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Fluoride Induce Haematological Alterations in Freshwater Asian Stinging Catfish, *Heteropneustes fossilis* (Bloch)

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Abstract: In present investigation, heamotoxic effects of fluoride were noticed in freshwater fish, *Heteropneustes fossilis* after exposing to sublethal concentrations (35 mg F/ L and 70 mg F/L) of fluoride for 15, 30 and 45 days. For this intention routine haematological parameters like total erythrocyte count (TEC), total leukocyte count (TLC), haemoglobin content (Hb), PCV%, erythrocyte indices, blood clotting time and O_2 carrying capacity were estimated.

The findings of present study revealed that the fluoride had a negative impact on haematological parameters of *Heteropneustes fossilis*. The fish *H. fossilis* showed a significant decrease in TEC, Hb %, and PCV % while significant increase in WBC count in all exposure durations and concentrations at different level of significant ($p < 0.05$, $p < 0.01$ and $p < 0.0001$). The erythrocyte indices like MCV, MCH and MCHC values were significantly reduced in a dose-dependent manner in fluoride exposed fishes ($p < 0.05$ and $p < 0.01$). This might be due to the possible disruption of haemopoietic tissue or impairment of liver and kidney functions. Significant increase in blood clotting time and decreased O_2 carrying capacity were also noticed in present study. Based on the results, we conclude that fluoride is toxic to *Heteropneustes fossilis* and its occurrence in the environment may threaten the health of aquatic species.

Keywords: Fluoride toxicity, Haematological parameters, Blood clotting, O_2 carrying capacity, Stinging catfish Fish, *Heteropneustes fossilis.*

I. INTRODUCTION

The contamination of aquatic resources with pollutants has become a major concern not only the threat to public health but it also cause to damage aquatic life. Every year, several ponds and lakesacross various Indian states become sites of mass fish deaths. The primary cause for thisphenomenon is water pollution, most often stemming from anthropogenic activities. Among waterpollutants, fluoride is a potentially toxic element and widely distributed in earth's crust and naturally contaminates groundwater consistently due to weathering and volcanic eruption activities (Kabita and Pendias, 1984). Fluoride is an ionic form of Fluorine. It is most electronegative and highly reactive element, belonging to the 7th group of periodic table. In India, most of fish farming depends on groundwater due to unequal pattern of rain. Although ground water is suitable for fish farming but in some areas groundwater containing higher concentration of fluoride than the recommended permissible limit (>1.5) by WHO (1984). Several workers have been reported excess fluoride concentration in groundwater in different states in India (Das, 1996; Susheela, 1999; Lal, 2002; Choubisa et al., 2009). The ingestion of excess fluoride via food and drinking water causes several adverse effects in human beings (Kumar, 2022), domestic animals (Singh and Kumar, 2023) and experimental fish species inhabiting in fluoride contaminated water(Saxena, et al., 2001; Gupta, 2002; Gupta, 2003, Tripathi et al., 2004; Tripathi et al., 2006; Kumaret al., 2007a; 2007 b; Kumar, 2020; Kumar and Swami, 2022). Earlier workers have reported hemotoxic effects of fluoride in different experimental animals (Gujarathi et al., 1991; Saxena et al., 2001; Machalinska et al., 2002, Kant et al., 2009; Sayeed & Khan, 2010; Kumar et al., 2010; Abbas et al., 2014; Guru et al., 2014; Sajja et al., 2018; Taygi et al., 2024). Blood is considered most sensitive indicator of animal health in toxicological studies. Recently, the culture of catfisheshas been more emphasize due to its taste and medicinal values. Therefore, present study has beenplanned to observe the haemotoxic effect of fluoride on freshwater Asian stinging catfish, *Heteropneutes fossils.*

II. MATERIALS AND METHODS

Experimental Animal: Live healthy specimens of *Heteropneustes* fossilis were collected with the help of fisherman's from the Gomti river of Lucknow city and transported to the laboratory in plastic containers. Prior to the experimentation fishes were acclimatized under standard laboratoryconditions for 15 days.

Experimental Toxicant: Sodium fluoride (ER grade, M.W. 41.99) is white colour powdered compound easily soluble in water, obtained from Qualigens Fine Chemical, Ltd. Mumbai, India used as source of fluoride. The stock solution of fluoride was prepared by dissolving 11.55 g of sodium fluoride in 500 ml of distilled water in volumetric flask. This stock solution contains 10 mg F ion / ml, which was further diluted according to the desired concentrations needed for experimentation. This solution could be kept at room temperature for one month.

Experimental Design: The after acclimatized fish were divided into three groups each group contains 15 fish. The selection of sublethal concentrations of fluoride for this experiment is based on earlier study (Kumar, 2020). One control group and two exposed groups containing two concentrations, i.e., 35 mg F/L (1/10th), 70 mg F/L (1/20th) and respectively of LC_{50} value (349.75 mg F/L) (Kumar, 2020). Both control and fluoride treated groups were maintained under the sameenvironmental conditions during the experimental period. No fish mortality was recorded during the experimental period. The fish were exposed under normal laboratory conditions for 15, 30 and45 days. Aquarium water along with fluoride was entirely substituted once every two days by transferring the fish into freshly prepared fluoride solutions. During experimentation all fish werefed with goat liver.

At the end of experimental duration i.e.15, 30, 45 days fish were used for blood sampling. Blood samples were collected from 3 fish of each experimental group randomly by puncturing the caudal veins with the help of a heparinized 2 cm disposable syringe. The blood sample was mixed lightly and used for the estimation of haematological parameters like TEC, TLC, Hb%, PVC%. The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated by using standard methods (Dacie & Lewis,1991). For estimation of blood clotting time (CT) non-heparinized blood was used and clotting time was determined by capillary tube method of Wright. Oxygen carrying capacity was calculated by multiplying haemoglobin content with 1.25 oxygen combining power of Hb/g by using standard methods (Johansen, 1970).

$$O_2 \text{ carrying capacity of blood (ml } O_2 \text{ / g Hb)} = \text{Hb \% X 1.25}$$

III. RESULTS

In present study concentration and duration dependent depletion in TEC, Hb%, PVC%, MCV, MCH, MCHC and O_2 carrying capacity indices were recorded. But increased TLC and blood clotting time was noticed in fluoride exposed fish, *Heteropneustes fossilis*. The alteration of values in all these haematological indices was found to be time dependent and the significance of variation were more prominent with the elapsed duration of exposure. The variation patterns in different blood indices has been given in the (Table-1, Figure 1-9). The results obtained in present investigation discussed below-

1. ***Total Erythrocyte count (TEC)*:** Compared to control group the concentration dependent reduction in RBCs count were recorded significant ($p < 0.05$) in lower concentration in all three exposure durations and highly significant ($p<0.01$) in higher concentrations after 30 and 45 days. (Table-1, Fig. 1).
2. ***Total Leukocyte count (TLC):*** Compared to control increased in TLC count were found significant ($p<0.05$) in lower concentration in all exposure periods and highly significant ($p<0.01$) in all fluoride exposure durations in higher concentration (Table-1, Fig. 2).
3. ***Haemoglobin (Hb%):*** Compared to control group duration and concentration dependent depletion in Hb% were recorded significant ($p<0.05$) in lower concentration and highly significant ($p<0.01$) in higher concentration (Table-1, Fig.3).
4. ***Packed Cell Volume (PCV%):*** Similarly concentration and duration dependent reduction in PCV % were recorded significant ($p<0.05$) in lower concentration and highly significant ($p<0.01$) in higher concentration (Table-1, Fig. 4).
5. ***Mean Corpuscular Haemoglobin (MCH):*** Compared to control group decrease MCH values were calculated significant ($p<0.05$) after 15 days in both concentrations and after
   30 days it was found more significant ($p<0.01$) in lower concentration and highly significant ($p<0.001$) in higher concentration (Table-1, Fig. 5).

6. ***Mean Corpuscular Volume (MCV):*** Compared to control group reduced MCV were recorded significant ($p<0.05$) in lower concentration and highly significant ($p<0.001$) in higher concentration in all exposure durations (Table-1, Fig. 6).
7. ***Mean Corpuscular Haemoglobin Concentration (MCHC):*** Compared to control group decrease values of MCHC were estimated significant ($p<0.05$) after 30 days in lower concentration and thereafter it was observed highly significant ($p<0.01$).
8. ***Blood Clotting Time:*** Compared to control group significant increased blood clotting time were recorded after 30 days and highly significant ($p<0.01$) after 45 days in lower concentration exposed fishes. Fish exposed to higher concentration of fluoride showed highly significant ($p<0.01$, $p<0.001$) clotting time in all durations (Table-1, Fig. 8)
9. ***Oxygen Carrying Capacity:*** In compared to control group decreased O_2 carrying capacity were estimated significant ($p<0.05$) in lower concentration in all exposure durations and highly significant ($p<0.01$, $p<0.001$) in all exposure durations in higher concentration (Table-1, Fig. 9)

Tabel-1 Haematological parameters of *Heteropneustes fossilis* under fluoride toxicity

(Values are in Mean ± S.E., N=5)

S,No.	Haematological Parameter	Exposure duration (days)	Control	Lower Concentration (35 mg F/L)	Higher Concentration (70 mg F/L)
1.	TEC (10^6/mm^3)	15	1.34±0.06	1.18±0.06*	1.10±0.07*
		30	1.30±0.08	1.12±0.06*	1.03±0.04**
		45	1.32±0.05	1.08±0.03**	0.92±0.05***
2.	TLC (10^3/mm^3)	15	180.6±20.11	208.4±21.20*	231.8±15.32**
		30	184.8±18.10	215.8±16.50**	235.4±16.40***
		45	180.9±16.15	216.7±18.30**	241.6±17.34**
3.	Hb (dl/g)	15	11.6±0.6	9.3±0.3*	7.5±0.6**
		30	10.3±0.4	9.4±0.6*	7.0±0.4***
		45	10.9±0.8	8.8±0.7*	6.5±0.8**
4.	PCV (%)	15	35.5±4.2	30.1±1.3*	26.5±1.6***
		30	33.3±4.4	28.4±3.2*	24.6±1.4***
		45	34.9±0.8	26.5±1.7**	22.5±1.5***
5.	MCV (fl/cell)	15	262.96±6.8	255.08±7.3*	240.90±6.4**
		30	254.61±7.5	251.76±8.2*	238.68±6.4**
		45	259.09±8.2	243.52±8.4*	245.65±7.2**
6.	MCH (pg/cell)	15	82.96±2.1	78.81±3.3*	68.18±6.4*
		30	79.32±2.5	83.93±4.2	67.96±6.4*
		45	75.00±3.2	81.45±3.2*	70.65±7.2*
7.	MCHC (g/dl)	15	31.54±1.4	30.89±3.2	28.30±2.4
		30	31.11±1.6	33.34±1.5	28.45±3.4*
		45	28.95±1.2	33.46±1.8**	28.76±2.2
8.	Blood clotting time (second)	15	26.5±0.51	27.05±0.52	32.0±0.62**
		30	25.6±0.43	30.6±1.02*	51.2±0.80***
		45	26.2±1.00	38.7±1.12***	62.5±0.95***
9.	O_2 carrying capacity (ml O_2/g Hb)	15	14.00±1.4	11.63±1.5*	9.38±1.3**
		30	12.88±1.6	11.75±1.4*	8.76±1.4***
		45	12.38±1.6	11.00±1.2*	8.16±1.2***

*p<0.05, **p<0.01, ***p<0.001 Significance in comparison to control

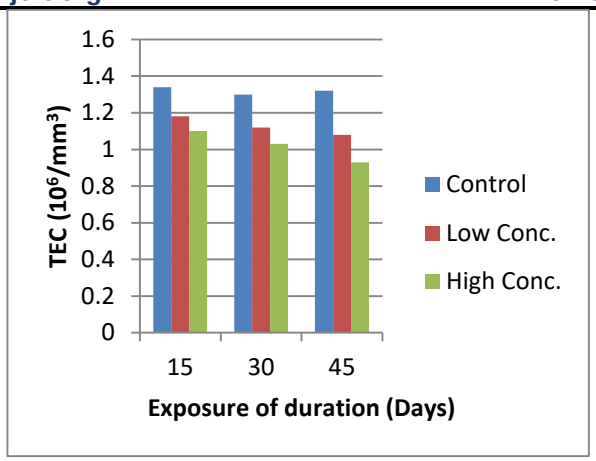


Figure- 1

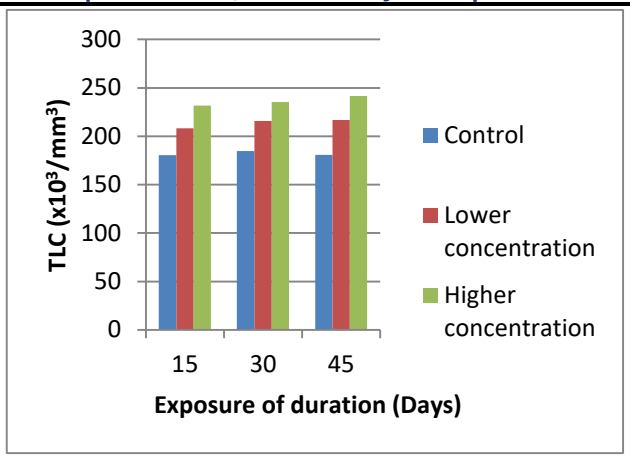
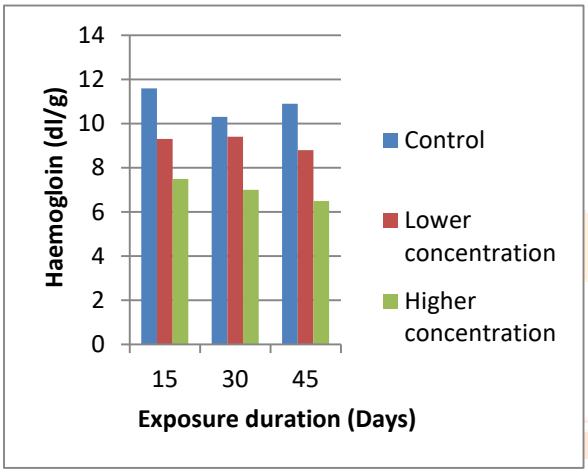


Figure- 2



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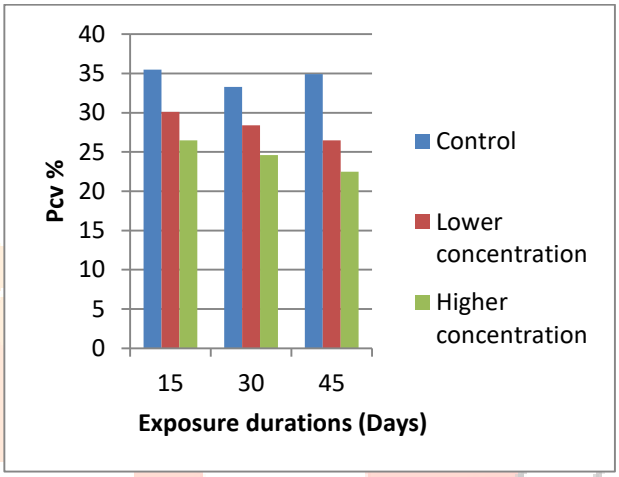

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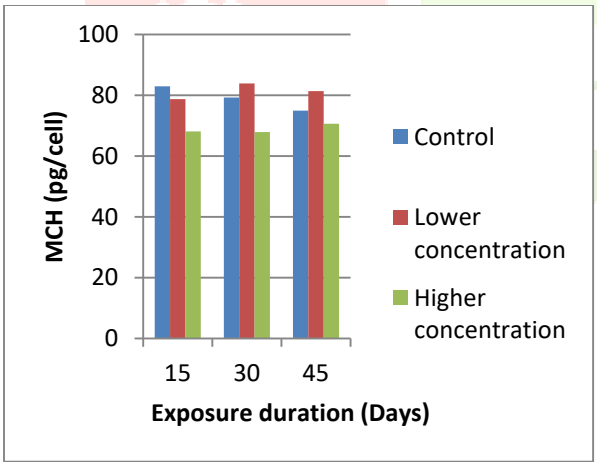

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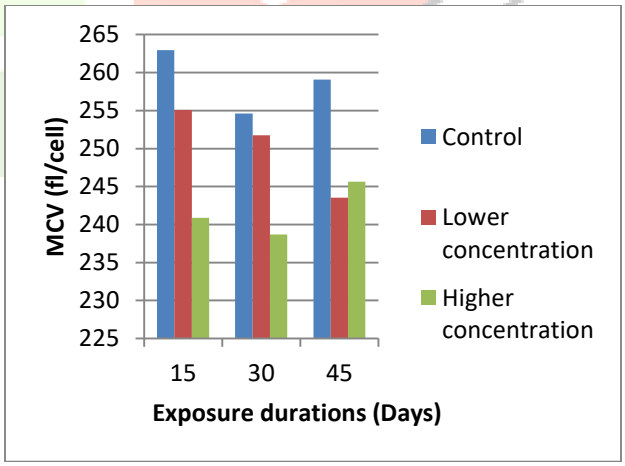
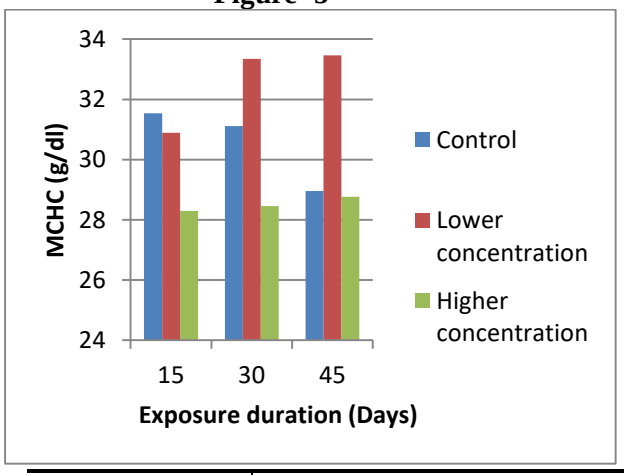


Figure- 6



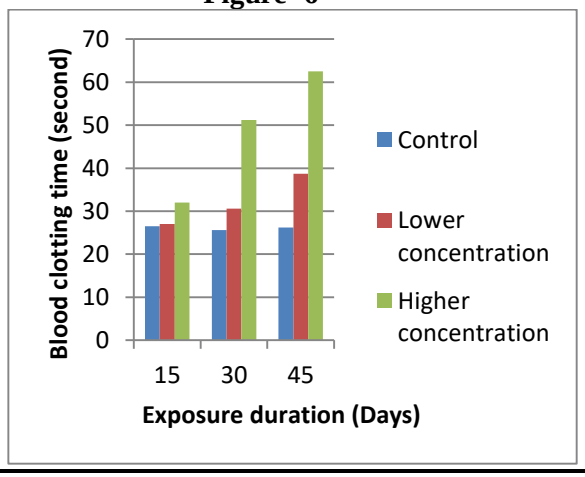


Figure- 7 **Figure- 8**

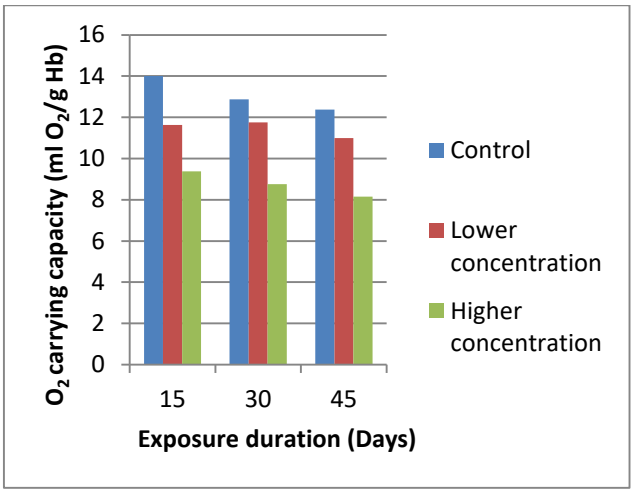


Figure- 9

IV. DISCUSSION

Literature suggested that the fluoride ion caused alteration in the haematological profile in experimental animals. Blood is a very sensitive indicator of the functioning of almost every systemof the body. The result of fluoride toxicity recorded during this investigation is similar to the results of fluoride toxicosis study conducted on rabbits by Abbas et al. (2017). The declining values of RBC, haemoglobin and PCV were noted that led to lowering in the values of MCV, MCH, and MCHC. In contrast to our result, there was enhancement in the TLC in all exposure periods. The loweringin the erythrocytes and haemoglobin related values may be due to the lysis and less production of RBCs in bone marrow as a result of decline haemoglobin production (Sayeed & Khan, 2010). Similar observations and findings were also recorded by earlier researchers in different fish species(Saxena et al., 2001, Kumar et al., 2010; Sujja et al., 2018; Tyagi et al., 2024). The histo- morphological findings in similar studies depicted the presence of macrophages in the spleen, causing more damage to erythrocytes in fluoride treated groups than control groups. This suggests the fluoride induced enhancement in phagocytosis in spleen localized macrophages, which consequently leads to the development of anaemia (Kahl et al., 1973; Danilov and Kasyanova, 1975). Moreover, fluoride also retards the normal process of erythropoiesis by interacting with the iron of haemoglobin (Abbas et al., 2017). Measurement of blood erythrocyte antioxidant enzyme activities shows that Fluoride from drinking water increases levels of superoxide dismutase, glutathione peroxidase and catalase (Akdogan et al., 2004). Therefore, theoxidative stress caused by fluoride ion in the blood may also be the reason for the resulted toxicity. Such reduction in haematological values has been recorded with mammals by other workers also (Atmaca et al., 2014). Reduction in PCV value on fluoride poisoning has been recorded by Swarupand Singh (1989) and Gujarathi et al. (1991). The decreased RBC count in the present study mayhave been associated with a decreased rate of erythrogenesis due to the negative effect of fluorideon erythropoiesis or to shortened life span of erythrocytes and membrane degeneration (Atmaca et al., 2014; Ozsvath, 2009). It has been reported that fluoride toxicity causes hematopoietic progenitor cells injury in humans (Machaliński et al., 2000; Machalinska et al., 2002). The studiesof Guru et al. (2014) and Saxena et al. (2001), fluoride toxicity on blood parameters conducted onother fish species are also in agreement with the findings of this study.

Due to toxicant effect there is decrease in the no. of RBC haemoglobin concentration and packed cell volume. As a result of haemoglobin reduction the amount of oxygen transported will be reduced and this affects the metabolism of fish at cellular level.

The gills are important organs with multiple functions including gaseous exchange, osmotic pressure regulation, acid base balance, ion transport and excretion of nitrogenous waste. Bhatnagar et al. (2007) visualised the fluoride induced histopathological changes in gills and kidney of freshwater fish *Labeo rohita*. In the fluoride exposed fish group with increasing severity with the time the gill tissue develop clubbed lamella, lamellar hyperplasia. On exposure to fluoride primary and secondary lamellar epithelium become swelled and clubbing of the secondary lamellae of gills fusion of secondary lamellae hyperplasia of gills will occur (Bajpai et al., 2012). The pathological changes in the structure of gills i.e. hyperplasia of mucous cells, which could inhibit oxygen intake and result in hypoxic condition of fish.

In the sodium fluoride exposed fish *Rita rita* there is significant decrease in RBC, HB, PCV, MCH which increase with increase in concentration of fluoride (Somnath et al., 2016), Kumar et al. (2010) reported significant decrease in RBC, HB, PCV and oxygen carrying capacity of blood in *Clarias batrachus*. Similar time and dose dependent decrease in RBC and increased WBC count was observed by Kamble and Velhal (2010).

In present study, the decrease in RBC count by fluoride may be responsible for reduction in Hb content, possibly from the destructive action of fluoride on the erythrocyte (RBC) membrane resulting in lower viability of the affected cells (Kumar et al., 2010). The damaging effect of any toxicant on erythrocytes may be secondary while primary action may be on erythropoietic tissues, causing either a decrease in RBCs production or an increased rate of erythrocyte destruction (Karuppasamy et al., 2005; Sujja et al., 2018). In this connection, a major concern is the effect on the ability of haemoglobin molecules to bind oxygen loosely and reversibly for their capacity to transport O_2. The observed decrease in O_2 carrying capacity in F-exposed fish may be due to the reduction of RBCs and Hb% (Kumar et al., 2010). This finding is supported by the observations of Chatterjee &. Ganguli (1993) who have also reported decreased O_2 carrying capacity of fish after exposure to Cu, Zn, and mahua oil cake. Another possibility for the decrease in O_2 carrying capacity in the present study may be due to damage of gills under fluoride stress leading to loss of respiratory area. This is clearly evident from histological examination of the gills (Kumar et al., 2010).

The decline in PCV (packed cell volume) observed in the present investigation may be the result of the reduction in RBC count and Hb content in *H. fossilis*. The findings are supported by the observations of Gupta et al.(2001), Saxena et al. (2001) and Kumar et al. (2010) they have reported similar changes in *Channa punctatus* and *C. batrachus* after exposure to fluoride. The slight variation in MCHC values may be due to changes in haemopoietic activity caused by fluoride.

All these changes were similar to those reported by Gupta (2003) in *Channa punctatus* and Kumar et al. (2010) in *C. batrachus* following exposure to fluoride. Hyperplasia of epithelium results in an increase of the diffusion distance affecting the exchange of gases. Fusion of lamellae may be considered to cause a decrease in the total respiratory area of the gills, thus resulting in a decreased O_2 uptake capacity of the gills. In this condition fish fail to get adequate oxygen for total metabolic activities, and they therefore visit the surface more frequently, as found in the present study. Earlier workers have also reported that increased thickness of the epithelial layers results from hyperplasia following exposure to the pesticide endosulfan (Nowak, 1992; Kumar et al., 2010).

V. CONCLUSION

We conclude that fluoride is haemooxic to *Heteropneustes fossilis* and its occurrence in the environment may threaten the health of aquatic species. Accordingly, it suggests that haematological parameters could be effectively used as reflective bioindicators in ecotoxicological studies.

VI. ACKNOWLEDGEMENT

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