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HAEMOTOXICITY OF LEAD (Pb) ON THE BLOOD PARAMETERS OF FRESH WATER TELEOST *HETEROPNEUSTES FOSSILIS* (BLOCH)

V. K. Verma ¹, N. Gupta ^{*2} and D. K. Gupta ³

Department of Environment Science ¹, Future Institute of Engineering and Technology, Bareilly - 243123, Uttar Pradesh, India.

Chhatrapati Shahu Ji Maharaj University ², Kanpur - 208024, Uttar Pradesh, India.

Department of Biotechnology ³, Bareilly College, Bareilly - 243001, Uttar Pradesh, India.

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Correspondence to Author:

Prof. Neelima Gupta

Vice-Chancellor,
Chhatrapati Shahu Ji Maharaj
University, Kanpur - 208024,
Uttar Pradesh, India.

E-mail: guptagrawal@rediffmail.com

ABSTRACT: Blood parameters are important indicators for assessing the health status of living organisms subject to changing environment. Blood parameters (Hb%, TEC, TLC, ESR, PCV, MCH, and MCV) of *Heteropneustes fossilis* exposed to lead were investigated at different time intervals. Acclimatized specimens were divided into four groups: Group-A (Control), Group-B (2.25 ppm), Group-C (2.65 ppm), Group-D (2.85 ppm) of Pb and examined at 7, 14, 21 and 28 days' time intervals. The maximum impact of the treated metal was observed on ESR (Erythrocyte Sedimentation Rate) [1.80 mm/h (Control) to 2.90 mm/h at 2.85 ppm of lead] during maximum time exposure (28 Days) whereas PCV (Packed Cell Volume) showed a fall [44.00 % (control) to 28.00% (2.85 ppm) at 28 Days pe (post-exposure). The other parameters also showed changes in their values when exposed to lead being highest at 2.85 ppm doses during maximum time exposure (28 Days). The above findings indicate that lead adversely affects the blood physiology as reflected in hematological values.

INTRODUCTION: Water is an integral constituent of all living things, and it is the universal biological solvent. Modern industrial and agricultural techniques require the use of many million tons of fertilizers, heavy metals and pesticides. These effluents find their way into aquatic systems through surface runoff from industries or as a result of the discharge of these wastes into streams and rivers. They pose a constant threat to non-target organisms such as fish and insects and tend to bio-concentrate the inorganic and industrial wastes ^{1,2}.

The industrial complexes have become the focus of environmental pollution ³. The main pollutant from these industrial complexes is effluent, which contains heavy metals such as Cu, Ni, Zn, Pb, Cr, Hg, Cd, and various organic compounds like phenols and formaldehyde. The recovery of these heavy metals using conventional techniques is neither economical nor eco-friendly ^{4, 5}. Once aquatic organisms accumulate heavy metals they can be transferred to higher trophic levels of the food chain. Carnivores at the top of the food chain including humans receive most of their heavy metal burden from aquatic ecosystems by way of their food, especially where fish are present ⁶.

Pb is used in lead-based paints, ceramic and petroleum industries, electronic products, electric storage batteries, pesticides, insecticides, electric cable insulation sheets, floor covering, pipes and ammunition.

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The important route of absorption of this heavy metal by man and other aquatic animals is by ingestion and inhalation. Lead accumulation results in various long and short term effects or diseases in humans and animals like memory loss, learning impairment and other diseases⁷. It is one of the most dangerous and insidious metallic poison⁸. The metal is known to impact the blood values in fish^{9, 10} to the extent of damaging chromosomal complements^{11, 12}. It has toxic effects on a variety of freshwater organisms with low sensitivity of 4 µg/L¹³. Acute toxicity of lead is mainly due to mucous induced respiratory asphyxiation and the disruption of Ca²⁺ and Na⁺ homeostasis¹⁴. LC₅₀ studies of lead in *Clarias batrachus* were reported¹⁵. The use of hematological techniques in fish culture for toxicological research, environmental monitoring and fish health conditions has grown rapidly in recent years^{16, 17, 18}. Hematology is an indicator of immunological status and can provide a definitive diagnosis of fish during toxicant exposure^{19, 20, 21}. Hematological indices are of different sensitivity to various environmental factors and chemicals²². Studies have shown that when the water quality is affected by toxicants, any physiological change is reflected in values of one or more hematological parameters of aquatic organisms^{23, 24}. However, some workers noted that there is a possibility that studies on fish blood might reveal conditions within the body of the fish long before there is an outward manifestation of disease²⁵. The effects of heavy metals including lead on hematology and other changes of catfish were observed by several investigators^{10, 26-30}.

The effluents of the pharmaceutical industry affected the blood of *Clarias gariepinus* indicating that Pb was present in effluents in undesirable amounts, which induced significant changes in hematological parameters (RBC, HCT, HGB, MCV, MCH, WBC and lymphocytes)³¹. In the present study, the hematological parameters (Hb, TEC, TLC, ESR, PCV, MCH and MCV) of catfishes, *Heteropneustes fossilis* subjected to Pb exposure were investigated. It is envisaged that the results will assist in providing base-line information on lead toxicity with respect to *H. fossilis*.

MATERIALS AND METHODS: *Heteropneustes fossilis* (20-30 gms) (n=16) of each group (one

control, three lead-exposed) were collected from different fish markets of Bareilly district and acclimatized for 7 days to laboratory conditions. The groups were designed as follows:

Group 'A': Control

Group 'B': Exposed to 2.25 ppm of Pb

Group 'C': Exposed to 2.65 ppm of Pb.

Group 'D': Exposed to 2.85 ppm of Pb

Blood was collected from the caudal vein of fish with the help of an anticoagulated syringe and Hb %, TEC, TLC, ESR, PCV, MCH and MCV were estimated according to established research methodology³².

RESULTS: When *H. fossilis* were exposed to different concentrations of lead, the minimum value of the hemoglobin percentage was 9.20% (21 and 28 days: 2.85 ppm of Pb) **Table 1**. Maximum percentage changes (33.33%) were observed during the 21 and 28 days exposure period at 2.85ppm of lead **Fig. 1**. A corresponding decrease in the total erythrocyte counts corresponded to an increase in the concentrations of lead. The minimum TEC value was 1.60×10^6 /Cumm (28 days: 2.85 ppm of Pb). The maximum percentage changes in TEC were 37.25% at a maximum concentration (Group D) during the maximum exposure period (28 Days) **Fig. 2**. TLC of *H. fossilis* increased to a maximum of 36,100/Cumm (28 days: Group D) and a minimum increase was 24,800/Cumm (7 days: Group C) **Table 1** as compared to the control values (21400/Cumm). TLC also recorded the maximum percentage increase of 68.69% in Group D **Fig. 3**.

ESR values increased in different groups showing the peak value (2.90 mm/h) during the highest concentration at higher time exposure (28 days: Group D) while the minimum value of ESR (2.10 mm/h) was recorded during 7 days in Group B **Table 1**. ESR showed the maximum percentage changes (68.69%) at highest concentration during 28 days exposure period **Fig. 4**. PCV value of control group was 44%, the lowest value (28.00%) of PCV was observed in group D (28 days: 2.85) while minimum reduction was observed at 7 days at the lowest concentration (2.25 ppm) **Table 1**.

36.36% changes in PCV were observed at 2.85 ppm of Pb during 28 days pe **Fig. 5**.

As indicated in **Table 1**, the control value of MCH was 54.11(Pg). In Group C, the minimum value 50.27 pg was observed during 21 days and 28 days pe. The maximum values in each treated group (B, C and D) were recorded as 60.00, 62.43 and 67.00 (Pg) at 7 days pe. A maximum decline of 7.09% change were observed in Group C (2.65 ppm of

lead) during 21 and 28 days **Fig. 6**. Fluctuations in the values of MCV were observed in all treated groups of *H. fossilis* as compared to the fishes of control groups. The minimum value of MCV was 162.16 μ^3 (28 days: Group C) and the maximum value of MCV was 195.12 μ^3 (7 days: Group B) **Table 1**. Percentage change in MCV was merely 1.42% for 28 days at 2.85 ppm of lead **Fig. 7**.

TABLE 1: HAEMATOLOGICAL PARAMETERS OF *H. FOSSILIS* EXPOSED TO VARIOUS CONCENTRATION OF Pb (LEAD)

| S. no. | Parameters | Group A (Control) | Group B (2.25 ppm Pb) | | | | Group C (2.65 ppm Pb) | | | | Group D (2.85 ppm Pb) | | | |
|--------|-------------------------|-------------------|-----------------------|---------|---------|---------|-----------------------|---------|---------|---------|-----------------------|---------|---------|---------|
| | | | 7 Days | 14 Days | 21 Days | 28 Days | 7 Days | 14 Days | 21 Days | 28 Days | 7 Days | 14 Days | 21 Days | 28 Days |
| 1 | Hb % | 13.80 | 12.30 | 10.40 | 9.50 | 9.40 | 12.80 | 11.00 | 9.30 | 9.30 | 13.20 | 11.40 | 9.20 | 9.20 |
| 2 | TEC $\times 10^6$ /Cumm | 2.55 | 2.05 | 1.89 | 1.80 | 1.78 | 2.05 | 1.88 | 1.85 | 1.85 | 1.97 | 1.90 | 1.65 | 1.60 |
| 3 | TLC /Cumm | 21400 | 27400 | 25000 | 26400 | 27800 | 24800 | 27100 | 30300 | 31000 | 26800 | 30000 | 35000 | 36100 |
| 4 | ESR (mm/h) | 1.80 | 2.10 | 2.20 | 2.20 | 2.30 | 2.50 | 2.40 | 2.50 | 2.60 | 2.40 | 2.20 | 2.60 | 2.90 |
| 5 | PCV % | 44.00 | 40.00 | 36.00 | 32.00 | 32.00 | 38.00 | 36.00 | 33.00 | 30.00 | 36.00 | 34.00 | 32.00 | 28.00 |
| 6 | MCH (Pg) | 54.11 | 60.00 | 55.02 | 52.77 | 52.80 | 62.43 | 58.51 | 50.27 | 50.27 | 67.00 | 60.00 | 55.75 | 57.50 |
| 7 | MCV (μ^3) | 172.54 | 195.12 | 190.47 | 177.77 | 179.77 | 185.36 | 191.48 | 178.37 | 162.16 | 182.74 | 178.94 | 193.93 | 175.00 |

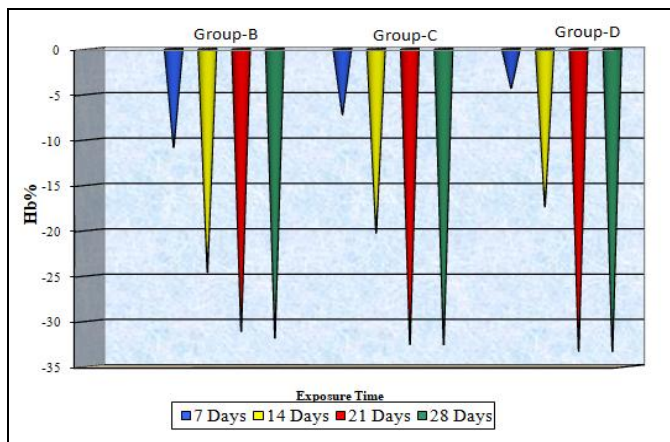


FIG. 1: PERCENTAGE CHANGES IN Hb % OF *H. FOSSILIS* EXPOSED TO VARIOUS CONCENTRATIONS OF Pb

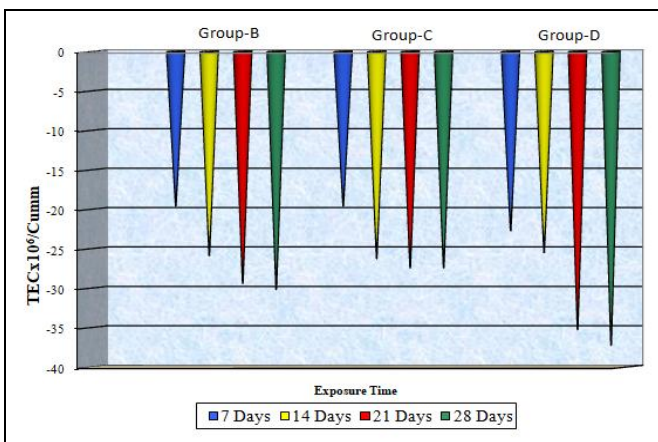


FIG. 2: PERCENTAGE CHANGES IN TEC % OF *H. FOSSILIS* EXPOSED TO VARIOUS CONCENTRATIONS OF Pb

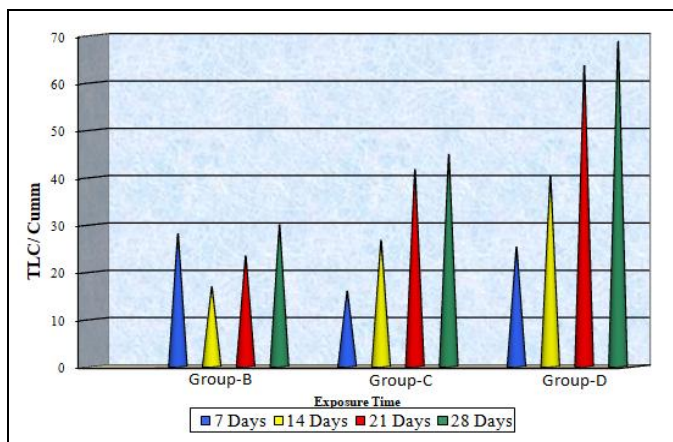


FIG. 3: PERCENTAGE CHANGES IN TLC/ CUMM OF *H. FOSSILIS* EXPOSED TO VARIOUS CONCENTRATIONS OF Pb

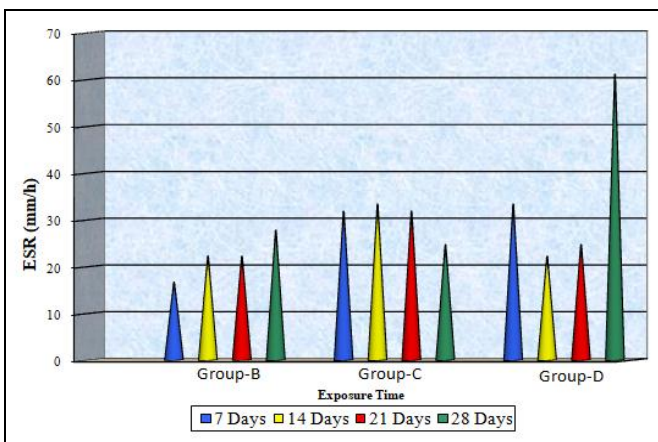


FIG. 4: PERCENTAGE CHANGES IN ESR (MM/H) OF *H. FOSSILIS* EXPOSED TO VARIOUS CONCENTRATIONS OF Pb

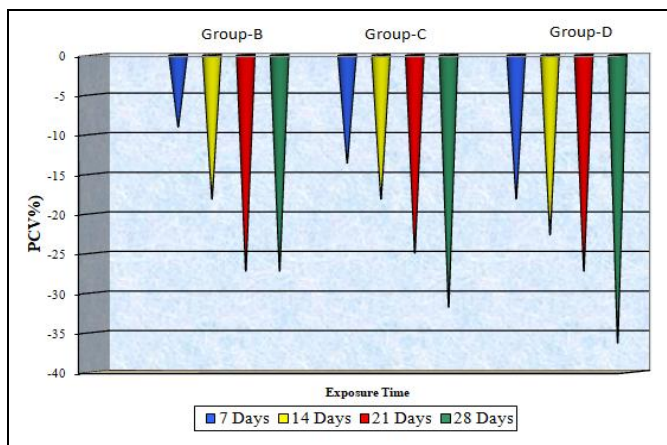


FIG. 5: PERCENTAGE CHANGES IN PCV% OF *H. FOSSILIS* EXPOSED TO VARIOUS CONCENTRATIONS OF Pb

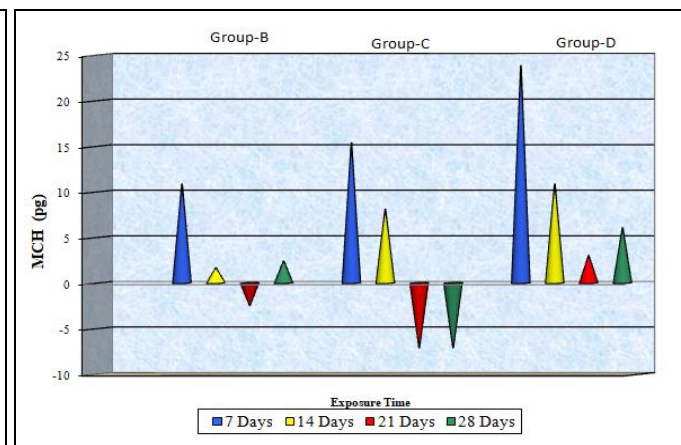


FIG. 6: PERCENTAGE CHANGES IN MCH (PG) % OF *H. FOSSILIS* EXPOSED TO VARIOUS CONCENTRATIONS OF Pb

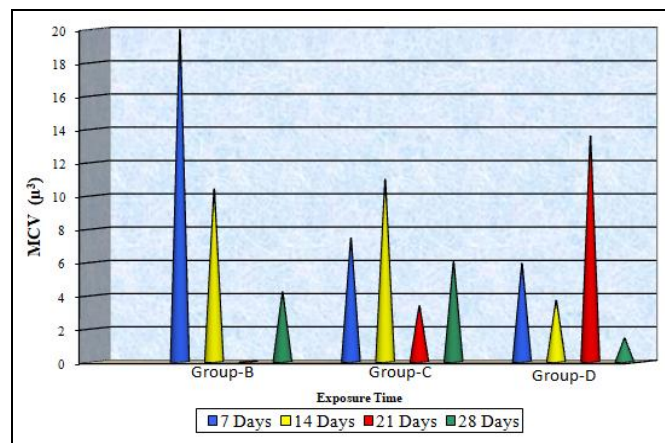


FIG. 7: PERCENTAGE CHANGES IN MCV (μ^3) % OF *H. FOSSILIS* EXPOSED TO VARIOUS CONCENTRATIONS OF Pb

DISCUSSION: The importance of hematology in the diagnosis of fish anomalies and for evaluating the effect of pollution has been accepted widely. The application of hematological techniques like Hb, TEC, TLC, ESR, PCV, MCH and MCV, have proved valuable for fishery biologists in determining the health status of fishes. *H. fossilis* exposed to various concentrations of lead (2.25 ppm, 2.65 and 2.85 ppm) showed variations in hematological parameters. Different fish species show differentiation in sensitivity and reaction to heavy metals including lead, which has been reported by many workers. The influences of heavy metals on hemoglobin reflect the O₂ carrying capacity of blood. Reduction in Hb% was recorded in the blood of *Clarias gariepinus* during short term exposure of lead³³. Consistent reduction in hemoglobin concentration in white fish *Coregonus* species poisoned with lead has been reported³⁴.

Hemoglobin percentage declined during lead exposure in (*T. tinca*³⁵, *C. gariepinus*,^{26, 35, 36}

*Cyprinus carpio*³⁷, and *Oreochromis niloticus*³⁸). Research indicated a significant reduction in the Hb% which may lead to anemic condition in *Channa punctatus*³⁹. Another research supported our findings on hemoglobin in the bunny fish *Mesopotamichthys sharpeyi* exposed to lead⁴⁰. In another experiment, the same catfish (*H. fossilis*) exposed to cadmium, reported a significant decrease in Hb% during 7, 14 and 21 days as compared to the control fish⁴¹.

The value of WBCs increased in the blood due to pollution and stress corresponding to the lead concentration and time exposure when *Anguilla anguilla* was exposed to different concentrations of lead⁴². A significant increase in immunological parameters (TLC) following lead exposure suggests that lead may weaken the immune system, resulting in increased susceptibility to infection⁴³. Increased WBC counts due to exposure to Pb in the blood of *Oreochromis niloticus*³⁸ and *Channa punctatus*³⁹ further support our findings.

The results fall on similar lines when the same species of experimental fish were treated with lead nitrate³⁰. WBC count and serum enzyme also increased in fish exposed to Dioatylphthalate⁴⁴.

Contrary to the findings of WBC, the total erythrocyte counts in the blood of the investigated catfish constantly depleted corresponding to the increases in concentration and time exposure to lead. These findings are in concurrence to those reported in *Oreochromis niloticus*⁴⁵ and also studies by other authors^{27, 46, 47, 48}. Various heavy metals and toxins enter the aquatic system and exert a specific toxic effect on fish blood⁴⁹. Similar results on RBC depletion are supported in other fish species as well, *Clarias gariepinus*³⁰, *Mesopotamichthys sharpeyi*⁴⁰ and *Channa punctatus*³⁰. ESR values showed an increase in different exposed groups. Heavy metal toxicity causes inflammatory lesions associated with tissue damage, anemia and neoplasia. Further, an increase in fibrinogen or serum globulins or a decrease in serum albumin may also cause an increase in the ESR. An increase in ESR values suggests that anemia was of macrocytic type⁵¹. These findings are supported by research^{52, 53, 54}.

The values of PCV showed a fall to 28% during the highest concentration and maximum exposure period. Anemia is a manifestation of acute and chronic in toxification by heavy metals. A decrease in PCV% treated with lead occurred in *Clarias gariepinus*⁵⁵ and the results are supported by other findings^{56, 53, 57}. PCV gradually increased up to 45 days but declined after 60 days of exposure to chromium in *Channa punctatus*⁵⁴. Contrarily, elevated values of PCV in the blood of Coruh trout occurred after treatment with Pb⁵⁸. The level of hematocrit in control fish was not significantly different but Pb exposed fish showed a significant difference when compared to the control fish⁵⁹.

MCH expresses the average hemoglobin concentration in individual erythrocyte counts. In the present study, MCH increased rapidly during the initial exposure of lead and values further reduced when lead concentration and exposure time increased. This is in agreement to the finding who reported a short term exposure of lead in *T. tinca*³⁵, *Clarias gariepinus*²⁶ and *Hetroclarias*⁵⁶ exposed to zinc and a significant fall in *Channa punctatus*

exposed to copper⁵³. The recovery in MCH values were also recorded who identified that lead toxicity affected the concerned hematological parameters and its recovery in long term exposure⁵⁷. Notable works on zinc⁶⁰ and Pb and Cd⁶¹ exposure in common carp have been reported. Pb showed a higher significant decrease in MCH values as compared to other heavy metals. Lower values of MCH occurred during 7, 14, and 30 days exposure period but these were recovered after 60 days of exposure to chromium trioxide in fresh water teleosts⁵⁴.

The decrease in hemoglobin concentration represents that the capability of the fish to supply adequate oxygen to the tissues gets limited considerably and this results in the decline of physical stir⁶². Elevation in MCV and MCH (Group D) shows macrolytic type anemia in *Clarias gariepinus*²⁷ and rainbow trout⁶³. High values of MCV subsequent to lead exposure were recorded in *O. niloticus*⁵⁷. Significant reduction in MCV in the blood of common carp exposed to lead⁶¹ was reported but no significant changes occurred in MCV when the same fish was exposed to cadmium⁶⁴. Most of the hematological parameters of the market fishes, *Channa punctatus* and *Heteropnestes fossilis* showed significant alteration from their control values when subjected to heavy metals⁶⁵.

CONCLUSION: The present study suggests that heavy metals are responsible for causing a variety of hematological anomalies. The studies also infer that blood may be utilized as a pathophysiological reflector of the whole body and therefore blood parameters may be regarded as important diagnostic tools for assessing the structural and functional status of fish exposed to toxicants.

The toxicity test helps in evaluating the nature and degree of the harmful effect produced on the aquatic organism, especially fish by toxic substances and provides valuable information that can be used for the protection of the aquatic ecosystem as a whole.

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