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Haemotoxic Assessment of Differential Leucocyte Counts (DLC) in Fresh Water Teleost, *Heteropneustes fossilis* Exposed to Lead

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BACKGROUND, in recent years, fish blood is being used for assessing the health and general condition of the fish subject to changing environmental conditions due to heavy metals which are regularly released by anthropogenic activities. The present experiment was set up to assess the effects of the heavy metal, lead (Pb) on the Differential Leucocyte Counts (DLC) of fresh water teleost *Heteropneustes fossilis.*

Results.The acclimatized fish were exposed to different concentrations of lead [2.25ppm (Group B), 2.65ppm (Group C), and 2.85ppm (Group D)] and the values were compared with the control(Group A). The time interval of examination was 7, 14, 21 and 28 days post exposure. DLC showed changes in small lymphocytes resulting in the fall of 41% to 31% at 2.65 ppm of Pb during 28 days of exposure period. The large lymphocyte counts also decreased from normal value of 39% to 27% at maximum concentration and maximum exposure period (2.85 ppm Pb; 28 days). The neutrophils increased from normal counts (14%) to maximum (26%) at 2.65 ppm of Pb during 28 days exposure period. The monocyte counts increased from normal counts of 3% to 7% during 7 and 28 days exposure period at 2.65 ppm of Pb. The counts of eosinophils increased against the toxicity of lead from 2% to 6% at maximum concentration (2.85 ppm) during 14 and 28 days exposure period. Basophils increased from normal range (1%) to 3% at 2.65 ppm of Pb at 28 days exposure period.

Conclusion, our results indicated that exposure to lead results in altered leucocyte counts.

Keywords: Differential Leucocyte Counts (DLC), *Heteropneustes fossilis,* Heavy metal, Lead.

Introduction

Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to their use in domestic, industrial, mining and agricultural activities as shown by Kalay and Canli, [1]. The rapid development of industry and agriculture has resulted in release of significant quantities of heavy metals thereby increasing pollution which acts as a significant environmental hazard for invertebrates, fish, and humans discussed by Uluturhan and Kucuksezgin, [2]. Almeida et al.[3] obtained that metals are discharged into rivers, through drainage (domestic, industry and agriculture) which can accumulate and biomagnify along water, sediment, and the aquatic food chain, resulting in sublethal effects or death in local fish populations. Mastan et al.[4] reported the haematological variables are extensively being used in clinical diagnosis of fish physiology in order to determine the effects of external stressors and diseased conditions in fish. Haematological abnormalities have also been studied undersublethal concentration of pollutants such as heavy metals exposed to fish: *H. fossilis*

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to lead by Gupta et al.[5] and *Channapunctatus* to cadmium by Karuppasamy et al.[6]. The introductions of toxicants into an environment where fishes were found, stuns them and or act as stressors of the fish and organisms found in such environment is reported by Oliveira-Ribeiro et al.[7]and Olatayo[8]. Differential leukocyte counts represent important characteristics of the health status of fish and in many cases they are also helpful in evaluating the immune system by Rowley et al.[9].

Lead is not necessary for the biological functions of animals, even at low concentration. It is discharged to aquatic systems, mainly from petroleum, dye and mining industries which have toxic effects and can cause mortality to aquatic animals [10; 11]. It is also regarded as immunotoxicant and its effects may be immunosuppressive, rendering an organism more susceptible to infectious diseases were obtained [12;13]. Also, Witeska et al.[14] observed that low toxicity of lead also suppresses the immune system of common carp. The increase in White Blood Cells (WBC), particularly lymphocytes observed in the study of Stanley et al. [15]; Dutta et al.[16] could be attributed to stimulation in the immune system in response to tissue damage in *Channapunctatus* through lead nitrate. Pandit and Sharma [17,18] reported that lead induced changes in the blood of the same fish, *Channapunctatus.* This work aimed to evaluate the differential leucocyte counts of fresh water air breathing fish, *Heteropneustes fossilis* exposed to different concentrations of lead in comparison with those in control.

Materials and Methods

The catfishes *Heteropneustes fossilis*(n=64) (10-20 gms.) were used as a test model for haemotoxic studies. The fishes collected from Rithora and Naryawal fish market, Bareilly district were acclimatized to laboratory conditions. Four groups were designed, one control and 3 experimental: exposed to three chronic levels of lead (Pb) as shown in Table 1. Low doses of lead were selected to observe the toxicity as high doses caused mortality of the fish.

The haematological investigations were conducted by collecting blood from the caudal vein of fish with the help of anticoagulated syringe and thin blood smears were prepared on clean slides, immediately air-dried and fixed in methanol for 2-5 minutes. They were subsequently stained in Giemsa (Qualigens) with phosphate buffer solution (pH 6.8). The solutions were mixed uniformly in the ratio 1:7 by means of a soft brush, stained for 40 minutes, washed in running tap water for 2-4 mins to wash off the excess stain, allowed to stand on end to dry and mounted in DPX[19].The cells were counted using high power microscope in a strip running along the whole length of the film by means of manual / electronic blood cell counter. At least 100 cells were counted and the leucocytes differentiated by the morphological characteristics of the nucleus and characterized as small lymphocytes, large lymphocytes, monocytes, neutrophils, eosinophils and basophils according to Wintrobe [19].

Results

The principleleucocyte components of the blood of *H. fossilis* consist of small and large lymphocytes, neutrophils, eosinophils, monocytes and basophils which were observed during the study period (Figs. A-F). Fishes when exposed to lead indicated a declined in the lymphocyte numbers as compared to the control group. The lowest values of small lymphocytes were 31% with maximum percentage changes -24.39 (28 days: 2.65 ppm of Pb) (Table-2; Graph1). The large lymphocytes depleted conspicuously during the experiment. The maximum fall was 27% with maximum percentage changes [–30.7% (28 days: 2.85 ppm)] (Table-2, Graph 2). On the other hand,

TABLE 1. Showingthe concentrations of Pb in all treated groups

No.	Group	Exposure to Pb (Lead)	Concentration /ppm
1	A	Control group.	0.00
2	B	Low concentration	2.25
3	C	Intermediate concentration	2.65
4	D	High concentration	2.85

the neutrophils increased conspicuously reaching to a maximum of 26% with maximum rise in percentage change being78.57% (28 days: 2.65 ppm of Pb) (Table-2; Graph 3). The concentrations of monocytes and eosinophils fluctuated, increases with percentage changes being 133.3% and 200% in Groups C and D, (Table-2; Graphs4 & 5). The number of basophils increased during the treatment days but they sometimes even disappeared with maximum 200% changes in Groups C and D (Table-2; Graph 6).

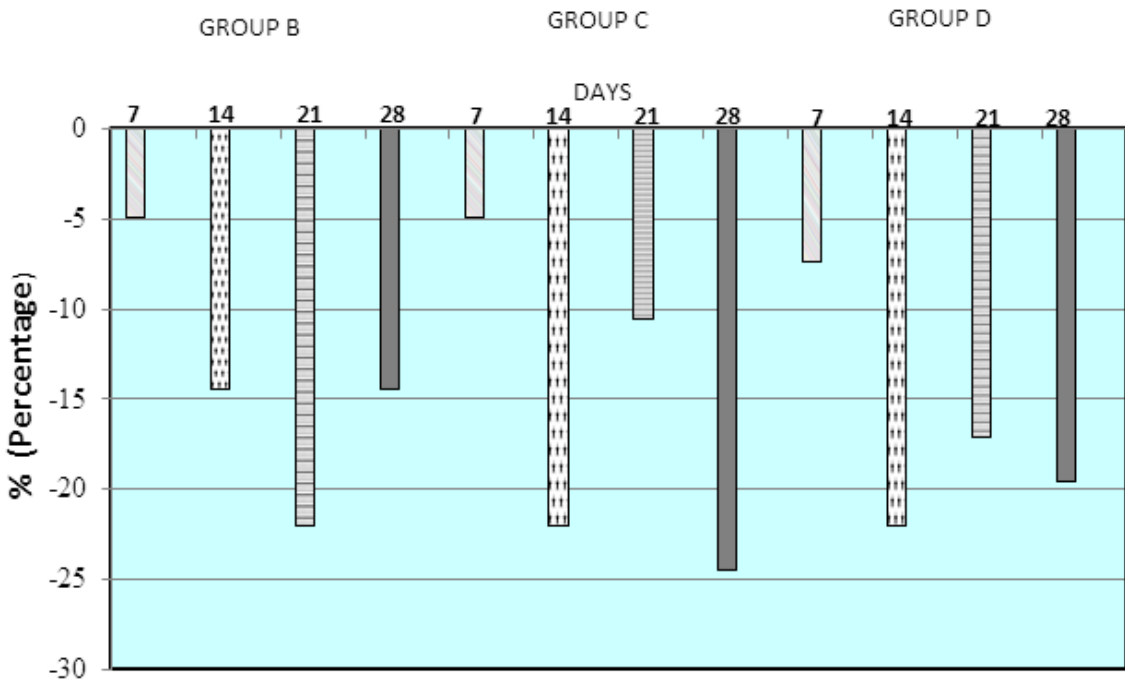
Discussion

Intense activity in industrial and agriculture sectors has inevitably increased the levels of heavy metals in natural environment[20-22]. Heavy metals are serious pollutants of the aquatic environment because of their persistent ability to be accumulated by aquatic organisms Pb is the blacklisted element by environmentalists and is released in to the environment by several resources [23].Adhikari et al. [24] investigated that blood parameters are considered to be pathophysiological indicators of the whole body and therefore are important in diagnosing the structural and functional status of fish exposed to toxicants. Also, Jones [25] discussed the immune parameters alteration in fishes experiencing heavy metal exposure to develop healthy management tools to support a rapidly growing aquaculture industry. The results of the present investigation indicate that sublethal concentration of lead inflicted drastic changes in differential leucocyte counts through changes in nuclear morphology in *Heteropneustes fossilis*. The granular leucocytes (small and large lymphocytes) decreased corresponding to the increase in lead concentrations and time, on the other hand, the other granular leucocyte (monocyte) increased correspondingly. The granular leucocytes, neutrophils and eosinophils increased but basophils did not show any significant changes. The reduction in lymphocyte counts after exposure to lead is attributable to lymphopenia representing a diminution in lymphocyte production. Ellis [26] suggested that in fish lymphopenia is commonly accompanied by neutrophilia and this was seen in the present study also. Our observations are also in concurrence to Mazon et al.[27] who exposed *Prchilodusscrofa* to different concentrations of copper, where lymphocytes frequently decreased while monocytes percentage showed a slight increase but the changes were insignificant.

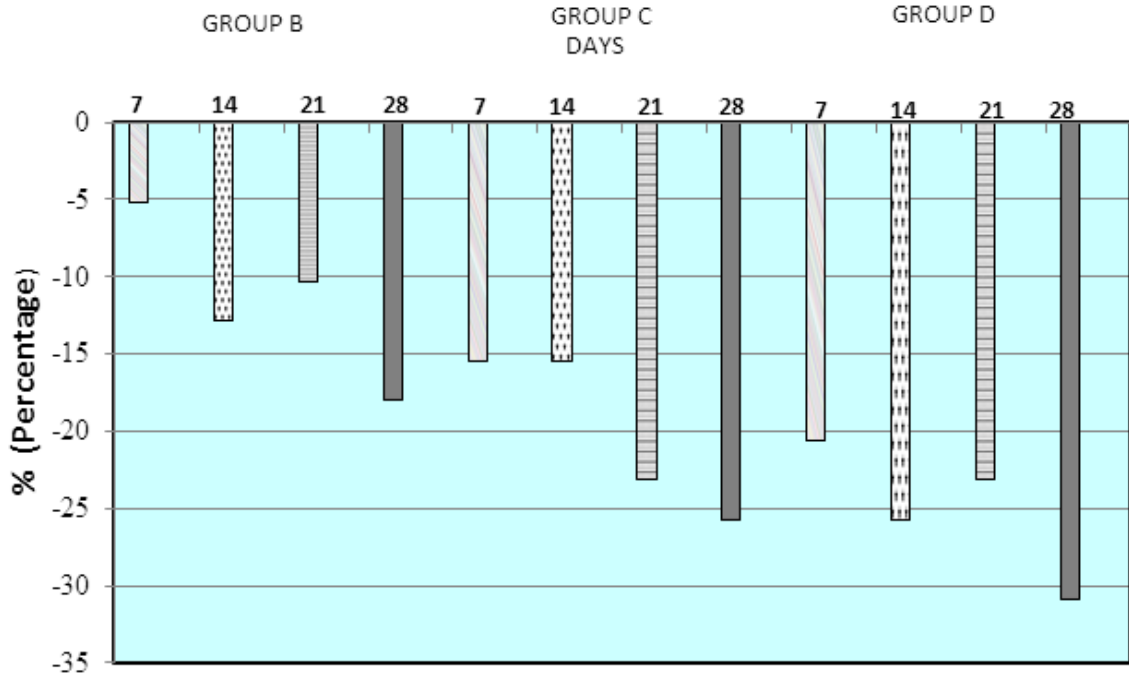
TABLE 2. Differentiate Leucocyte Counts of *H. fossilis* exposed to various concentration of Pb (Lead)#

Sl. 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	Small Lymphocytes	41	39	35	32**	35	39	32**	33*	31**	38	32**	34*	33*
2	Large Lymphocytes	39	37	34	35	32	33	33	30**	29**	31**	29**	30**	27**
3	Neutrophils	14	17	22*	14	23**	20*	22*	19	26**	23*	23*	22*	25**
4	Monocytes	3	4	4	5*	6**	7**	6**	5*	7**	5*	7**	7**	6**
5	Eosinophils	2	2	3	3	3	1	4**	2	4**	2	6**	5**	6**
6	Basophils	1	1	2*	1	1	0	3**	1	3**	1	3**	2*	3**

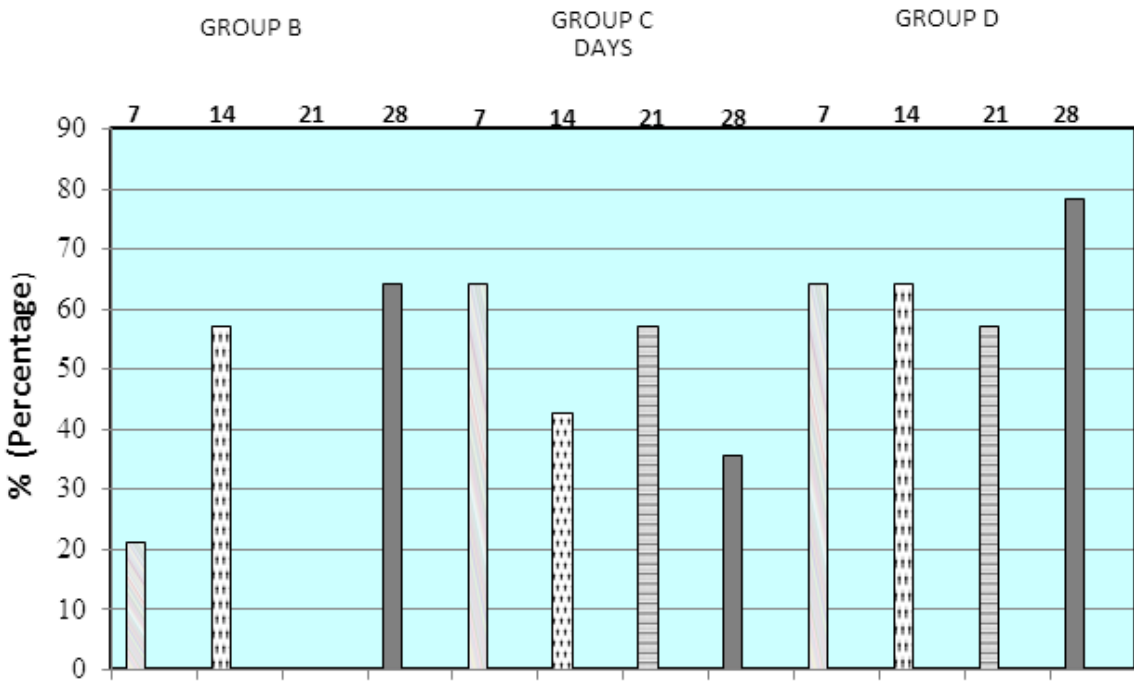
Values of significance *(P< 0.05); **(P <0.01)



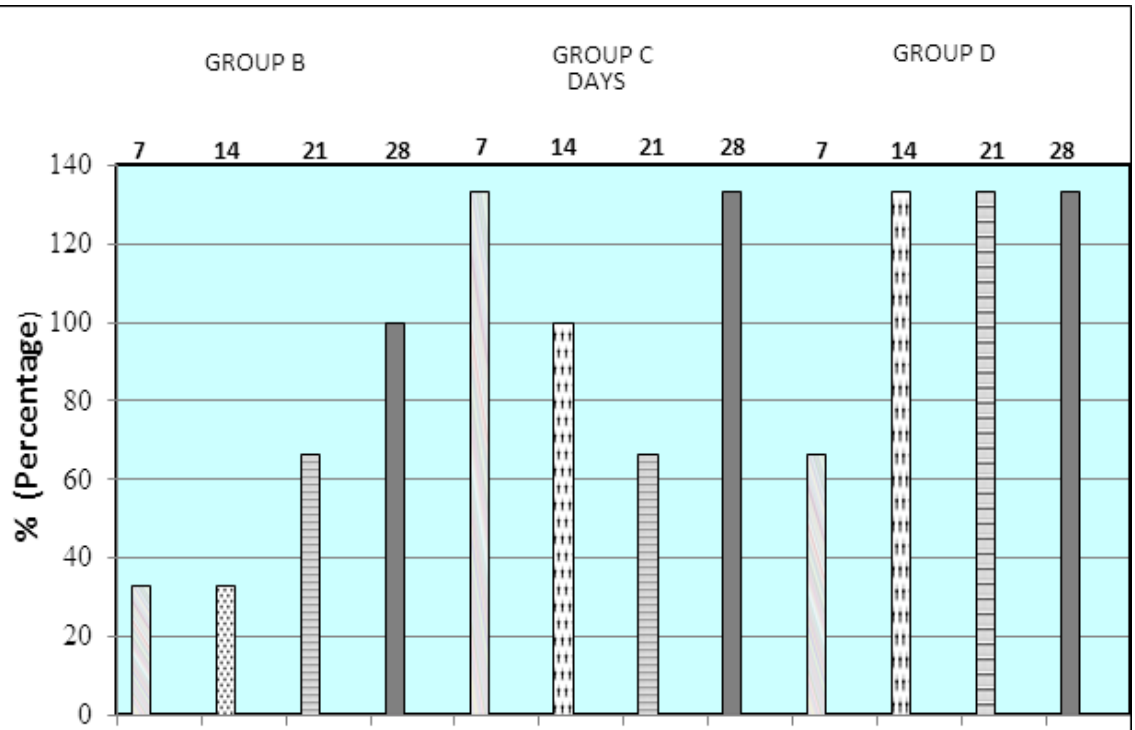
Graph 1: Percentage changes in small lymphocytes of *H. fossilis* to various concentrations of Pb



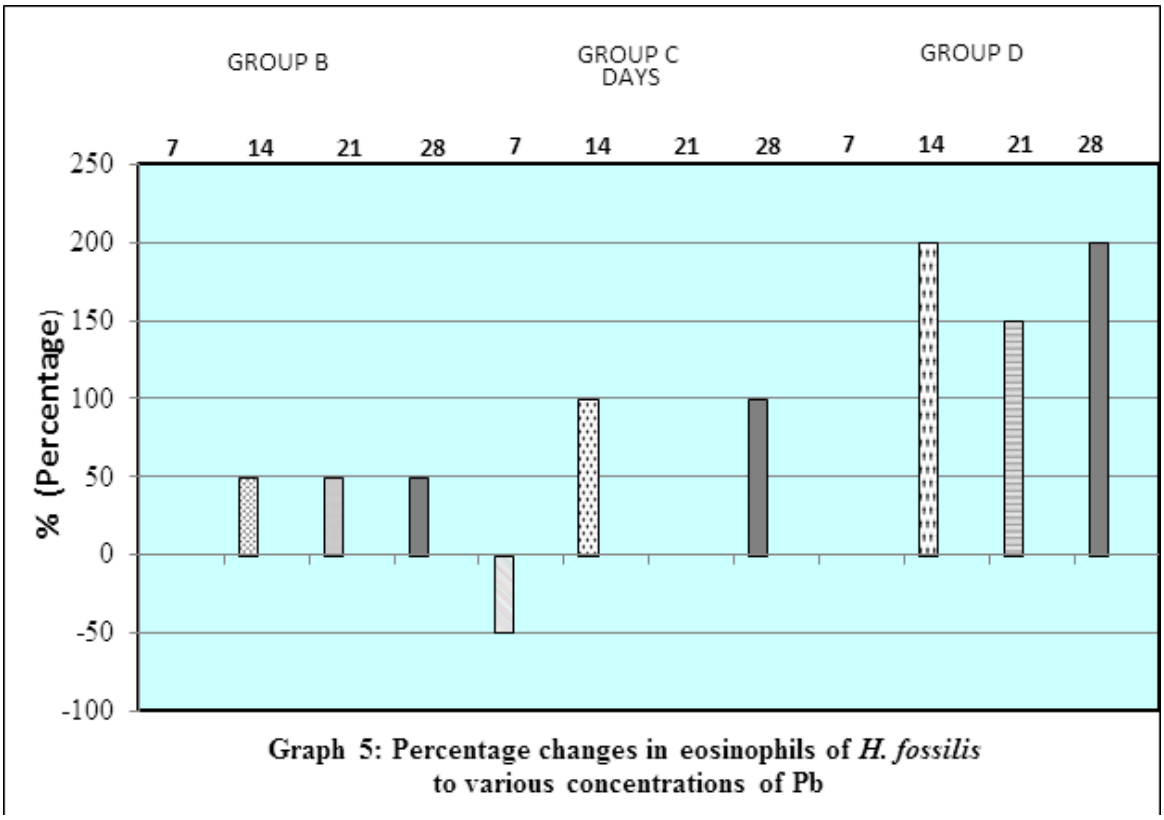
Graph 2: Percentage changes in large lymphocytes of *H. fossilis* to various concentrations of Pb



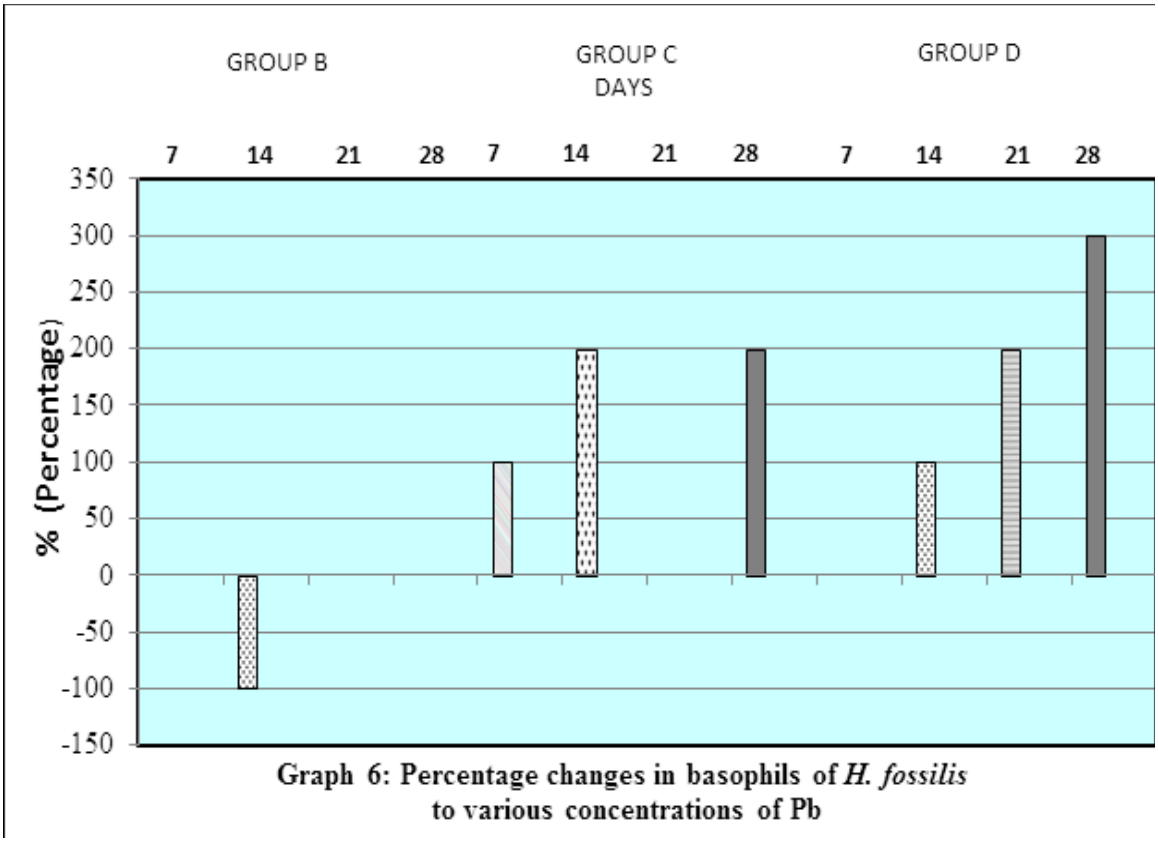
Graph 3: Percentage changes in neutrophils of *H. fossilis* to various concentrations of Pb



Graph 4: Percentage changes in monocytes of *H. fossilis* to various concentrations of Pb



Graph 5: Percentage changes in eosinophils of *H. fossilis* to various concentrations of Pb



Graph 6: Percentage changes in basophils of *H. fossilis* to various concentrations of Pb

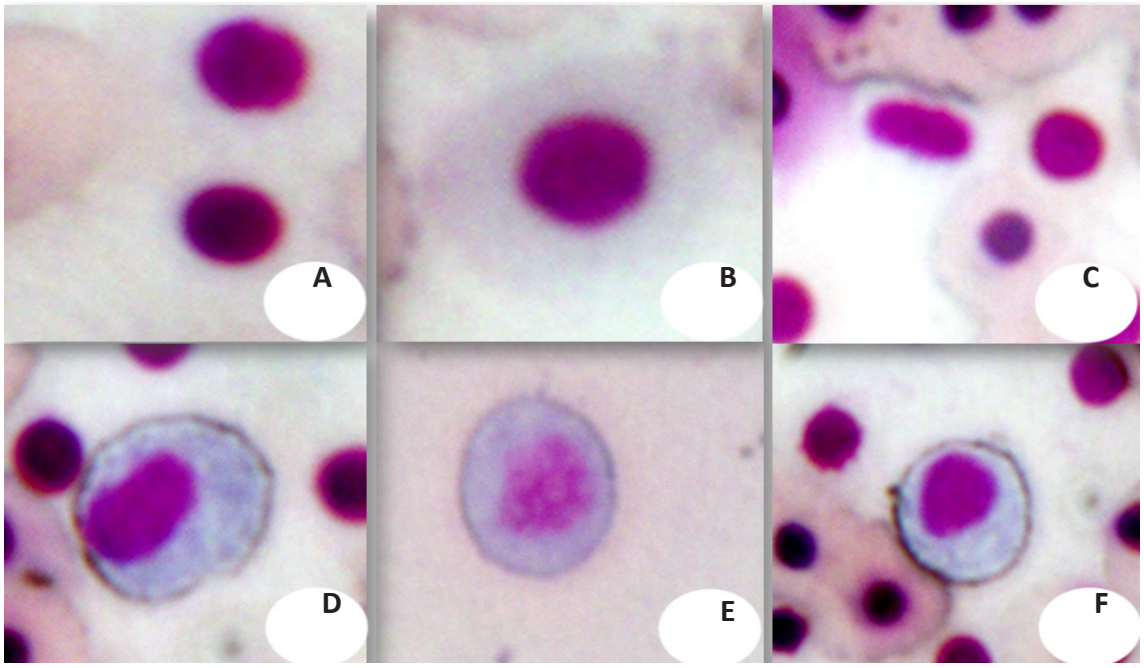


Fig. A. Blood film of Heteropneustes fossilis showing small lymphocytes, Fig. B: Blood film of Heteropneustes fossilis showing large lymphocytes, Fig. C: Blood film of Heteropneustes fossilis showing monocyte, Fig. D: Blood film of Heteropneustes fossilis showing neutrophil, Fig. E: Blood film of Heteropneustes fossilis showing eosinophils Fig. F: Blood film of Heteropneustes fossilis showing basophil

Our results are also in tune with Witeska et al. [14] indicating that lead caused changes in small and large lymphocyte, the percentage of neutrophils dropped because this metal induced tissue damage. The results on DLC were also presented by Bhatkar[28] in *Labeorohita* exposed to cadmium showed increased eosinophils and basophils percentage which play important roles in immunological defense system during exposure of toxicants like heavy metals induce phagocytosis but at the same time, lymphocytes and monocytes decreased in their concentration. Desai and Parkh[29] exposed *Oreochromismossambicus* to fungicides and recorded changes in lymphocytes. Hedayati and Ghaffari[30] investigated the effects of mercuric chloride on the blood of silver carp and their results also indicated a fall in lymphocytes and rise in eosinophils values corresponding to the increase in concentration and time period. The results of Wani and Sikdar-Bar [31] on *Clariasbatrachus* exposed to copper fall on similar lines reducing the lymphocyte and increasing the neutrophil percentage. In our results, the lymphocytes counts decreased consistently through Pb exposure while Dutta et al.[16] observed that the fish *Channapunctatus* exposed to $PbNO_3$, showed an increase in lymphocytes, basophils and eosinophils unlike neutrophils and monocytes. Abarghoei et al. [32] treated the gold fish to silver sulphate, the lymphocyte count decreased at 24 and 48 hours exposure period and increased in 96 hours, but the results of monocytes counts showed significant similarities. Sevcikova et al.[33]in the blood of common carp (*Cyprinuscarpio*)and Hedayati and Darabitabar[34] in Caspian roach (*Rutilusrutilus*) observed significantly greater lymphocyte reduction at the highest concentration of copper whereas monocyte and neutrophil counts increased as compared to the control group. The present studies indicate that exposure to lead alters the leucocyte counts in fish, *H. fossilis* when exposed to lead.

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Conflict of Interest: The authors have no conflict of interest regarding the publication of the paper

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