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Impact of Heavy Metal lead acetate on Hematology of Fresh Water Fish *Oreochromis niloticus*

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ABSTRACT

The current study examines the impact of lead acetate, a heavy metal pollutant, on the hematological changes in freshwater fish, *Oreochromis niloticus*, following prolonged exposure to significant changes at sub-lethal concentrations. The findings indicated that whereas WBC rose after a prolonged period of exposure, measures like RBC and Hb decreased.

Key words: *Heavy metal, lead acetate, freshwater fish,* Oreochromis niloticus*, hematological parameters,*

Introduction

One of the major countries that produce textiles and clothes is India. The threats posed by the material industry and ecological contamination are becoming major issues. In addition to a variety of artificial colors, material effluents also contain harmful synthetic compounds such as acids, sulfur, salts, naphthol, nitrates, hydrogen peroxide, surfactant-scattering agents, and toxic heavy metals (THM) like Cu, Cr, Cd, Zn, Ni, As, and Pb that are directly released into water streams and pose a threat to marine life (Akhter *et al.,* 2023). Over the past few decades, there has been growing worry about the wide range of pollutants that are contaminating freshwater. Pollutant levels, including those of heavy metals, have significantly increased as a result of increased human activity, particularly with the fast development of industry and agriculture (Al-Rudainy, 2015).

Furthermore, fish are frequently employed in bio monitoring and can enable the assessment of compounds that may be hazardous to people because they react to toxic agents similarly to higher vertebrates (Bolognesi and Hayashi, 2011). For many fish species, the main route of absorption begins in the water and continues through oral intake, the stomach, and dermal absorption, which is more effective (ILO, 1983; Sax and Lewis, 1989).

Hematological analysis is one of the most important methods for determining how organic and inorganic materials affect fish. It is therefore frequently employed in pharmacological and ecotoxicological research. Fish physiological status is frequently evaluated using hematological analysis. Blood indices are quick to react and sensitive biomarkers of a variety of environmental effects, such as toxic agent-contaminated water (Witeska *et al.*, 2023).

One of the most significant environmental issues of our day is heavy metal pollution. Fish are exposed to excessively high concentrations of lead among other elements. Lead (Pb) Mining and manufacturing processes produce lead and its by products, which are dangerous environmental contaminants (Moulis, 2010). Additionally, a number of studies have documented the accumulation of hazardous and non-biodegradable heavy metals, in-

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cluding lead, in several fish species, leading to toxicological consequences (Khoshnood *et al.,* 2011).

Lead is a heavy metal that causes pollution and disease in both the workplace and the environment. Ashour *et al*. (2007) have identified several target organs that are susceptible to adverse consequences from lead exposure, including the neurological, hematological, and renal systems. Lead directly affects the hematological system by shortening the life of red blood cells in circulation by causing their membranes to become more brittle (Flora *et al.,* 2012). Lead poisoning has been linked to neurological, gastrointestinal, reproductive, circulatory, immunological, histological, and histochemical alterations in animals, according to recent and noteworthy research (Park *et al.,* 2006; Berrahal *et al.,* 2007; Reglero *et al.,* 2009; Abdallah *et al.,* 2010; Mobarak and Sharaf, 2011).

On the other hand, nothing is known about the blood's reaction to stress in freshwater fish species found in India, like *Oreochromis niloticus.* Therefore, the current investigation sought to determine how lead acetate affected haematological measures such as hemoglobin (Hb), packed cell volume (PCV%), white blood cell (WBC), and red blood cell (RBC), as well as how exposure affected clinical symptoms (fish behavior).

Materials and Methods

Procurement and Rearing of Experimental Animal

Healthy *Oreochromis niloticus* having mean weight 20-25 gm and length 15 – 20 cm were collected from PSP fish farm, at Puthur and acclimatized to laboratory conditions (29 ± 1°C). The fish were fed daily on oil-less groundnut cake. The unused food was renewed after 2 hours and water was changed daily. Prior to experimentation the fish were acclimatized to experimental tanks for at least one week.

Heavy metal Lead acetate

Heavy metal lead acetate purchased from local chemist shop was used for the present study.

Definite Test

The LC_{50} values were determined by following the method of Litchfield and Wilcoxon (1949). Sublethal studies are helpful to assess the response of the test organisms under augmented stress caused by metals. 96 hr LC_{50} value obtained 30 ppm of lead acetate.

Experimental Design

A total of 40 fishes (10 fishes per aquarium) were separated as four groups. The following experimental groups were conducted in the freshwater fish *Oreochromis niloticus* for the period of 30 days. Sub lethal doses preferred based on IC_{50} values.

Group 1: *Oreochromis niloticus*, without any lead acetate exposure (control)

Group 2: *Oreochromis niloticus*, on exposure to **1.30** ppm lead acetate for a period of 30 days

Group 3: *Oreochromis niloticus*, on exposure to **1.60** ppm lead acetate for a period of 30 days

Group 4: *Oreochromis niloticus*, on exposure to **3** ppm lead acetate for a period of 30 days

Collection of blood

Blood samples were collected from the control and experimental fishes with the help of 24 gauge needle and stored in heparinized glass tube. The hematological parameters viz., total Red Blood Cells (RBC), White Blood Cells (WBC), Haemoglobin (Hb), Hematocrit (Ht) or PCV, Mean Cell Haemoglobin (MCH) and Mean Cell Haemoglobin Concentration (MCHC) were determined by adopting the method of Dacie and Lewis (1984).

Statistical Analysis

The data obtained were analyzed by applying analysis of variance DMRT one way ANOVA to test the level of significance (Duncan, 1957).

Results

Behavioural Responses

Oreochromis niloticus fish kept in normal freshwater conditions exhibit normal behavior, but fish exposed to lead acetate exhibit irregular swimming, aberrant posture, sluggishness, imbalance in posture, increased surface activity, opercular movement, gradual loss of equilibrium, and excessive mucus spreading over their body. These findings demonstrated lead acetate's high toxicity.

Hematological changes

The changes in hematological parameters of the fish *Oreochromis niloticus* exposed to acute toxicity of lead acetate 30 days exposure. In the present investigation showed on red blood cells (RBC), white blood cells (WBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) in the blood of *Oreochromis niloticus* appear to the affected by low, medium and high sublethal concentrations of lead acetate for a period of 30 days exposure. RBC, Hb, PCV and MCHC values were significantly decreased whereas WBC, MCV and MCH counts were significantly increased in sublethal concentrations of lead acetate exposed for a period of 30 days when compared to the control fish blood. However maximum changes were recorded in 30 days of high sublethal concentration of lead acetate exposure (Table 1 and Fig. 1 & 2).

Discussion

According to Al-Rudainy *et al*. (2015), hematological characteristics may serve as biomarkers of toxicity in fish studies. Research on fish blood reactions to long-term or short-term exposure to heavy metals is scarce. According to Salman (2014), hematological indices enable the quick identification of alterations in fish. In the current investigation, following a 30-day exposure to varying concentrations of lead acetate, hematological indicators showed substantial variation. Animals' blood cell reactions are crucial markers of alterations in their internal and/or external environments. In fish, exposure to chemical pollutants can induce either increases or decreases in hematological levels. Their changes depend on fish

Treatments	10 days	20 days	30 days
RBC			
Control	6.78 ± 0.03[a]	6.18± 0.90a	5.07± 0.91a
Low concentration	6.72 ± 0.06b	5.25 ± 0.26b	4.76 ± 0.06b
Medium concentration	5.82 ± 0.03c	4.91 ± 0.79c	3.76 ± 0.38c
High concentration	3.88 ± 0.05d	3.65 ± 0.54d	2.08 ± 0.43d
WBC			
Control	8.12 ± 0.03a	10.14 ± 0.53a	12.53 ± 0.32a
Low concentration	9.64 ± 0.34b	12.45 ± 0.04b	14.26 ± 0.29b
Medium concentration	10.46 ± 0.49c	13.40 ± 0.90c	15.36 ± 0.19c
High concentration	12.86 ± 0.59d	15.20 ± 0.44d	18.32 ± 0.39d
Hb			
Control	10.01 ±0.42a	8.88 ± 0.59a	7.52 ± 0.43a
Low concentration	9.22 ± 0.42b	7.10 ± 0.15b	6.35 ± 0.57b
Medium concentration	7.18 ± 0.77c	6.22 ± 0.53c	3.25± 0.98c
High concentration	6.13 ± 0.40d	5.26 ± 0.40d	2.88 ± 0.52d
PCV			
Control	30.63 ± 2.35a	30.52 ± 2.45a	30.33 ± 2.15a
Low concentration	29.38 ± 2.56b	28.44 ± 2.43b	26.35 ± 2.36
Medium concentration	28.892 ± 2.42c	28.40 ± 2.38 c	27.38 ± 2.41c
High concentration	27.71 ± 2.15d	26.42 ± 2.12	23.22 ± 2.05d
MCV			
Control	90.41 ± 7.43a	89.49 ± 7.25a	88.83 ± 7.18a
Low concentration	92.18 ± 7.79a	92.64 ± 7.90ab	93.52 ± 8.02b
Medium concentration	96.32 ± 8.19ab	93.32 ± 7.82ab	86.72 ± 8.82c
High concentration	103.62 ± 8.85ab	101.25 ± 8.62b	106.68 ± 8.85d
MCH			
Control	34.42 ± 2.52a	32.93 ± 2.28a	31.85 ± 2.32a
Low concentration	34.22 ± 2.61a	33.58 ± 2.60ab	31.30 ± 2.69b
Medium concentration	35.68 ± 2.62a	35.40 ± 2.71b	34.42 ± 2.91c
High concentration	37.13 ± 2.78a	36.38 ± 2.40b	37.71 ± 2.81d
MCHC			
Control	36.72 ± 2.38a	36.65 ± 2.41a	35.21 ± 2.36a
Low concentration	35.32 ± 2.32a	34.88 ± 2.28a	33.72 ± 2.31a
Medium concentration	33.61 ± 2.52a	32.51 ± 2.65a	31.10 ± 2.51b
High concentration	30.53 ± 2.40a	30.31 ± 2.52b	29.14 ± 2.48c

species, age, the cycle of the sexual maturity of spawners and diseases (Adeyemo, 2007).

In the present research exhibit RBC, Hb, PCV and MCHC values were significantly reduced whereas WBC, MCV and MCH counts were significantly enlarged in sublethal concentrations of lead acetate exposed for a period of 30 days when compared to the control fish blood. *O. mykiss* treated to Pb and Cu similarly showed a similar outcome (Ates *et al*., 2008). According to the authors, a drop in these values shows that the fish are anemic, which prevents blood from developing in blood-producing organs. Another possible explanation for the observed decrease in Hb content could be the production of reactive oxygen species brought on by metals (Larsson *et al.,* 1985). Reduced generation of red blood cells, poor Osmoregulation, or internal bleeding during stressful situations can all cause an RBC reduction. (Kavitha *et al.,* 2010). Previous studies also reported a decline in RBCs, Hb and Hct content in freshwater fishes exposed to Cd and Ni showing anemia, leucopoiesis and erythropenia (Vincent *et al.,* 1996; Nanda and Behera, 1996).

Researchers who subjected *Oreochromis niloticus* to Cd and discovered a substantial decrease in RBCs, Hb, and Hct compared to the control (Al-Asgah *et al*., 2015). Cadmium exposure in *Oreochromis niloticus* resulted in a considerable decrease in RBC, Hb, and HCT (Kaoud *et al.,* 2011). When fish *Cyprinus carpio* were exposed to Cr(VI), Shaheen and Akhtar (2012) likewise observed a significant drop in Hb content and TEC levels. A lower rate of erythropoiesis or an accelerated rate of red blood cell breakdown could be the cause of a reduction in red blood cell count (Mc Leay, 1973).

The corpuscular indices MCV, MCH, and MCHC are particularly significant in characterizing anemia in the majority of animals and are useful for both diagnosis and treatment (Coles, 1986). Red blood cell swelling can be accurately predicted by the MCHC; anemia or oligohaemia would be indicated by a low PCV (Wepener *et al.,* 1992). The PCV levels are helpful in assessing how stresses affect fish health and are also used to calculate blood's ability to carry oxygen (Larsson *et al.,* 1985). The increased values of MCV and MCH may indicate a condition of macrocytic anaemia (Bomford *et al.,* 1975).

Conclusion

Thus, the current study concludes that haematological parameters are critical to Oreochromis niloticus's ability to maintain excellent health. The current study shows that changing some factors puts a significant and edible fish at danger of dying.

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Conflict of Interest: None

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