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# Haematological Changes in Freshwater Catfish (*Clarias Batrachus*) Exposed to Zinc Sulphate (Linn 1758)

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ABSTRACT

Heavy metals are one of the contaminants of fresh water which harbour several aquatic species. In the present study efforts have been made to understand the sub-lethal toxic effects of zinc sulphate on Haematological changes. Hematological indices, such as red blood cell number, hemoglobin (Hb), platelets, mean corpuscular hemoglobin concentration were significantly declined following the exposure to zinc sulphate. hematocrit (Ht), mean corpuscular volume. and mean corpuscular hemoglobin increased significantly depending on the dose–concentration. Efforts have been made to investigate the effect of zinc sulphate on the haematological changes of sublethal concentrations of zinc sulphate (2.9 ppm) on blood of the freshwater catfish *Clarias batrchus* 28days of exposure periods.

**Keywords:** Zinc sulphate, freshwater fish, Haematology, *Clarias batrachus* .

## *INTRODUCTION*

Zinc has been chosen for the present study because it is a widespread trace metal pollutant of high toxicity not only to warm-blooded vertebrates but also to aquatic animals including fishes (OSPAR, 2002). Zinc, in its essential form, occurs in nature in the earth's crust. ZnSo4 is a trace metal, nutritionally essential and helps for the normal functioning of several enzymes. It is second-hand in batteries, construction materials, pigments, and printing processes. It is also used as a protecting coating over iron, steel, brass, etc. The major sources of environmental Zinc include the smelting of ores, municipal refuses, auto mobile exhausts, etc. In fishes, high levels of Zn decrease oxygen consumption, damage gills, retard growth and reduce reproductive potential. Contamination of water bodies by the heavy metals is a serious problem recognized in the world. Heavy metal toxic waste of the sea flora and fauna have slow been recognized as a grave problem. Heavy metal infectivity can have a devastating impact on the recipient environment's ecological equilibrium and aquatic species diversity (Charjan, 1997; Farombi et. al., 2007). Such emissions from heavy metals pose a most important hazard to fish. If fish are exposed to high metal concentrations in polluted aquatic ecosystems, they tend to remove these metals from their natural environment (Hoo et. al., 2004). From the standpoint of human consumption, fish is a valuable commodity. Thus, from the point of view of human health, metal pollution of freshwater sources and aquatic biota becomes a serious concern. While considerable attention has been given to the studies of Zinc Sulphate intoxication on the different species and information on the sub-lethal effects of Zinc, especially with respect to the fish *Clarias batrachus*, as an environmental pollution monitor is scanty. The present study is, therefore, an effort to determine the toxic effect of zinc sulfate on the *C. batrachus* of fish. Zinc has been chosen for the present study because it is a widespread trace metal pollutant of high toxicity not only to warm-blooded vertebrates but also to aquatic animals including fishes (OSPAR, 2002). Zinc, in its essential form, occurs in nature in the earth's crust. ZnSo4 is a trace metal, nutritionally essential and helps for the normal functioning of several enzymes. It is second-hand in batteries, construction materials, pigments, and printing processes. It is also used as a protecting coating over iron, steel, brass, etc. The major sources of environmental Zinc include the smelting of ores, municipal refuses, automobile exhausts, etc. In fishes, high levels of Zn decrease oxygen consumption, damage gills, retard growth and reduce reproductive potential. Contamination of water bodies by the heavy metals is a serious problem recognized in the world.

## MATERIALS AND METHODS

The fresh water fish *Clarias batrachus* (17 ± 2 cm length and 38 ± 2 g weight) were collected locally from Villupuram district, Tamilnadu, India, were brought to the laboratory and kept in a tank size of 60 x 30 x 30 (l x b x h) cm, filled with tap water for acclimatization for about two weeks. During the acclimatization the fish were fed with minced goat liver on every alternate days. Water in the tank was renewed, three or four times in a daily and aerated to ensure sufficient oxygen supply. For the fish used in experiments, feeding was stopped two days before the start of the experiments to reduce the quantum of excretory products in the tank.

***Total RBC Count:***

The RBC counts were made by Neubauer Haemocytometer (Shah and Altindag 2004a). Blood was diluted 1:20 with Hayem's solution (Mishra et al., 1977). Erythrocytes were counted in the loaded haemocytometer chamber and total numbers were reported as 106 mm-3 (Wintrobe, 1967). Counting was done in the five smaller squares i.e. in the 1st, 5th, 13th, 21st and 25th. The RBC's on the lower and right sides of a square were added in the total, while those on the upper and left sides were rejected.

***Total WBC Count:***

WBC counts were made by Neubauer Haemocytometer (Shah and Altindag 2005). Blood was diluted 1:20 with Turk's diluting fluid and placed in haemocytometer. Four large (1 sq mm) corner squares of the haemocytometer were counted under the microscope (Nikon microscope 80i) at 100X. The cells touching the boundary lines were not counted. The total number of WBC was calculated in mm3 x 103 (Wintrobe, 1967).

***Estimation of haemoglobin:***

The principle of Sahli's method is simple: "the haemoglobin contained in a known quantity of blood is converted into acid haematin by means of hydrochloric acid. The colour is then compared with a standard tube containing acid haematin of known strength" (Whitby and Britton, I935). N/10 HCl solution was filled in the graduating tube up to 2 gms mark. The micropipette was filled up by sucking fresh blood upto 20 cmm marks. The blood of micropipette was then added to the N/10 solution in the graduated tube. The acid haematin solution was thoroughly stirred with the help of a glass rod and then allowed to stand for 10 minutes. Afterwards the acid haematin solution was gradually diluted by adding distilled water in a drop wise manner. This was continued till the colour of the acid haematin solution matched with that of the standard sealed tubes. The reading before the colour just fades was taken as the correct and final reading.

***Determination of haematocrit value (Ht or Packed Cell Volume):***

Haematocrit value of blood was estimated by centrifuging blood in heparinised haematocrit tubes (Germany) at 7000 rpm/min for 30 minutes. From the volume of blood taken packed cell volume after centrifugation haematocrit percent was calculated.

***Mean Corpuscular Haemoglobin (MCH):***

The mean corpuscular haemoglobin (MCH) content was calculated from the values of haemogloin content and erythrocyte count by using the formula and was expressed in picograms.

$$\text{Mean corpuscular haemoglobin} = \frac{\text{Haemoglobin (g/100 mL)}}{\text{Red blood count (million/mm}^3\text{)}} \times 10$$

***Estimation of Mean corpuscular Haemoglobin concentration (MCHC):***

Estimation of mean cell haemoglobin concentration was calculated from the values of haemoglobin and the haematocrit percentage by using the formula and was expressed in percentage.

$$\text{Mean cell haemoglobin concentration} = \frac{\text{Haemoglobin (g/100 mL)}}{\text{Haematocrit percentage}} \times 100$$

## STATISTICAL ANALYSIS

The data obtained in biochemical parameters, haematology and genotoxicity were subjected to standard statistical analysis each sampling time and their respective control groups in different groups. Duncan's multiple range test (Bruning and Kintz, 1968) was performed to determine whether the parameters altered significantly by exposure periods.

## RESULTS

***Total count of red blood corpuscles:***

The mean total number of RBC in control fish was 5.15 million/mm$^3$. A non-significant increase was noted after 7 d (4.05 million/mm$^3$) of exposure and 14 d of exposure is noted by 3.95 million/mm$^3$. After 21d of exposure, a decrease of 3.82 million/mm$^3$ in the RBC count was noted from the respective control group. The value again decreased non significantly as 3.75 after 28 d respectively (Table 1 and Fig. 1).

***Total count of white blood corpuscles (WBC):***

The total count of white blood corpuscles showed a non-significant increase up to 28 d of exposure from control group (6.40 million/mm$^3$). The maximum increase is noted after 28 d of exposure (8.92 million/mm$^3$).

***Haemoglobin:***

Haemoglobin content in control fish were measured as 8.72 □ 1.06 g/dL. After 7d of exposure the value decreased as 8.02 g/dL. After 14d of exposure 7.15 g/dL was noted. Non-significant reduction in the haemoglobin content was recorded after 21 d of exposure (6.28g/dL). Continuous depletion of haemoglobin content was noted upto28 d (5.67 g/dL) of exposure (Table 1 and Fig. 1).

***Packed Cell Volume (Haematocrit):***

Haematocrit value of control fish was recorded as 42.68%. The values decreased non-significantly as 38.12%, 36.18% and 34.16% after 7, 14 and 21d of exposure respectively. But after 28d of exposure a significant decrease in haematocrit value of 32.00% ($p < 0.05$) was noted (Table 1 and Fig. 1).

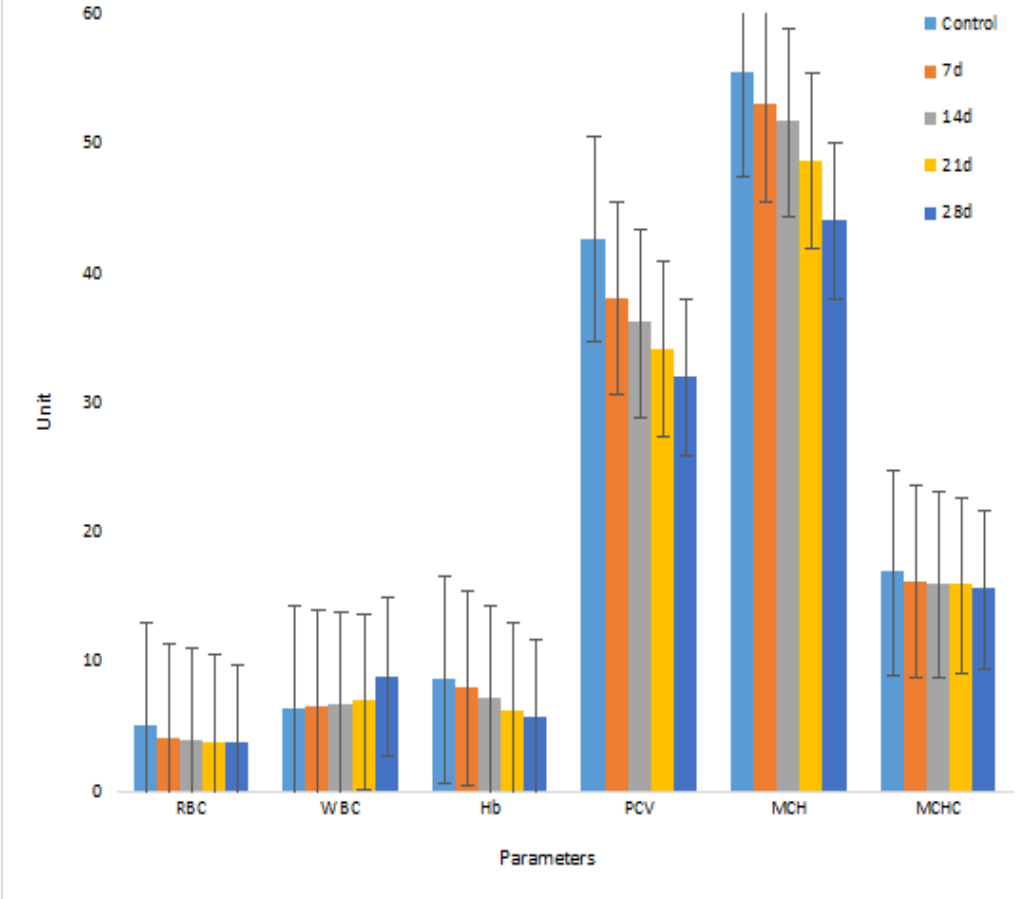
***Mean corpuscle haemoglobin (MCH):***

In control fish the mean corpuscular haemoglobin was recorded as 55.54 picograms cell$^{-1}$. In general a significant variation was noted after 7 d of exposure periods. Maximum significant reduction in mean corpuscular haemoglobin was recorded after 28 d of exposure period, (44.12 picograms cell$^{-1}$) was noted (Table 1 and Fig. 1).

***Mean corpuscles haemoglobin concentration (MCHC):***

Mean corpuscles haemoglobin concentration of control fish is 16.93%. A non -significant decrease was noted at all stages of exposure as 16.25, 16.01, 15.97 and 15.63 respectively after 7, 14, 21 and 28 d of exposure (Table 1 and Fig. 1).

**Fig 1. Showing the haematological parameters of C. batrachus in different experimental groups for 7, 14, 21 and 28 days**



| Parameters | Control | Duration (in days) | | | |
|---|---|---|---|---|---|
| | | 7d | 14d | 21d | 28d |
| RBC ($10^6$/mm$^3$) | 5.15 ± 0.05 | 4.05 ± 0.02 NS | 3.95 ± 0.00 NS | 3.82 ± 0.25 NS | 3.72±0.15 NS |
| WBC ($10^3$/mm$^3$) | 6.40 ±1.15 | 6.53 ± 1.08 NS | 6.74 ± 1.15 NS | 6.98 ± 1.00 NS | 8.92 ±1.05 NS |
| Haemoglobin (g/dL) | 8.72 ± 1.06 | 8.02 ± 0.75 NS | 7.15 ± 1.00 NS | 6.28 ±1.12 NS | 5.67 ±0.06 NS |
| PCV (%) | 42.68 ± 1.25 | 38.12± 0.79 NS | 36.18±1.28 NS | 34.16±1.65 NS | 32.00 ±0.99* |
| MCH (picograms cell$^{-1}$) | 55.54 ± 1.25 | 53.00± 1.00 NS | 51.68 ±1.25 NS | 48.72 ± 0.98* | 44.12±1.15* |
| MCHC | 16.93 ± 0.78 | 16.25± 1.00 NS | 16.01± 0.98 NS | 15.97± 0.75 NS | 15.6 ± 1.00 NS |

**Table 1. Showing the haematological parameters of *C. batrachus* in different experimental groups for 7, 14, 21 and 28 days**

Average Values ± SE; n=3; *p > 0.05; NS = Non significant.

## DISCUSSION

The importance of heamatology in the diagnosis of fish diseases and assessment of the effect of pesticides has been widely accepted. Hematology has been generally recognized to be basic when diagnosing fish infections and evaluating the effect of pesticides. In light of the sub-deadly focus from the zinc sulfate arrangement, an abatement of erythrocyte (RBCs), hemoglobin tally (Hb) and hematocrit (HCT) can be ascribed to the corruption of develop RBCs and a hindrance of erythrocyte generation, because of a lessening in metal toxicosis of haem union. What's more, a drop in RBCs, Hb and HCT could be identified with the disposal from course of RBCs. Such results are reliable with Ramadevi and so forth (1998), who saw that the blood of grill chicks has diminished from the danger of pesticides in RBCs, Hb and HCT. In Nil tilapia harmed with mercury, Shalaby (2001) found that the measure of RBCs, Hb and pressed cell (PCV). In the African catfish blood (Clarias gariepinus) after ochratoxin harming Mousa and Khattab (2003) have found declining RBCs, Hb and Hct. In the conclusion of pallor in most by far of creatures, the blood files PCV, MCH and MCHC are particularly significant (Coles, 1986). The variations from the norm in these blood records can be because of a zinc-dangerous defensive reaction by incited erythropoiesis and can likewise be connected to a diminishing in RBC, Hb and Hct in light of overpowered interruptions in the hemopoietic capacity of fish presented to sub-deadly contamination focuses (Mousa, 1999). (Mousa, 1999) Some specialists have additionally affirmed that numerous toxicants adjust circulatory strain (Saeedi et al., 2012; Rauf Abdul and Naeemuddin Arain, 2013; Lakshmanan et al.,2013; Venkataraman and Sandhya Rani, 2013) while, concentrating the harmfulness of various pesticides on angles Change erythrocytes profile incited by intense impacts of dichlorvos, malathion and organophates in numerous fishes (Benarji and Rajendranth, 1990; Khattak and Hafeez, 1996; Sibel et al., 2006). Svoboda et al.,(2001) exhibited that red platelets include diminished impressive in like manner carp presented to pesticide. In the present examination, zinc brought down the Hb rate and RBC include in *Clarias batrachus* after various introduction periods.

The white platelets in fish react to different stressors including dangerous substantial metals, diseases and concoction aggravations. Expanding number of white platelets is ordinary response to the synthetic substances, for example, zinc as in the present examination. It is shown as the response of the insusceptible framework under harmful conditions, subsequently the expanded number of white platelets (leukocytosis) might be the consequence of worry of the test substance. Khoshbavar-Rostami et al.,(2005) detailed a lessening in RBC, Hb, and WBC esteems just as an expansion in MCV and MCH in Acipenser stellatus presented to various centralizations of diazinon. Diminished number of white platelets may likewise be identified with the expanded degree of corticosteroid hormones, whose entertainment has a vague reaction to any natural stressor. The expansion in the quantity of white platelets may likewise be ascribed to incitement of the invulnerable system of the fish to dispense with the impacts of the contaminations. Magar and Dube (2012). additionally found that the expanded WBC against malathion poisonous quality in crisp water fish Channa punctatus.

## SUMMARY

Efforts have been made to investigate the effect of zinc sulphate on the haematological changes of sublethal concentrations of zinc sulphate (2.9 ppm) on blood of the freshwater catfish *Clarias batrchus* 28days of exposure periods. Haematological alterations include reduction in the RBC count, haemoglobin content, PCV, (Packed Cell Volume) MCH (Mean Corpuscle Haemoglobin) and MCHC ( Mean Corpuscle Haemoglobin Concentration) were noted. In general the total white blood corpuscle count show variation in all the exposure periods. All these alterations in the haematological parameters in the fish *Clarias batrchus* exposed to sublethal zinc solution, may be due to physiological stress caused by the irritant present in the aquatic medium.

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