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HAEMATOLOGICAL PROFILE OF FRESHWATER FISH, *CHANNA STRIATUS* (BLOCH) UNDER THE STRESS OF ZINC SULPHATE

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ABSTRACT: In the present investigation, Zinc Sulphate toxicity was evaluated on various haematological parameters in a fresh water air-breathing fish, *Channa striatus*. When exposed to sublethal concentrations (15, 20 and 30mg/L) of Zinc Sulphate in water for a period of 10, 20, 30 and 40days. At the end of 10, 20, 30 and 40days exposure periods, blood samples were collect from the control and experimental fish. The fish blood was assayed for selected haematological parameters, Haemoglobin (Hb), Red blood cells (RBC), Haematocrit (Hct) and White blood cells (WBC). The derived haematological indices of Mean cell volume (MCV), Mean cell Haemoglobin (MCH) and Mean cell Haemoglobin concentration (MCHC) were calculated. The fish, showed a haematological response after exposure (10, 20, 30 and 40days) to 15, 20 and 30mg/L of Zinc Sulphate. There was a decrease in Haemoglobin content (Hb), Red blood cells (RBC), Haematocrit value (Hct) and Mean cell haemoglobin (MCH). A significant increase was observed in WBC and Mean cell volume, Mean cell haemoglobin concentration. In conclusion, the alterations were observed indicate that haematological parameters can be used as an indicator of Zinc related stress in fish on exposure to elevated Zinc Sulphate levels.

KEYWORDS: Channa striatus, Zinc Sulphate, sublethal, Haematological parameters.

INTRODUCTION

The pollution of the environment with toxic substances has been increased in recent years as a result of the rapid growth of industries[1]. The industrial discharges containing toxic and hazardous substances, including heavy metals contribute tremendously to the pollution of aquatic ecosystem[13]. The aquatic environment has been always subjected to different types of pollutants of industrial, agricultural and domestic wastes and severely affect the aquatic organisms[7].

The contamination of the aquatic systems with heavy metals from natural, anthropogenic sources has become a global problem which poses a threat to ecosystems and natural communities[12]. Increased discharge of heavy metals into natural aquatic ecosystems can expose aquatic organisms to unnaturally high levels of these metals[26]. The heavy metal toxicants were accumulated in the fish through general body surface which affects their life support system. Once these toxic substance enters into body, they damage and weaken the mechanism concerned leading to physiological, pathological and biochemical disorders[3].

Zinc is the second most abundant trace element after Fe and is an essential trace element and micronutrient in living organisms, found almost in every cell and being involved in nucleic acid synthesis and occurs in many enzymes. Additionally, Zn is involved in more complicated functions, such as the immune system, neuro-transmission and cell signalling[20]. Although, small quantity of Zinc are required for the normal development and metabolism, but if its level exceeds the physiological requirements, it can act as a toxicant[27]. The deficiency of Zinc has many symptoms including hindered growth, hypogonadism in males, neurosensory impairments and cell-mediated immunological malfunction [10].

Haematology of fish is often determined as an index of their general health status. Haematological parameters are important patho-physiological indices and are important in diagnosing the structural and functional status of fish exposed to toxicants[17]. In recent years, for determining the effect of toxic substances during clinical diagnosis haematological parameters are used because of close association between the circulatory system and external environment[33,28]. Thus haematological alterations could be used as a warning system to indicate the presence of chronic toxicity of a toxicant in natural water.

MATERIALS AND METHODS

Experimental Animals

In the present study, *Channa striatus* weighing 26-28g used in the present study were procured from State Fisheries culture tanks. They were transported to the laboratory in oxygenated containers and treated with $KMnO_4$ to avoid dermal infection and acclimatized to laboratory conditions for 15 days. The fish were fed with commercial feed once a day at a rate of 2% of body weight and temperature was maintained at 28±1°C. Water in the containers was replaced by fresh water at every 24 h. Before starting the experiment, LC_{50} value was calculated by Finney Probit Analysis method[8]. The LC_{50} was found as 120 mg/ L.

Analytical Techniques

Determination of LC_{50} at 96 hours by Finney Probit Analysis method[8]. The sublethal concentrations of Zinc Sulphate were selected for the investigation were $1/8^{th}$ of LC_{50} (15mg/L), $1/6^{th}$ of LC_{50} (20mg/L) and $1/4^{th}$ of LC_{50} (30mg/L). Haemoglobin (HB) was estimated by Cyanomethemoglobin method[17]. Red blood cell count (RBC) and White blood cell count (WBC) were estimated by Haemocytometer method[25]. Haematocrit (Hct) was estimated by Wintrobe tube method[32]. Red Cell Indices were calculated from Hb Concentration, Hct percentage and RBC count[6].

Statistical Analysis

Effects of Sublethal Concentrations (15mg/L, 20mg/L and 30mg/L) of Zinc Sulphate for 10, 20, 30 and 40days of exposure on haematological parameters of fish, *Channa striatus*, were studied. The alterations in haematological parameters such as haemoglobin, red blood cells, white blood cells, haematocrit, red cell indices in terms of SEM(Mean ± SE) along with Control. The results were statistically analysed by Student's t-test, all the data was presented as Mean ± SE. For each experimental treatment, three replicates were taken. For each replicate, under each treatment mean was calculated. The mean values thus obtained were used to calculate experimental treatment Mean and standard errors of mean. P-values were determined using the t-statistics and denoted as NS-Not Significant; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

RESULTS AND DISCUSSIONS

Table-1 and Figure-1 Revealed that the changes in haemoglobin of the blood of fish channa striatus exposed to sublethal concentrations of $ZnSO_4$ upto 40 days. The haemoglobin content was decreased throughout the study period showing a minimum percent decrease of -2.39 at the end of 10th day and a maximum percent decrease of -19.49 at the end of 40th day. The decrease was more at higher concentration (30mg/L) and higher duration (40days) when compared to the control. The alterations in red blood cell count (RBC) of the blood of fish, *Channa striatus* exposed to sublethal concentration of $ZnSO_4$ upto 40days, shown in table-2 and figure-2. The RBC count was decreased throughout the study period when compared to the control showing a minimum percent decrease of -12.21 at the end of 10th day and a maximum percent decrease of -31.88 at the end of 40th day. The decrease was more at higher concentration (30mg/L) and higher duration (40days).

Table-3 and Figure-3 expressed the changes in White blood cell count was elevated showing a minimum percent increase of 6.72 at the end of the 10th day and a maximum percent increase 24.07 at the end of 40th day. The significant increase was more at higher concentration (30mg/L) and higher duration (40days) when compared to control. Table-4 and Figure-4 represented the data on changes in the haematocrit values of the fish channa striatus exposed to sublethal concentrations of $ZnSO_4$ upto 40days. The haematocrit values showing a minimum percent decrease of -1.19 at the end of 10th day and a maximum percent decrease -15.10 at the end of 40th day. The decrease was more at higher concentration and higher duration when compared to control.

Table-5 and Figure-5 revealed the changes in Mean cell volume (MCV) of the blood of fish, *Channa striatus* exposed to sublethal concentration of $ZnSO_4$ upto 40days. The MCV was increased throughout the study period when compared to the control showing a minimum percent increase of 1.14 at the end of 10th day and a maximum percent increase of 5.01 at the end of 40th day. The increase was more at higher concentrations (30mg/L) and higher duration compared to control.

Table-6 and Figure-6 represented the changes in the Mean cell haemoglobin (MCH) was decreased throughout the study period shows a minimum percent decrease of -1.01 at the end of 10th day and a maximum percent decrease of -11.53 at the end of 40th day. The decrease was more at higher concentration and higher duration when compared to control. Table-7 and

Figure-7 revealed that the changes in Mean cell haemoglobin concentration (MCHC) of the blood of fish, C*hanna striatus* exposed to sublethal concentrations of $ZnSO_4$ upto 40days. The MCHC was increased throughout the study period when compared to control showing a minimum percent increase of 3.33 at the end of 10th day and a maximum percent increase of 16.56 at the end of 40th day. The increase was more at higher concentration (30mg/L) and higher duration (40days).

Table-1: Alterations in Haemoglobin (HB) of *Channa striatus* exposed to sublethal concentrations of Zinc Sulphate compared to control (Mean ± SE)

Concentration of $ZnSO_4$		Experimental Days			
		10 days	20 days	30 days	40 days
Control	Mean SE	13.190 ± 0.158	13.200 ± 0.219	13.210 ± 0.236	13.210 ± 0.243
15mg/L	Mean SE %V	12.880^{NS} ± 0.314 -2.39	12.580* ± 0.182 -4.69	12.470* ± 0.22 -5.54	12.420* ± 0.216 -5.92
20mg/L	Mean SE %V	12.610* ± 0.220 -4.44	11.960** ± 0.173 -9.38	11.920** ± 0.189 -9.69	11.880** ± 0.263 -10.01
30mg/l	Mean SE %V	11.670** ± 0.191 -11.55	10.880*** ± 0.171 -17.57	10.740*** ± 0.208 -18.62	10.630*** ± 0.251 -19.49

Each value is the Mean ± SE of six individual observations.
The Hb values are expressed as gram / deciliter (g/dL) in fish blood.
SE- Standard Error; %V-Percent variation; NS: Not Significant;* $P<0.05$; ** $P<0.01$; *** $P<0.001$

Figure-1: Haemoglobin content in *Channa striatus* exposed to sublethal concentrations of $ZnSO_4$ compared to control

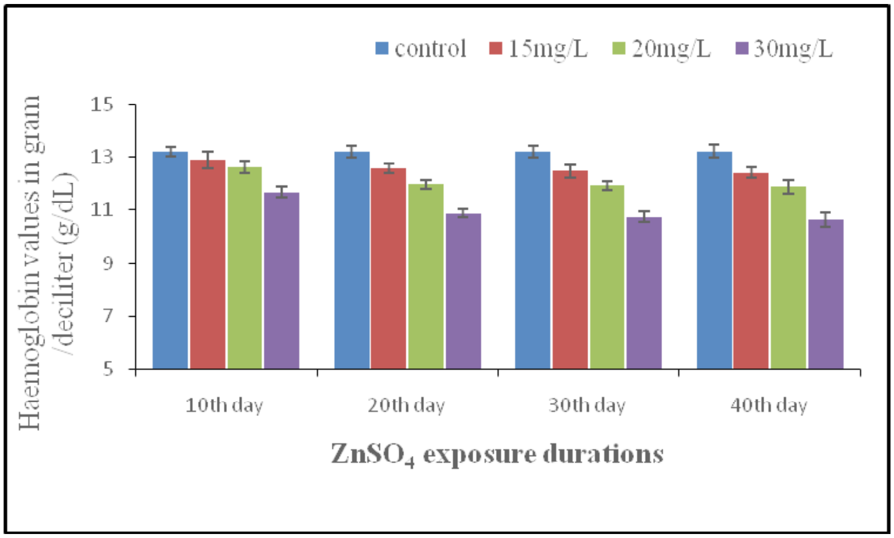


Table-2: Red Blood Cells (RBC) Count of *Channa striatus* exposed to sublethal concentrations of Zinc Sulphate compared to control. (Mean ± SE)

Concentration of $ZnSO_4$		Experimental Days			
		10 days	20 days	30 days	40 days
Control	Mean SE	3.660 ± 0.196	3.630 ± 0.243	3.600 ± 0.153	3.600 ± 0.148
15mg/L	Mean SE %V	3.220^{NS} ± 0.294 - 12.21	3.110^{NS} ± 0.177 - 14.49	3.060* ± 0.212 - 15.11	2.980* ± 0.103 - 17.45
20mg/L	Mean SE %V	2.810* ± 0.222 - 23.39	2.710** ± 0.196 - 25.44	2.630** ± 0.179 - 27.04	2.590** ± 0.149 - 28.14
30mg/l	Mean SE %V	2.690* ± 0.211 - 26.70	2.600** ± 0.162 - 28.52	2.520** ± 0.150 - 30.14	2.460*** ± 0.145 - 31.88

30mg/L Mean SE %V

Each value is the Mean ± SE of six individual observations.

The RBC values are expressed as millions/mm^3 (10^6/mm^3) of fish blood. SE- Standard Error; %V-Percent variation; NS: Not Significant;* P<0.05; ** P<0.01; *** P<0.001

Figure-2: Red Blood Cells Count in *Channa striatus* exposed to sublethal concentrations of $ZnSO_4$ compared to control

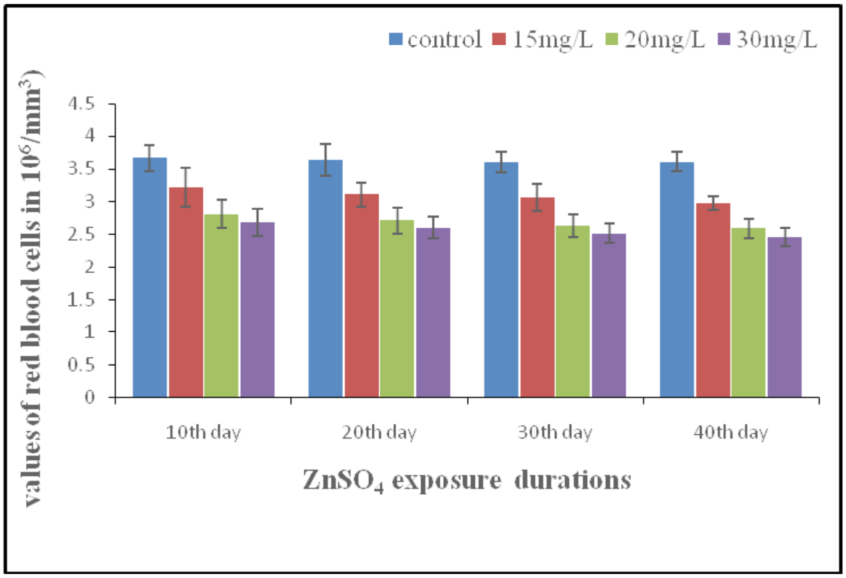


Table-3: White Blood Cell (WBC) count of *Channa striatus* exposed to sublethal concentrations of Zinc Sulphate compared to control. (Mean ± SE)

Concentration of $ZnSO_4$		Experimental Days			
		10 days	20 days	30 days	40 days
Control	Mean SE	4.280 ± 0.109	4.320 ± 0.104	4.360 ± 0.102	4.380 ± 0.109
15mg/L	Mean SE %V	4.570^{NS} ± 0.167 6.72	4.680^{NS} ± 0.217 8.41	4.780* ± 0.212 9.81	4.840* ± 0.236 10.66
20mg/L	Mean SE %V	4.650* ± 0.147 8.66	4.830* ± 0.219 11.97	5.050** ± 0.194 16.01	5.190** ± 0.201 18.55
30mg/l	Mean SE %V	4.840* ± 0.222 13.12	5.010** ± 0.198 16.03	5.200** ± 0.207 19.30	5.430*** ± 0.124 24.07

Each value is the Mean ± SE of six individual observations.
The WBC values are expressed as thousands/mm^3 ($10^3/mm^3$) of fish blood.
SE- Standard Error; %V-Percent variation; NS: Not Significant;* P<0.05; ** P<0.01; *** P<0.001

Figure-3: White Blood Cells Count in *Channa striatus* exposed to sublethal concentrations of $ZnSO_4$ compared to control

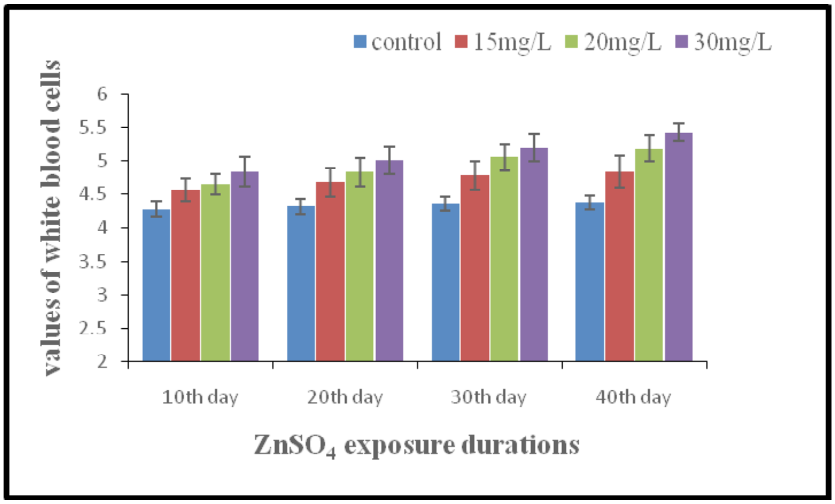


Table-4: Changes in Haematocrit (Hct) of *Channa striatus* exposed to sublethal concentrations of Zinc Sulphate compared to control. (Mean ± SE)

Concentration of $ZnSO_4$		Experimental Days			
		10 days	20 days	30 days	40 days
Control	Mean SE	39.470 ± 0.185	39.390 ± 0.263	39.340 ± 0.180	39.210 ± 0.108
15mg/L	Mean SE %V	39.250^{NS} ± 0.151 -1.19	38.540* ± 0.213 -2.15	38.370** ± 0.289 -2.97	36.160*** ± 0.199 -5.23
20mg/L	Mean SE %V	38.110** ± 0.206 -3.45	37.180** ± 0.199 -5.61	36.910*** ± 0.154 -5.85	35.050*** ± 0.301 -10.61
30mg/l	Mean SE %V	36.220*** ± 0.201 -8.23	35.480*** ± 0.396 -9.93	34.200*** ± 0.139 -12.68	33.290*** ± 0.181 -15.10

Each value is the Mean ± SE of six individual observations.
The Haematocrit values are expressed as percentage (%) of fish blood.
SE- Standard Error; %V-Percent variation; NS: Not Significant; * $P<0.05$; ** $P<0.01$; *** $P<0.001$

Figure-4: Haematocrit values (%) in *Channa striatus* exposed to various period treated at various sublethal doses of $ZnSO_4$ compared to control

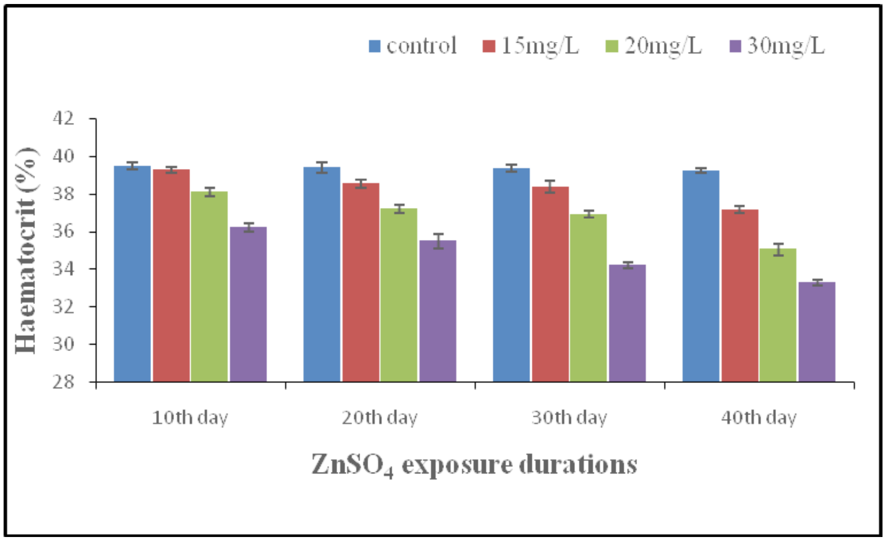


Table-5: Changes in Mean Cell Volume (MCV) of *Channa striatus* exposed to sublethal concentrations of Zinc Sulphate compared to control. (Mean ± SE)

Concentration of $ZnSO_4$		Experimental Days			
		10 days	20 days	30 days	40 days
Control	Mean SE	118.800 ± 0.182	118.580 ± 0.200	118.910 ± 0.199	119.650 ± 0.209
15mg/L	Mean SE %V	120.150* ± 0.592 1.14	120.540** ± 0.478 1.65	120.680** ± 0.288 1.49	123.220*** ± 0.376 2.98
20mg/L	Mean SE %V	120.360** ± 0.343 2.15	122.310*** ± 0.300 3.34	122.910*** ± 0.367 3.36	124.690*** ± 0.349 4.21
30mg/l	Mean SE %V	122.140*** ± 0.260 2.81	124.290*** ± 0.370 4.82	124.720*** ± 0.234 4.89	125.650*** ± 0.262 5.01

Each value is the Mean ± SE of six individual observations.
The MCV values are expressed as (μm^3) in fish blood.
SE- Standard Error; %V-Percent variation; NS: Not Significant; * P<0.05; ** P<0.01; *** P<0.001

Figure-5: Mean Cell Volume in *Channa striatus* exposed to sublethal concentrations of $ZnSO_4$ compared to control

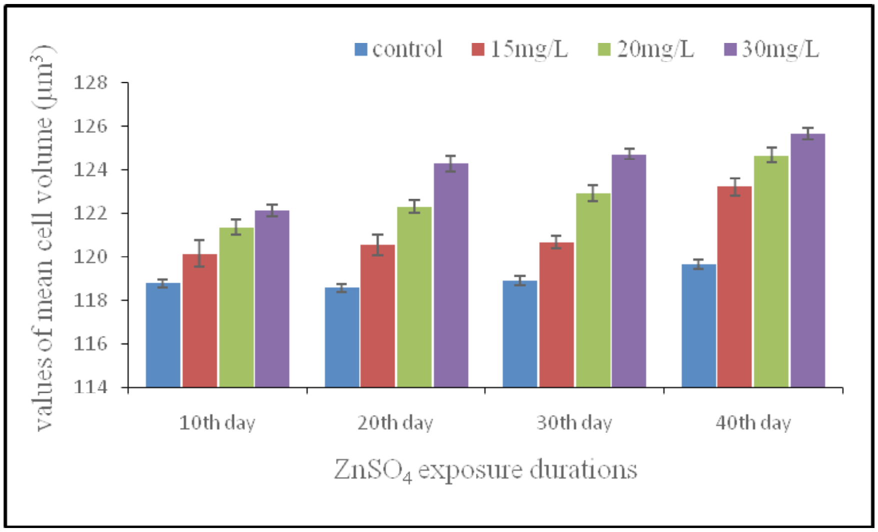


Table-6: Changes in Mean Cell Haemoglobin (MCH) of *Channa striatus* exposed to sublethal concentrations of Zinc Sulphate compared to control. (Mean ± SE)

Concentration of $ZnSO_4$		Experimental Days			
		10 days	20 days	30 days	40 days
Control	Mean SE	39.550 ± 0.216	39.430 ± 0.203	39.560 ± 0.387	39.450 ± 0.398
15mg/L	Mean SE %V	39.150 NS ± 0.245 -1.01	38.880 NS ± 0.347 -1.39	38.640 NS ± 0.296 -2.33	37.800* ± 0.433 -4.18
20mg/L	Mean SE %V	37.660** ± 0.371 -4.78	37.350** ± 0.311 -5.28	36.910** ± 0.374 -6.69	35.910*** ± 0.367 -8.97
30mg/l	Mean SE %V	35.560*** ± 0.483 -10.09	35.240*** ± 0.357 -10.63	35.170*** ± 0.256 -11.11	34.900*** ± 0.342 -11.53

Each value is the Mean ± SE of six individual observations.
The MCH values are expressed as picogram (pg) in fish blood.
SE- Standard Error; %V-Percent variation;
NS: Not Significant;* P<0.05; ** P<0.01; ***P<0.001

Figure-6: Mean Cell Haemoglobin in *Channa striatus* exposed to sublethal concentrations of $ZnSO_4$ compared to control

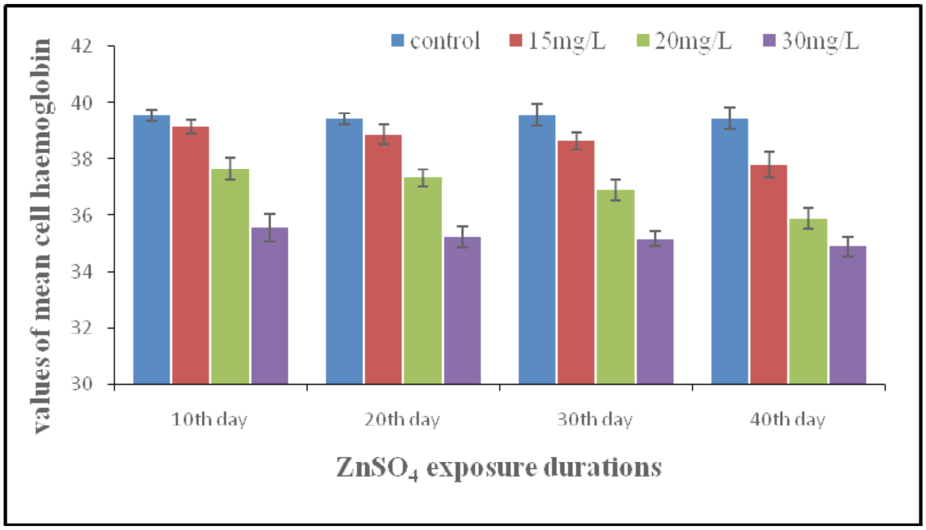
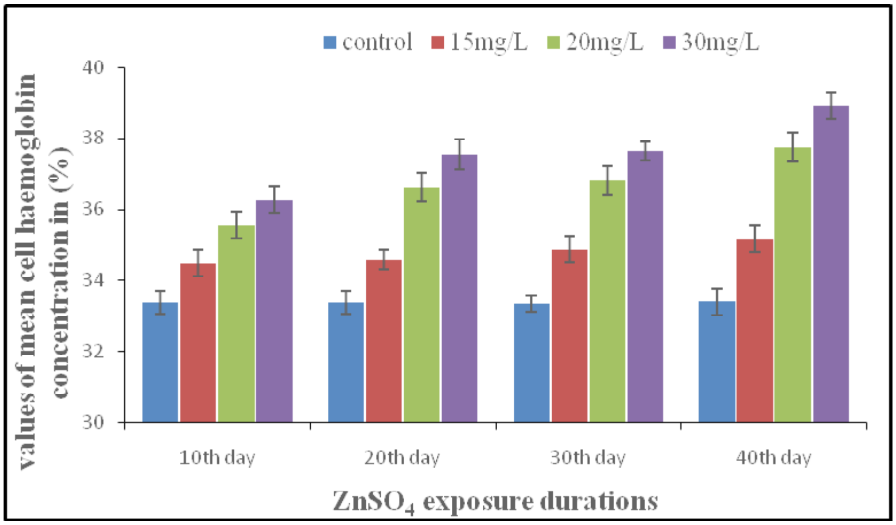


Table-7: Changes in Mean Cell Haemoglobin Concentration (MCHC) of *Channa striatus* exposed to sublethal concentrations of Zinc Sulphate compared to control. (Mean ± SE)

Concentration of $ZnSO_4$		Experimental Days			
		10 days	20 days	30 days	40 days
Control	Mean SE	33.370 ± 0.32	33.360 ± 0.32	33.340 ± 0.22	33.390 ± 0.37
15mg/L	Mean SE %V	34.480* ± 0.37 3.33	34.580** ± 0.28 3.66	34.870** ± 0.36 4.59	35.160** ± 0.37 5.30
20mg/L	Mean SE %V	35.550** ± 0.37 6.53	36.620** ± 0.40 9.77	36.810*** ± 0.41 10.41	37.750*** ± 0.41 13.06
30mg/l	Mean SE %V	36.260*** ± 0.37 8.66	37.550*** ± 0.42 12.56	37.640*** ± 0.25 12.91	38.920*** ± 0.36 16.56

Each value is the Mean ± SE of six individual observations.
The MCHC values are expressed as percentage (%) in fish blood.
SE- Standard Error; %V-Percent variation;
NS: Not Significant;* $P<0.05$; ** $P<0.01$; *** $P<0.001$

Figure-7: Mean Cell Haemoglobin Concentration in *Channa striatus* exposed to sublethal concentrations of $ZnSO_4$ compared to control



The contamination of aquatic environment by heavy metals are severely hazardous of their toxicity. Whether as a consequence of acute and chronic events constitutes additional source of stress for aquatic organisms.[19] The alterations in haematological parameters of fish, Channa striatus exposed to different concentrations of Zinc Sulphate upto 40 days. [23] Reported that toxicants and pollutants have significant effects, which can result in several physiological dysfunctions, in fish. Dysfunction in the fish induces changes in blood parameters possible as a result of blood water content. In the present investigation, similar alterations were observed in blood, Haemoglobin, RBC, Haematocrit and MCH levels of *Channa striatus*. The decrease was more at higher concentrations (30mg/L) and higher duration (40days). The significant decrease in haemoglobin of Clarias gariepinus to sublethal concentration of Zinc has been reported by [2]. The reduction in red blood cell counts and haemoglobin (%) was found to cause macrocytic anaemia as noticed in fishes by [30].

The fall in red blood cell counts seemed to be the major factor for the reduction of haematocrit and mean cell haemoglobin[4]. The total erythrocytes count, haemoglobin and packed cell volume exhibited a steady and significant decrease with an increase in the duration of exposure to 10ppm nickel[11,5]. Chaturvedi and Agrawal (1993) reported that in fish's reduction of haematocrit (PCV) was due to lower red blood cell counts and this resulted in significantly higher ESR value. Haematological indices such as haemoglobin, haematocrit and red blood cell count have been reported to indicate secondary responses of an organism to irritants[24].

The reduction in the haemoglobin and haematocrit values in the fish could also be attributed to the lysing of erythrocytes[26]. Thus significant reduction in these parameters is an indication of severe anemia. [20] Have reported that the decrease in total RBC, Hb% and PCV in the, *Heteropneustes fossilis* after nickel sulphate treatment for 15days. The reduction in some haematological values indicated anaemia and it may be due to erythropoisis, haemosynthesis and osmaregulatary dysfunction or due to an increase in the rate of erythrocyte destruction in the haemopoitic organs[15,27]. The significant reduction in the mean cell haemoglobin (MCH) may be due to the reduction in cellular blood iron, resulting in reduced oxygen carrying capacity of blood and eventually stimulating erythropoiesis[12].

White Blood Cells (WBC), Mean cell Volume (MCV) and Mean Cell Haemoglobin Concentration (MCHC) were increased throughout the experimental durations. The increase was more at higher concentration (30mg/L) and higher duration (40days). The increase in number of WBC may play an important role in immunological defence systems during exposure to toxicants like heavy metals[16]. The White Blood Cells (WBC) showed a gradual increase on exposure to chronic, sublethal dose of nickel for 60 days was reported by [11]. The increase in WBC on exposure to various heavy metals caused by liver dysfunction and depression.[9] The values of mean cell volume (MCV) and mean cell haemoglobin concentrations (MCHC) were found to increase during macrocytic anaemia. [26] Observed increased Mean cell volume (MCV) levels up to 30days exposure to different concentrations of Copper in *Oreochromis mossambicus* and suggested that macrocytic anemia due to reserve of erythropoiesis and in the rate of erythrocyte destruction in the haemopoietic organs. The values of MCHC and MCV content were higher in zinc exposed fish, *Claris batrachus*, when compared with the control[21]. The values of MCV and MCHC were found to increase during macrocytic anemia[4].

CONCLUSION

The present study revealed that the alterations in haematological parameters of *Channa striatus* due to intoxication of Zinc Sulphate. Since majority of heavy metals are released cumulatively and regularly, through the industrial and human activities their residues are known to bio accumulate in the tissues of fish and other animals and transfer via food chain to the human bodies, they cause risk to the health of the people who consume these fish seems to be considerable. It is concluded that the utilization of Zinc Sulphate should be minimize and should create awareness among the people about the toxicity of Zinc Sulphate.

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