocytes increased but later it decreases. The occurrence of similar hematologic anomalies has also been already reported in *C. fasciatus* after acute exposure to sub lethal concentrations of nickel and chromium by (15). Erythrocyte sedimentation rate (ESR) in copper treated fish *H. fossilis* reflected the elevated erythrocyte count.

The total erythrocyte, packed cell volume (PCV) and MCV indicated the RBC destruction thus manifestation of anemia associated with erythropenia. They are pre determined both by concentration of heavy metal in water and time exposure, and both these factor can cause reversible and irreversible changes in the homeostatic system of fish. The damaged intestinal villi have been observed in *Heteropneustes fossils* following exposure of fish to Zinc and Chromium mixture (16) thus resulting in poor iron absorption which is solely essential for erythropoisis, may be another reason of decreased level of hemoglobin. The decreased RBC count in present study can also be correlated with the reduction in

oxygen carrying capacity due to gill damage by metals a parallel finding, with (6). Gradual decrease in total RBC count, hematocrit and Hb% reflected the anemic condition of fish and disruption of young blood cells due to toxicity of heavy metal mixtures in the Tawa reservoir, present study is in conformity with the result of *C. carpio* exposed to Malathion, (17) in cat-fish *Clarias batrachus* exposed to copper, Srivastava (11) in *H. fossilis* induced to chlorodecon, (18). Heavy metals exposure to *labeo rohita in* present study induced leucocytosis as advocated by increased WBC count. The number of WBC increased regularly in the monsoon period of exposure, which seems to be associated to malfunctioning of hemopoietic system stressed by heavy metals intoxication. During recovery of number of WBC began to normalize but still remained above the control value (i.e. in post monsoon), this might be due to gradual automatic repair of damaged hemopoietic tissue to tide over stressful condition caused by mixture of heavy metal exposure.

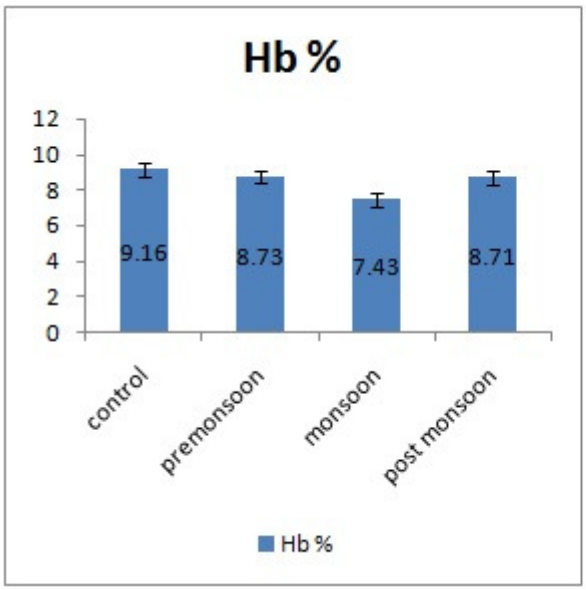
White blood corpuscles play a major role in the defense mechanism of fish which may be directly proportional to the severity of the causative stress condition and may be attributed to an increase in leucocytes mobilization. The leucocytosis is for the removal of cellular debris of necrosed tissue at a quicker rate, also evidenced by (19) in *H. fossilis* exposed to manganese. In this study the WBC count increased from normal values. Many investigators have demonstrated that the leukocytes of teleost are extremely sensitive to toxicant. The number of circulating WBCs in *C. fasciatus* decreases following exposure to heavy metal (15). Leucopenia has also been reported in other teleosts exposed to heavy metal (20) and pollutants (21). Thrombocytosis and decreased blood clotting time was observed in *C. fascoatis* following exposure to copper and cobalt (15).

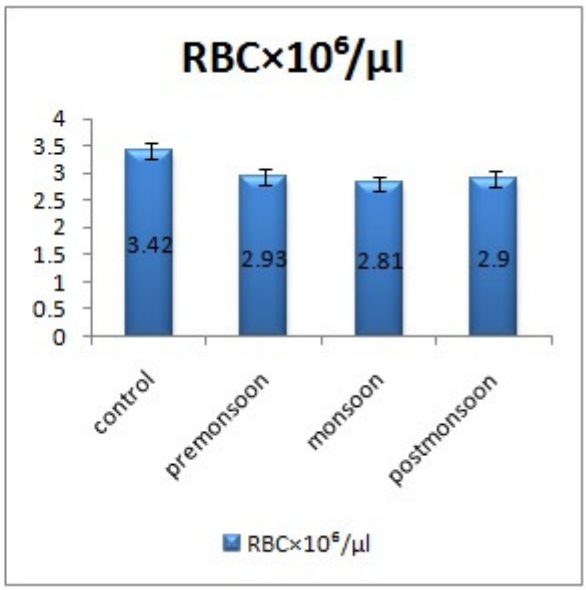
Significant increased value of the MCV to *H. fossilis* after exposure to copper and arsenic in present study also supported by (22-23) in fresh water teleost fish against various toxicants. Increase in MCV values in both sub lethal concentration of both copper and arsenic in this study may be considered as good index of RBC destruction, swelling of RBC, due to endosmosis and thus disturbed osmoregulations.

Table 1: Haematological parameters of *Labeo rohita*, caught from Tawa reservoir during pre monsoon, monsoon and post monsoon seasons:

Serial. No	BLOOD Parameters	Control	Pre monsoon	Monsoon	Postmonsoon	Significance
1	RBC×10⁶/µL	3.42±0.47	2.93±0.49^{NS}	2.81±0.47^{NS}	2.90±0.50^{NS}	P >0.05
2	Hb%	9.16±0.75	8.73±0.14*	7.43±0.35*	8.71±0.16*	P<0.05
3	WBC×10³µL	4.63±0.37	5.03±0.54*	5.80±0.32*	5.47±0.54*	P<0.05
4	MCH	27.13±1.73	28.01±2.36^{NS}	23.45±1.80^{NS}	27.73±2.7^{NS}	P>0.05
5	PCV%	28.06±0.84	26.33±1.06*	23.86±0.84*	24.96±0.75*	P<0.05
6	MCHC%	31.11±2.13	30.80±2.05^{NS}	29.78±1.71^{NS}	30.26±1.49^{NS}	P>0.05
7	MCV%	84.8±12.58	82.94±8.95^{NS}	49.37±13.13^{NS}	85.26±9.30^{NS}	P>0.05

Values are Mean ± SEM, n =3, * = Significant at p <0.05, ^{NS} = Not Significant, RBC = Red blood cells, Hb = Haemoglobin, WBC = White blood cells, MCH =Mean corpuscular volume, PCV% = Packed cell volume, MCHC = Mean corpuscular Haemoglobin concentration, MCV = Mean corpuscular Haemoglobin.





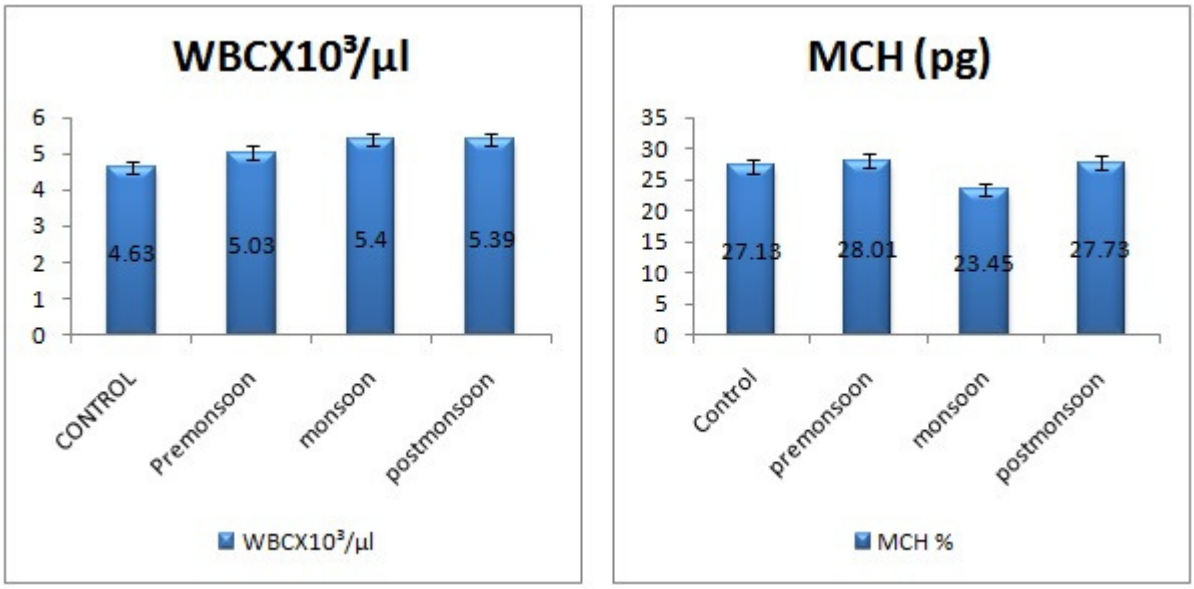
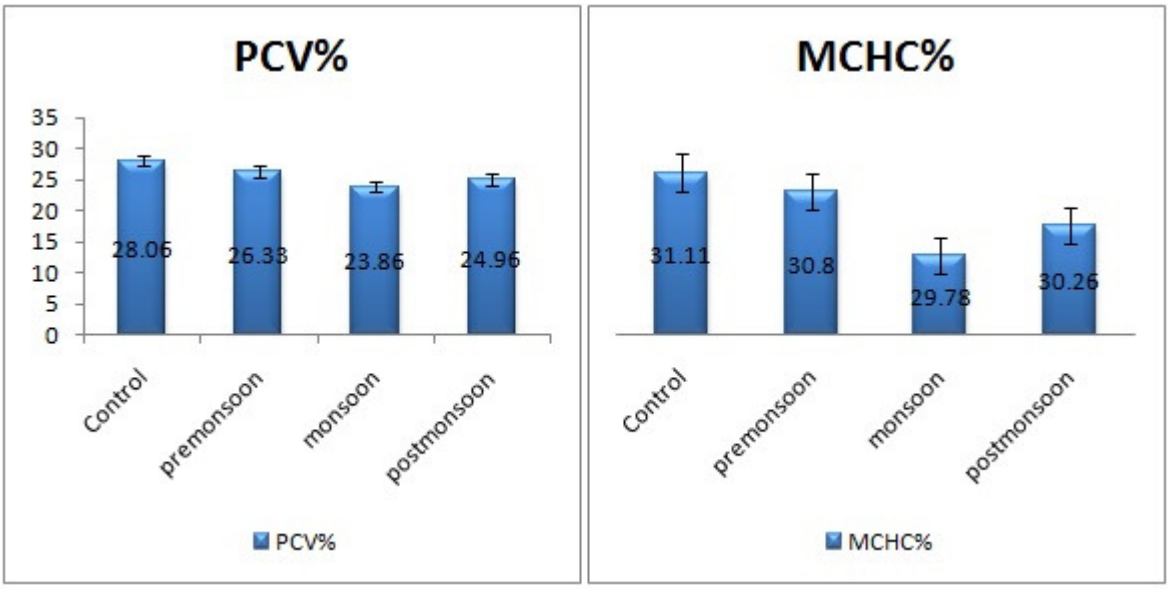


Fig 1-4: Haematological responses in *Labeo rohita* showing % increase (+) or decrease (-) captured during pre monsoon, monsoon and seasons in Tawa reservoir.



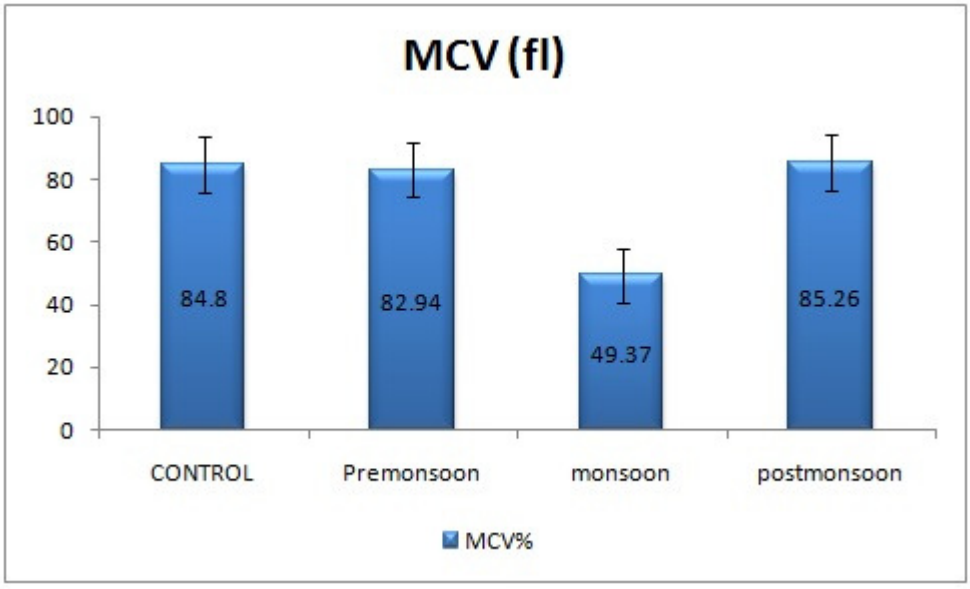


Fig 5-7: Haematological responses in *Labeo rohita* showing % increase (+) or decrease (-) captured during pre monsoon, monsoon and seasons in Tawa reservoir.

Conclusion

Considerable changes were observed in Haematological parameters of *Labeo rohita* during premonsoon, monsoon and postmonsoon seasons due to accumulation of mixture of various Heavy metals. These variations represent disturbances in the metabolic processes of the organism. Measurement of these haematological parameters indicates the effects of stress and abnormality in Labeo rohita which confirms the increasing concentration of heavy metals and threat to the aquatic biota.

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