ALUMINIUM INDUCED CHANGES IN THE HEAMATOLOGICAL PARAMETERS OF THE FRESHWATER FISH, *CYPRINUS CARPIO* var. *COMMUNIS*

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ABSTRACT: *In the present investigation, aluminium induced toxicity in haematological parameters such as haemoglobin content HCT, RBC, and WBC count in Cyprinus carpio var. communis exposed to 24 hours* LC_{50} *concentration. To assess the haematological profile of the control and treated fish, haemoglobin, haematocrit levels and RBC and WBC and counts were measured in the whole blood of Cyprinus carpio var. communis. Erythrocyte indices of fish viz., MCHC (Mean Cell Haemoglobin Concentration), MCH (Mean Cell Haemoglobin) and MCV (Mean Cell Volume) were also calculated. It was observed that the aluminium sulphate induced remarkable changes in the various haematological parameters of the selected fish.*

Key Words: *Aluminium toxicity, Haematological parameters, Haemoglobin, HCT, RBC, WBC, MCHC, MCH, MCV.*

1. INTRODUCTION

Blood constituents have been well established for many of the higher vertebrates as diagnostic tools in human and animal medicine. Changes in blood parameters are often quick to respond environmental or physiological alterations. Furthermore, they are easily measurable and provide an integrated measure of the physiological status of organisms which lives in a particular polluted niche. Emphasized the need for the study on haematological and physiological variables because of their relationship with respiration (haemoglobin content and related parameters), defence mechanisms (leucocyte values) and energetics (metabolite levels). Changes in haematological parameters such as haemoglobin content, HCT, RBC and WBC count can be induced by incidental factors such as capture and sampling as well as chronic factors such as exposure to disease and environmental contaminants. Haematological techniques including measurements of haematocrit, erythrocyte and leucocyte counts have proved valuable for fishery biologists in assessing the health of fish and in monitoring stress responses.

Aluminium exposure in waters reduced the haemoglobin content in *Salmo gairdneri* in brook trout, *Salvelinus* fontinalis, and in females of *whitefish, Coregonus* wartmanni (Vuorinen *et al.,* 1990). Whereas, the haemoglobin concentration in the males of whitefish, *Coregonus* wartmanni was increased by aluminium exposure at low pH (Vuorinen *et al.,* 1990). The studies in Witters *et al.,* (1986) rainbow trout, *Oncorhynchus mykiss,* Wood *et al.,* (1988a, b) in brook trout *Salvelinus fontinalis,* in white fish, *Coregonus wartmanni,* and in *Perca fluviatilis* have indicated that in acidic waters aluminium toxicity did not alter the haemoglobin content of blood. The Haematocrit (HCT) of blood is the ratio of the volume of erythrocytes to that of the whole blood. Decrease in HCT by cadmium exposure was recorded in Tilapia, *Oreochromis mossambicus,*, in flounders, in *Salmogairdneri* and in *Tilapia zillii*. Significant increase in HCT values in different fishes exposed to chromium, copper, mercury and Zinc was also reported by earlier workers. Similar increase in MCV was reported in fish exposed to mercury. In general, MCHC value was lowered in fish due to aluminium exposure in acidic waters (Milligan and Wood, 1982; Goss and Wood, 1988; Wood *et al.,* 1988a, Vuorinen *et al., 1990;* Vuorinen and Vuorinen, 1991). The aim of the present investigation is to ascertain the impact of aluminium toxicity in neutral pH on the haematological parameters of fish *Cyprinus carpio* var. *communis.*

2.MATERIALS AND METHODS

2.1 Haematological Studies

To assess the haematological profile of the control and treated fish, haemoglobin, haematocrit levels and RBC and WBC counts were measured in the whole blood of *Cyprinus carpio* var. *communis.* Erythrocyte indices of fish *viz.,* MCHC (Mean Cell Haemoglobin Concentration), MCH (Mean Cell Haemoglobin) and MCV (Mean Cell Volume) were also calculated.

2.2. Haemoglobin

Haemoglobin content was analysed by using standard procedure as mentioned below.

2.3 Haematocrit (Packed Cell Volume):

Haematocrit was estimated by micro haematocrit (Capillary) method by sodium heparinised micro haematocrit capillaries (Rakta Mucaps, Top Exports Private Ltd., Bombay, India), as described by Nelson and Morris (1989) using RM 12C micro centrifuge (Remi Centrifuge, Remi Motors, Bombay, India) and a micro haematocrit reader.

2.4 RBC Count

Erythrocytes were counted by the method of Rusia and Sood (1992) using haemocytometer.

2.5 Leucocytes count

Leucocytes were counted by the method of Rusia and Sood (1992) using haemocytometer. The Mean Cell Haemoglobin concentration (MCHC) is the average concentration of haemoglobin in a given volume of packed red cells. It is calculated from the haemoglobin concentration and the haematocrit. Mean Cell Haemoglobin (MCH) The MCH is the content (weight) of haemoglobin of the average red cell. It is calculated from the haemoglobin concentration and the red cell count. Mean Cell Volume (MCV) is the average volume of red cells and is calculated from the Haematocrit (HCT) and Red Cell Count (RBC).

3. RESULTS

Changes in haematological profiles of fish, *Cyprinus carpio* var. *communis* during acute aluminium exposure are given in Table 1 and Fig. 1. Haemoglobin content and haematocrit value decreased from the control level by -11.41% and -7.57%, respectively. However, their decrease was not significant. Erythrocyte and leucocyte counts were altered significantly from the control value by registering -15.40% decrease and +25.01% increase, respectively. Erythrocyte indices such as MCHC declined in the experimental groups when compared to that of control giving a percent decrease of -3.87%, while MCH and MCV increased compare to control by +5.74% and +9.62%, respectively. However, statistical analysis revealed that these changes were not significant. Table 2 and Fig. 1 give the data on changes in haemoglobin content of the fish blood during sublethal aluminium treatment. At the end of 7th day a pronounced decline in haemoglobin content -57.16% was observed in the experimental fish which gradually recovered during the subsequent exposure periods showing a decrease of -43.20%, -34.77%, -29.59% and -21.90% after 14th, 21st, 28th and 35th day, respectively.

Exposure of fish to sublethal aluminium sulphate resulted in a +11.65% increase in the haematocrit value of fish at the end of 7th day (Table 3, Fig. 12). However, after 7th day, the haematocrit value declined giving a percent decline of -30.02%, but at the end of the 21st day, the haematocrit reached a value equal or -0.002% to that of the control. At the end of the 28th day the haematocrit value again decreased showing -17.67%. The end of the 35th day, haematocrit value increased by +50.02% Compare to the control. Red blood cell count of the blood samples of *Cyprinus carpio* var. *communis* after 7th day treatment was reduced drastically by -45.71%. However, there was a gradual recovery during subsequent exposure periods giving a percent decline of -38.22%, -30.78%, -27.27% and -21.56% after 14th, 21st, 28th and 35th day, respectively (Table 4 and Fig.13). Control groups alone were tested 14th and 35th, 28th and 35th days were on par with each other. The rest of the periods varied significantly among themselves. When treatment groups alone were tested 14th and 28th day, 21st and 35th day were on par with each other. The rest of the combinations varied from the above-mentioned periods and also among themselves. When period means were analysed irrespective of the treatments 14th and 28th, 14th and 35th day were on par with each other while 7th and 21st day varied among themselves and varied from that of 14th, 28th and 35th day. Analysis of treatment means showed that the experimental groups varied from the control depicting the aluminium impact on the red blood cell count of the blood.

Table 5 and Fig. 14 present the data on the changes in WBC count in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate. The WBC count increased than that of the control by +80.99% on 7th day treatment and further increased on 14th and 21st day giving a value of +133.35% and +130.25%, respectively. However, the WBC count declined gradually in the following weeks (28th and 35th day) showing +83.00% and +50.51% increase from the control, respectively. The changes occurred due to the sublethal aluminium exposure in the MCHC value is given in Table 6 and Fig. 15. MCHC value declined by -61.01% after 7th day exposure and in the subsequent weeks the percent decline was reduced giving a value of -16.40%, -34.56% and -14.39% on 14th, 21st and 28th day, respectively. However, after the 35th day the percent decline of the MCHC value again increased showing -49.43% declined to relative to the control. When period means were analysed irrespective of the treatments, only 21st day varied from tile rest of the exposure periods, which were on par with each other. When treatment means were analysed irrespective of the exposure period, the means of MCHC values of aluminium exposed fish varied significantly compared to the control.

Table 7 and Fig. 16 illustrate the changes occurring in the MCH value due to aluminium stress in fish blood. The MCH value decreased than that of the control after 7th day recording -21.67%. During following periods, MCH value reduction was very low registering -7.30%,-2.20% and -2.56%, on the 14th, 21st and 28th day, respectively. Whereas on 35th day, MCH value increased compared to the control by +9.66%. Table 8 and Fig. 17 give the data on changes in the treatment groups with regard to MCV value in fish. There was a conspicuous percent increase of +105.55% in the MCV value in aluminium treated fishes after 7th day. However, the increase in MCV value decreased in the following weeks giving +16.11%, +46.11% and +14.11% on 14th, 21st and 28th day, respectively. However, on 35th day, the percent change in MCV value again arose to +94.73% relative to the control.

DISCUSSION

Several metals inflict anaemia in fishes (Larsson, 1975; Johansson- Sjobeck and Larsson, 1978; Gill and Pant, 1987; Ruparelia *et al*., 1990; Gill *et al*., 1991). Haux and Larsson (1984), Tewari *et al*., (1987) and Gill *et al*., (1991) reported that anaemia accompanied with reduction in haematocrit, haemoglobin and red blood cell is the most prominent haematological effect of metal intoxication in fishes. Anaemia may be due to an increase of plasma volume caused by a disturbed water balance, decreased rate of RBC production, loss or destruction of red blood cells, as suggested by Larsson *et al*., (1976). In the present study also, aluminium exposed fishes exhibited anaemic condition by recording lower content of haemoglobin, haematocrit and red blood cells than the control fish. This may be due to destruction of RBC or inhibition of erythropoiesis or interference of the metabolism or disturbance in fluid volume balance produced by aluminium ions which may find support from the above workers. Gill and Pant (1987) recorded in *Puntius conchonius* after mercury exposure, an initial fall in RBC and haemoglobin count, but after 2 or 3 weeks, increase occurred in both these indices. Folmar (1993) stated that increases in haematocrit values were associated with osmotic shifts; as the pH of the blood decreased, erythrocytes swelled and plasma volume decreased, the findings of McKim *et al.* (1970), Vander Putte *et al. (1982)* and Hilrny *et al.* (1987) support the above view, that RBC swelling is the reason for haematocrit increase in fishes after exposure to chromium, copper, mercury and zinc, respectively.

The above concept is further strengthened by the observation of higher value of haematocrit in fishes by aluminium exposure in acidified waters (Goss and Wood, 1988; Wood *et al.,* 1988b; Dietrich and Schlatter, 1989a; Playle *et al., 1989;* Witters *et al.,* 1986, 1990; Reid *et al.,* 1991). Higher values of haematocrit observed in the present study after 7th and 35th week may find support of the above author's explanation that the RBC swelling consequent to fluid volume disturbance might have increased the haematocrit value. Rupareba *et al.* (1991) suggested that red blood cell swelling is the cause for the decrease in MCHC value in *Oreochromis mossambicus* after cadmium exposure. Nikinmaa (1982) and Vuorinen *et al.* (1992) reported that lowered MCHC, elevated haematocrit denotes the swelling of erythrocytes, a mechanism by which blood O2 transport capacity is increased when fishes were subjected to less effective gas exchange (Vuorinen *et al.,* 1992). Besides, lowered MCHC might result from the release of young erythrocytes containing less haemoglobin than the older ones (Lehmann and Sturenberg, 1975) into the circulation (Vuorinen *et al., 1990).* Ghazaly (1992) reported reduction in MCH value in *Tilapia zillii* after cadmium poisoning. Further, the author stated that these haematological changes in fish compensate for impaired oxygen uptake due to gill damage caused by cadmium.

In the present study, similar haematological changes might have occurred due to aluminium exposure. The increased mean cell volume (MCV) during stress (capture) was due to the swelling of red blood cells or to the release of large red blood cells into the general circulation as reported by Fletcher (1975). The anaerobic condition (hypoxia) probably developed during sublethal aluminium stress (Poleo, 1995) may be the reason for increased mean cell volume as indicated by Soivio and Nyholm (1973) that anaerobic storage of trout red blood cells caused them to swell. Anthropogenically, chromium is released into the environment through sewage and fertilizers Ghani (2011). Aluminium is the third most abundant element found in the earth's crust (Gupta *et al*., 2013). Aluminium has no biological role and is a toxic non-essential metal to microorganisms (Olaniran *et al*., 2013). Accumulation of as in the muscles was the least in the all experimental groups when compared to other soft tissues. It is not an active site with detoxification, and hence, as is not transported from other tissues to muscles. Navarez *et al*., (2011) found positive correlation of as content in muscles of channel Catfish: Ictalurus punctatus and green sunfish: Lepomis cyanellus. Lam *et al*., (2006) and Li *et al*., (2016) found metabolic and histopathological liver when the

Zebra fish exposed with arsenic. Selamoglu Talas *et al*., (2012) also found during their investigation that granulocyte, erythrocyte, haemoglobin, haematocrit values were decreased in Cyprinus carpio by the use of as in comparison with control group. Although for ex. the role of immune cells as mediators of secondary or idiosyncratic liver damage in fish is as yet uncertain (Vliegenthart *et al*., 2014). In particular Wolf *et al*., (2015), which was co-authored by a team of fish pathlogistics, provides many illustrated examples of morphologic misdiagnosis and non- lesions in fish used for toxicological bio assay. Fish exposed to as exhibited anxiety in breathing due to the clogging of gills by coagulated mucous and suffered direct damage of as ions to blood vessels, resulting in vascular collapse in the gills and anoxia (Mondal and Samantha (2015)). Towards that end, several recent articles further describe issues that lead to and are caused by, poor quality fish histopathology data (Wolf (2011); Wolf *et al*., (2015); Wolf and maack (2017); Wolf (2018).

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Table 1 Changes in the haematological profiles of *Cyprinus carpio* var. *communis* exposed to acute aluminium sulphate toxicity.

S. no	Parameters	Control	Experimental	Percent change	Calculated 't'
01.	Haemoglobin (g/dl)	3.952± 0.1434	3.502 ± 0.1583	-11.41	2.1098
02.	Haematocrit (%)	5.302± 0.2552	4.902 ± 0.2917	-7.57	1.0330
03.	Erythrocytes (million / cubic m)	1.302 ± 0.0243	1.102 ± 0.0608	-15.40	3.0681
04.	Leucocytes (thousand cubic mm	20.152 ± 791.7015	25.182 ± 574.9261	+25.01	5.1472
05.	MCHC (g / dl)	75.442 ± 5.6628	72.536 ± 5.576	-3.87	0.3659
06.	MCH (Pico grams)	30.410± 1.1453	32.150 ± 2.0367	+5.74	0.7449
07.	MCV (cubic micra)	40.742 ± 1.4391	44.652 ± 1.6544	+ 9.62	1.7845

Values are means ± S.E of five individual observations
- Denotes per cent decrease over control
+ Denotes per cent increase over control
Values are significant at 5% level
Degrees of freedom at 8t 0.05 = 2.306.

Table 2 Haemoglobin content in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods.

S.no	Exposure period (in days)	Blood haemoglobin content in g/dl		Percent change
		Control	Treatment	
01.	07	02.802 ± 0.166	1.202 ± 0.102	-57.16
02.	14	02.642 ± 0.602	1.502 ± 0.034	-43.20
03.	21	02.822 ± 0.134	1.842 ± 0.083	-34.77
04.	28	02.302 ± 0.132	1.622 ± 0.039	-29.59
05.	35	02.562 ± 0.053	2.002 ± 0.034	-21.90

Values are means ± S. E. of five individual observations
'-' Denotes per cent decrease over control

Table 3 Haematocrit values of *Cyprinus carpio* var. *communis* exposed to sublethal aluminium sulphate toxicity for varying periods.

S.no	Exposure period (in days)	Blood haematocrit (%)		Percent change
		Control	Treatment	
01.	07	4.302 ± 0.124	4.802 ± 0.257	+.11.65
02.	14	5.002 ± 0.356	3.502 ± 0.356	-30.02
03.	21	4.002 ± 0.356	4.002 ± 0.356	-0.002
04.	28	5.102 ± 0.102	4.202 ± 0.124	-17.67
05.	35	4.002 ± 0.356	6.002 ± 0.160	+50.02

Values are mean ± S. E. of five individual observations
'-' Denotes per cent decrease over control
'+' Denotes per cent increase over control

Table 4 Erythrocytes count in the blood of *Cyprinus carpio* var. *communis* exposed to sub lethal concentration of aluminium sulphate for varying periods.

S.no	Exposure period (in days)	Erythrocytes counts in million / cu.mm		Percent change
		Control	Treatment	
01.	07	0.593 ± 0.019	0.323 ± 0.015	-45.71
02.	14	0.803 ± 0.027	0.497 ± 0.032	-38.22
03.	21	0.912 ± 0.018	0.632 ± 0.034	-30.78
04.	28	0.724 ± 0.111	0.528 ± 0.028	-27.27
05.	35	0.785 ± 0.034	0.616 ± 0.288	-21.56

Values are mean ± S. E. of five individual observations
'-' Denotes per cent decrease over control

Table 5 Leucocyte count in the blood of ***Cyprinus carpio*** var. ***communis*** exposed to sublethal concentration of aluminium sulphate for varying periods.

S.no	Exposure period (in days)	Leucocyte counts in thousands / cu.mm		
		Control	Treatment	Percent change
01.	07	18.502 ± 0.804	33.482 ± 1.319	+80.99
02.	14	19.202 ± 0.666	44.802 ± 1.039	+133.35
03.	21	21.602 ± 1.979	49.732 ± 1.178	+130.25
04.	28	27.902 ± 1.236	51.053 ± 0.808	+83.00
05.	35	19.512 ± 0.656	29.362 ± 0.760	+50.51

Values are mean ± S. E. of five individual observations
'-' Denotes per cent decrease over control
'+' Denotes per cent increase over control

Table 6 MCHC value of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

S. no	Exposure period (in days)	Mean cellular haemoglobin concentration in g/dl		Percent change
		Control	Treatment	
01.	07	65.058 ± 3.0484	25.380± 2.6310	-61.01
02.	14	53.928 ± 4.163	45.096 ± 5.658	-16.40
03.	21	73.526 ± 8.868	48.132 ± 6.303	-34.56
04.	28	45.129 ± 2.588	38.669± 2.652	-14.39
05.	35	65.992 ± 5.810	33.385 ± 0.817	-49.43

Values are mean ± S. E. of five individual observations
'-' Denotes per cent decrease over control

Table 7 MCH value of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

S.no	Exposure period (in days)	Mean Cellular Haemoglobin value in picograms		Percent change
		Control	Treatment	
01.	07	47.574 ± 3.387	37.273 ± 2.375	-21.67
02.	14	33.153 ± 1.668	30.739 ± 1.932	-7.30
03.	21	30.974 ± 1.231	30.298 ± 2.274	-2.20
04.	28	31.911 ± 2.092	31.112 ± 1.725	-2.56
05.	35	32.906 ± 1.459	36.077 ± 1.042	+9.66

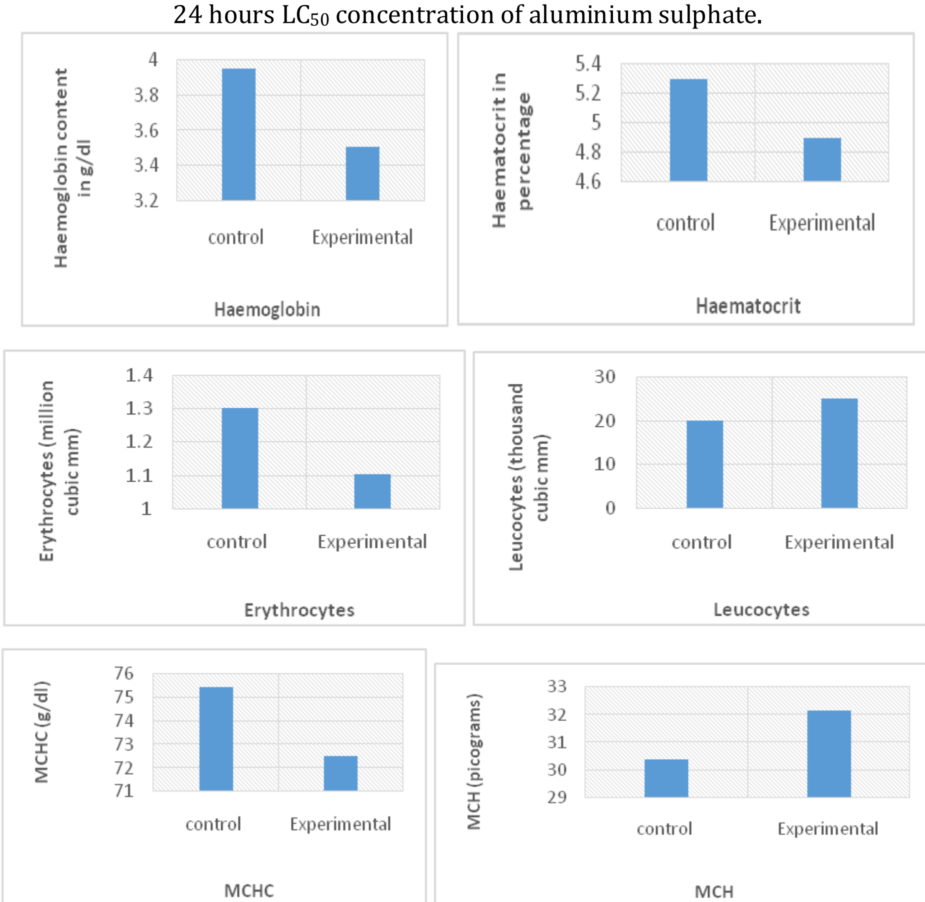
Values are mean ± S. E. of five individual observations
'-' Denotes per cent decrease over control
'+' Denotes per cent increase over control

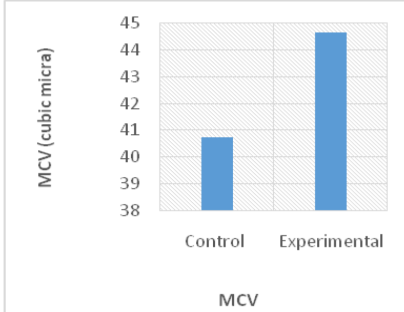
Table 8 MCV value of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate toxicity for varying periods.

S.no	Exposure period (in days)	Mean cellular volume value in cubic micra		Percent change
		Control	Treatment	
01.	07	73.094 ± 3.962	150.224 ± 9.084	+105.55
02.	14	62.652± 4.780	72.732 ± 10.025	+16.11
03.	21	44.278± 4.621	64.982 ± 4.641	+46.78
04.	28	70.700 ± 2.275	80.660 ± 4.566	+14.11
05.	35	50.736 ± 2.800	98.782 ± 6.149	+94.73

Values are mean ± S. E. of five individual observations
'+' Denotes per cent increase over control

Fig 1. Changes in the haematological profiles of *Cyprinus carpio* var. *communis* exposed to 24 hours LC_{50} concentration of aluminium sulphate.





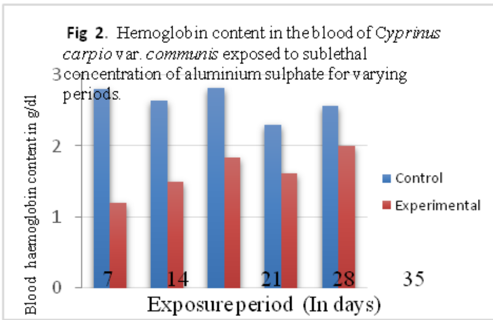


Fig 2. Hemoglobin content in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods.

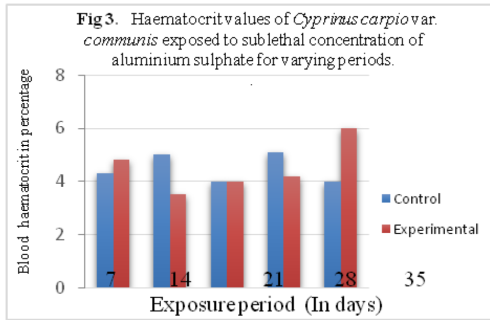


Fig 3. Haematocrit values of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods.

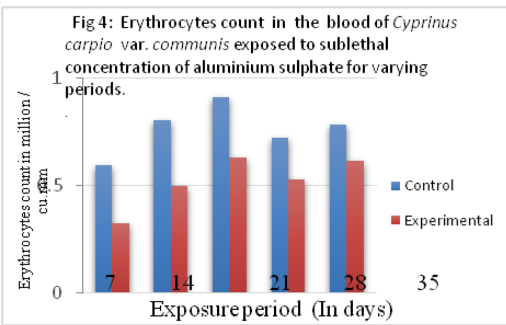


Fig 4: Erythrocytes count in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods.

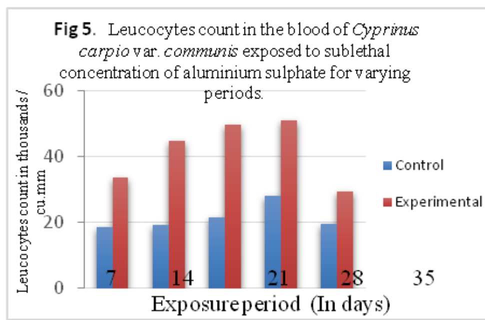


Fig 5. Leucocytes count in the blood of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods.

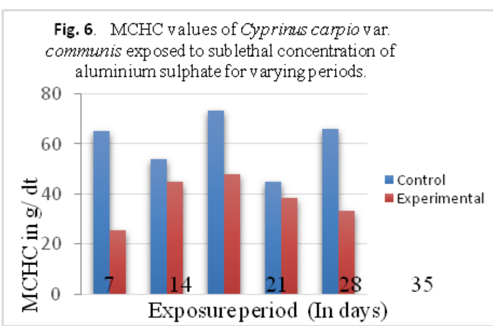


Fig. 6. MCHC values of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods.

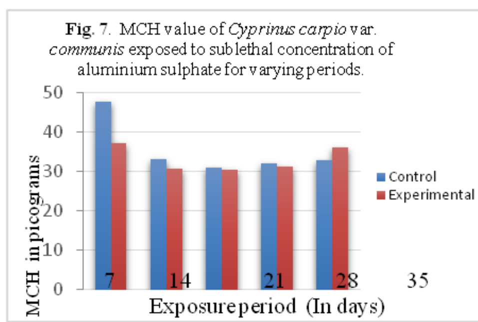


Fig. 7. MCH value of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods.

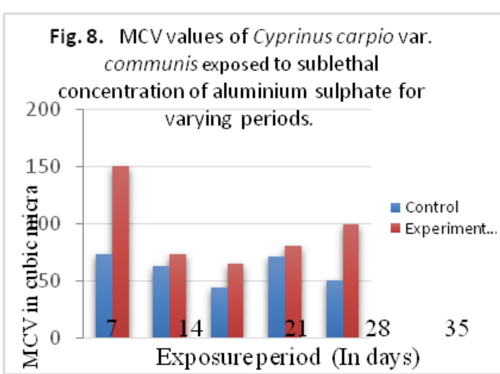


Fig. 8. MCV values of *Cyprinus carpio* var. *communis* exposed to sublethal concentration of aluminium sulphate for varying periods.