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ASSESSMENT OF SUBLETHAL COPPER TOXICITY ON BLOOD PARAMETERS IN ROHTEE OGILBII

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ABSTRACT

This research set out to evaluate the haematological profile of the freshwater fish species Rohtee Ogilbii by comparing the levels of hemoglobin, red blood cells (RBCs), and white blood cells (WBCs) in both normal and copper-treated fish. Collecting specimens of Rohtee Ogilbii from Andhra Pradesh's Kolleru Lake and acclimatizing them in a controlled environment for 20 days yielded fish that weighed 5.0-6.0 grams and measured 7-8 cm. Experiments on sublethal toxicity were conducted with exposure sessions spaced 5 days apart, using a concentration of 0.024 ppm, or 1/10th of the 24-hour Lc50 value. Red blood cell (RBC), white blood cell (WBC), and hemoglobin levels were found to be significantly lower in the fish group that was exposed to sublethal quantities of copper as compared to the control group. Copper exposure has negative effects on Rohtee Ogilbii haematological parameters, according to the data. This might have consequences for the health of fish and the stability of aquatic ecosystems.

Keywords: Copper, Red blood cells, White blood cells, Hemoglobin Content, Sublethal

I. INTRODUCTION

Anthropogenic activities including mining, farming, and industrial runoff may significantly contaminate the environment with copper, a trace metal necessary for many biological processes. While trace quantities of copper are required for physiological processes, aquatic life, especially fish, is severely endangered when copper concentrations in the water exceed safe levels. Enzymes that include copper are essential for many biological functions, such as cellular respiration, antioxidant defense, and the formation of connective tissues. For these metabolic processes, copper is essential, and fish are no exception. But copper causes oxidative stress, upsets cellular homeostasis, and harms tissues when concentrations go over the normal limit. Concerns about copper's toxicity in aquatic environments are significant because of how common it is as a contaminant. Changes to the biochemical and physiological processes of aquatic creatures result from interactions between copper ions and a variety of biological components.

The ecologically significant and pollutant-sensitive Rohtee Ogilbii is a great model organism for research on environmental stresses since it is widespread in freshwater habitats like Kolleru Lake in Andhra Pradesh, India. Changes in the physiological characteristics of this fish species—which is vital to the local aquatic ecosystem—can indicate the state of its surroundings. Learn more about the effects of heavy metal pollution on freshwater ecosystems by studying the effects of copper toxicity on Rohtee Ogilbii.

Important indications of fish health are haematological measures, which include hemoglobin content, white blood cell count, and red blood cell count (RBC). When fish are under stress, such when exposed to toxicants, these metrics reveal a lot about their physiological status. Red blood cell (RBC) counts show how well the blood can carry oxygen, white blood cell (WBC) counts show how well the immune system is working, and hemoglobin levels show how well the blood can transport oxygen. The degree to which contaminants like copper alter physiological processes may be better understood by keeping an eye on these metrics.

Impact of Copper on Hematological Parameters

- **RBC Counts**

Red blood cells are essential for fish because they transport oxygen throughout the body. If copper poisoning causes hemolysis (decay) of red blood cells (RBCs), the overall number of RBCs will decrease. As a result, fish experience hypoxia and other stress symptoms as their ability to transfer oxygen is hindered. The red blood cell count drops dramatically when exposed to copper levels below the lethal threshold, according to research. This phenomenon was seen in Rohtee Ogilbii. Further impairment of red blood cell (RBC) function occurs as a result of morphological changes, such as cell membrane damage, altered size and shape, and reduced quantity.

- **WBC Counts**

The immune system relies on white blood cells to fight off illnesses and infections. Fish immunological responses may be dampened by copper poisoning, which lowers white blood cell numbers. Because of this decrease in immune system function, fish are at increased risk of contracting various illnesses and infections, which threatens their very existence. Increasing copper exposure significantly decreased white blood cell counts in Rohtee Ogilbii. Increased illness incidence and death rates in fish populations are among the severe ecological repercussions that might result from a compromised immune system, as shown by this reduction.

- **Hemoglobin Content**

Keeping tissues supplied with oxygen is essential, and hemoglobin plays a vital role in this process. Fish hemoglobin levels drop when copper poisoning impacts hemoglobin production and function. This reduction worsens hypoxia by reducing the fish's capacity to transport oxygen to different tissues. Copper has a negative effect on the respiratory efficiency and general physiological status of Rohtee Ogilbii, as shown by the fact that its exposure drastically lowers hemoglobin content.

II. REVIEW OF LITERATURE

Sasikumar, S. & Leon, J.. (2022) Pollution from heavy metals has been a major issue for the planet's ecosystems in recent times. Anthropogenic (human-caused) activities, industrial processes, volcanic eruptions, and household activities all contribute to higher concentrations of heavy metals in the environment. When employed as an algaecide and fungicide, copper sulfate is released into water bodies by aquaculture and agricultural areas, where it may harm aquatic life, particularly fish. Copper sulfate's impact on many hematological parameters in the experimental fish Oreochromis niloticus is the focus of this investigation. Commercial aquaculture relies heavily on Oreochromis niloticus, a kind of freshwater fish. One of the most important components of human diet is fish. To detect heavy metal pollution, fish are the best bio-indicators to use. As indicators of water pollution, heavy metals cause oxidative stress in fish. The current investigation set out to examine the impact of the heavy metal CuSO4 on the hematological functions of the freshwater fish O.niloticus. Copper sulfate had an LC50 value of 39.2 mg/L. For 30 days, researchers studied the hematological alterations in Oreochromis niloticus fish exposed to sublethal amounts of copper sulfate (1/10, 1/20, and 1/30). As the sub-lethal quantity of heavy metal Copper sulfate rose, the results showed that the Red Blood Cell (RBC) count and hemoglobin percentage (Hb) declined dramatically, while the levels of white blood cells (WBC), mean corpuscular volume (MCV), and mean corpuscular hydrogen (MCH) increased significantly. Investigating the treated fish's gill and liver histologically allowed researchers to detect the effects of copper sulfate. Using a variety of sublethal concentrations administered to freshwater fish O. niloticus over the course of 30 days, this research aims to examine the hematological and histopathological impacts on different tissues.Comprehensive dietary information on tilapia O.niloticus eating was gathered via in-person interviews. The calculation of fish intake (g) was done. The SPSS program for Windows was used to perform the statistical analyses, and all tests were deemed significant at a significance level of $p<0.05$.

Shah, Nazish et al., (2020) Copper, chromium, and lead exposure groups (LC50 and LC85) caused hemodynamic changes in Ctenopharyngodon idella. We compared the reference group with the treatment group after various exposure hours by analyzing the hematological changes in the blood samples that had been exposed to metals after collection. The levels of hemoglobin, red blood cells, hematocrit, total leucocytes, and lymphocytes were found to decrease significantly ($p < 0.05$) as the concentrations of metals increased, while the levels of granulocytes and monocytes showed a significant increase ($p < 0.05$) in relation to the doses of toxic metals given, in comparison to the values from the control group. Hematological indicators for red and white blood cells showed more significant alterations at the greatest dosage of metal groups supplied via LC85. There was no correlation between the exposed duration pattern and the resultant hematological alterations. Comprehensive study of dose-response relationships is the basis of the current work, which has substantially advanced our understanding of metal toxicity.

Padrilah, Siti et al., (2018) All living things need copper, a major trace metal, for proper development and metabolism. A harmful accumulation of continuous metal compounds that may enter water and disrupt biological systems is a possible consequence of using this element beyond its safe limits. Rising levels of pollution in the environment might have an impact on aquaculture as well. When examined at the molecular and structural levels of an organism, copper is shown to have a poisonous impact that may harm fish. This is due to the fact that fish are capable of accumulating heavy metals in their tissues, like other aquatic creatures. Metal concentration, exposure duration, metal absorption mechanisms, environmental conditions (pH, water temperature), and inherent characteristics (fish size, age) are the main determinants of this accumulation. Copper accumulates differently in various fish organs. Because of the histopathological changes seen in fish and the buildup of copper in their organs, this review set out to investigate the negative impacts of copper on fish.

Soydemir Çiftçi, Nuray et al., (2015) When Oreochromis niloticus were exposed to 4 ppm Cu and 0.2 ppm Pb for 7, 15, and 30 days, the effects of sub-lethal doses of copper and lead on hematocrit and mean cell volume (MCV) levels, as well as on erythrocyte counts, erythrocyte size, and erythrocyte nucleus areas, were assessed. For hematocrit levels, micro-hematocrit techniques were utilized, and for MCV, erythrocyte counts, erythrocyte sizes, and erythrocyte nucleus areas, microscopic methods were used. Both metals had a negative impact on erythrocyte counts throughout the measured exposure durations, with the exception of 30 days of copper exposure, whereas hematocrit and MCV levels, as well as erythrocyte and nucleus areas, rose considerably ($P<0.05$). As exposure times increased, lead caused a rise in MCV levels, while both metals increased erythrocyte and nucleus areas. Various additional hematological indicators were shown to decline with increasing exposure durations. Except for the erythrocyte area, all of the hemological parameters measured in the studies showed that lead-exposed fish performed better than copper-exposed fish. Results showed that copper and lead significantly altered O. niloticus hematological parameters, which impacted fish physiological states.

Azarin, Hajar et al., (2012) Many harmful substances end up in aquatic environments because of human interference. Copper sulphate (CuSO4) and other contaminants are present in high concentrations in the water that many fish swim in. Both the water quality and the quantity of CuSO4 determine its harmful effects on fish. So, the purpose of this research was to find out how some hematological parameters of Rutilus frisii kutum fingerlings (mean weight ±SD: o.4±0.1 g and mean length ±SD: 3.9±0.3 cm) were affected by sublethal doses of CuSO contaminants. Over the course of 60 days, Rutilus frisii kutum fingerlings were subjected to three doses of CuSO4 that were below the fatal threshold: 0.4, 0.04, and 0.004 mg L•1. The results showed that the control, 0.4, 0.04, and 0.004 treatments differed significantly in hematocrit, mean corpuscular volume (MCV), and mean corpuscular hemoglobin concentration (MCHC) ($p<0.05$). The control group had higher levels of hematocrit and MCV concentration. In addition Only the control group and the groups treated with 0.04, 0.004 mg L•1 showed a statistically significant change in white blood cell (WBC) count. Negligible differences were found in the concentrations of hemoglobin (Hb), red blood cells (RBCs), and mean corpuscular hemoglobin (MCH) in fish exposed to sublethal concentrations of CuSO ($p<0.05$), while an increase in neutrophil concentration was observed in fish exposed to 0.04 mg L•1 CuSO4 compared to the control group.

III. MATERIAL AND METHODS

The haematological profile of the control and treated fish was evaluated by measuring the hemoglobin, red blood cell, and white blood cell counts in the entire blood of Rohtee Ogilbii. Rohtee ogilbii, a freshwater fish species native to Andhra Pradesh, was caught at Kolleru Lake. Its average weight was 5.0-6.0 gram, and its average length was 7-8 cm. Ensuring their safe arrival to the laboratory, they were stored in enormous cement tanks in airtight polythene bags. Prior to the start of the experiment, the fish were allowed to acclimate for around twenty days. The fish were placed in a clean, laboratory-maintained glass tank after being introduced. Different amounts of copper were introduced to separate circular plastic tubs with a capacity of 50 liters of water. Ten fish in good health were placed in each aquarium. In addition, ten fish were kept in a 50-liter tub that did not contain any toxicant. Every concentration group had three separate experiments. After 24 hours, we recorded the survival rate and death rate of the fish in the control and copper-treated tubs. We used the concentration at which 50% of the fish died as the median lethal concentration (Lc50) for 24 hours, which was 0.24 ppm. In order to find the median fatal concentration of copper to the Rohtee Ogilbii fish, an identical experimental setup was also used for 96 hours. To keep the copper content consistent, the test water was replaced after 24 hours with newly produced solution. Copper sulfate had a median lethal concentration (Lc50) of 0.12 ppm for 96 hours. Using the Probit analysis approach, we were able to determine the median copper fatal concentration. One tenth of the Lc50 value after 24 hours (0.024 ppm) was used to perform the sublethal toxicity test. In this investigation, exposure times ranging from 5 to 25 days were recorded.

IV. RESULTS AND DISCUSSION

Table 1: Red blood cell count changes in Rohtee Ogilbii exposed to sublethal copper for 25 days

S. No.	Exposure Period	Control	Experiment	Change %	Calculated T Value
1	5	0.92 ± 0.69	0.85 ± 0.69	-2.21	-1.5
2	10	0.95 ± 0.91	0.88 ± 0.91	-6.55	0.04
3	15	0.89 ± 1.05	0.81 ± 0.70	-10.67	0.32
4	20	0.98 ± 0.70	0.87 ± 0.70	-14.52	-0.06
5	25	0.92 ± 0.28	0.78 ± 0.70	-18.40	-0.05

The following table shows the effects of sublethal copper concentrations on Rohtee Ogilbii red blood cell (RBC) counts throughout a 25-day period. Compared to the experimental group, which exhibits a steady decrease in RBC counts as exposure duration rises, the control group keeps their RBC numbers relatively unchanged. At first, after 5 days, the experimental group's RBC count is 0.85 ± 0.69, which is 2.21% lower than the control group's count of 0.92 ± 0.69. A computed T value of -1.5 indicates that this difference is statistically significant. On the tenth day, the red blood cell (RBC) count in the experimental group falls even lower to 0.88 ± 0.91, which is 6.55% lower than the control group. A t-value of 0.04 indicates that there was no statistically significant change. After 15 days, the RBC count in the experimental group dropped to 0.81 ± 0.70, indicating a decline of 10.67%. However, the T value was only 0.32, thus the trend was once again not statistically significant. On the twentieth day, the red blood cell count drops even lower to 0.87 ± 0.70, showing a decrease of 14.52% with a t-value of -0.06. The experimental group's RBC count drops to 0.78 ± 0.70 after 25 days, which is an 18.40% drop from the control group and a T value of -0.05. These results show that copper has a harmful effect on Rohtee Ogilbii's haematological health, with a clear downward trend in RBC counts over time; however, not all changes are statistically significant according to the T values given.

Table 2: White blood cell count changes in Rohtee Ogilbii exposed to sublethal copper for 25 days

S. No.	Exposure Period	Control	Experiment	Change %	Calculated T Value
1	5	20.58 ± 0.76	10.59 ± 0.56	-48.49	-0.55
2	10	19.05 ± 0.69	8.94 ± 0.58	-53.11	-0.32

3	15	18.54 ± 0.48	5.99 ± 0.40	-67.65	0.08
4	20	19.02 ± 0.69	5.62 ± 0.49	-70.44	0.21
5	25	18.21 ± 0.60	4.59 ± 0.56	-74.83	0.30

The table shows the changes in white blood cell (WBC) counts of Rohtee Ogilbii during a 25-day period when exposed to sublethal doses of copper. While white blood cell (WBC) counts in the control group remain continuously high, they fall sharply and steadily in the experimental group as exposure time rises. The WBC count in the experimental group decreases significantly by 48.49% after 5 days of exposure, from 20.58 ± 0.76 to 10.59 ± 0.56, compared to the control group, with a computed T value of -0.55. In comparison to the control group's WBC count of 19.05 ± 0.69 on the 10th day, the experimental group's WBC count drops to 8.94 ± 0.58, a 53.11% decline, with a T value of -0.32. By day 15, the trend of declining white blood cell counts has intensified, with the experimental group's count reaching 5.99 ± 0.40, a reduction of 67.65% from the control group's count of 18.54 ± 0.48, and a corresponding T value of 0.08. Twenty days later, the white blood cell count in the group that underwent the experiment drops even lower to 5.62 ± 0.49, indicating a decrease of 70.44% compared to the control group's 19.02 ± 0.69 count, and a corresponding T value of 0.21. A substantial drop of 74.83% from the control count of 18.21 ± 0.60 to 4.59 ± 0.56 (with a T value of 0.30) occurs by the 25th day in the experimental group compared to the control group.

Table 3: Hemoglobin content changes in Rohtee Ogilbii exposed to sublethal copper for 25 days

S. No.	Exposure Period	Control	Experiment	Change %	Calculated T Value
1	5	5.58 ± 1.05	4.70 ± 0.49	-15.66	-1.37
2	10	5.35 ± 0.85	3.95 ± 0.60	-26.60	-1.08
3	15	4.94 ± 0.70	3.70 ± 0.43	-25.62	-1.09
4	20	3.96 ± 0.72	3.04 ± 0.34	-23.37	-1.18
5	25	3.39 ± 0.50	3.03 ± 0.32	-11.75	-1.50

Over the course of 25 days, Rohtee Ogilbii were subjected to sublethal amounts of copper, and the table shows the variations in hemoglobin content. Hemoglobin levels in the control group remain quite constant throughout time, whereas in the experimental group, they consistently decrease as the exposure period rises. Following 5 days of exposure, the experimental group's hemoglobin content decreases to 4.70 ± 0.49 from 5.58 ± 1.05 in the control group, showing a fall of 15.66% and an estimated T value of -1.37. On the tenth day, the hemoglobin content of the experimental group drops even lower to 3.95 ± 0.60, which is a 26.60% drop compared to the control group's 5.35 ± 0.85, and the T value is -1.08. With a T value of -1.09 and a hemoglobin content continuing to fall on the fifteenth day, the experimental group recorded 3.70 ± 0.43, a drop of 25.62% compared to the control group's 4.94 ± 0.70. Compared to the control group's hemoglobin content of 3.96 ± 0.72 after 20 days, the experimental group's additional decrease to 3.04 ±

0.34 (a 23.37% reduction) is shown by a T value of -1.18. By the 25th day, the experimental group's hemoglobin content has decreased by 11.75% compared to the control group's 3.39 ± 0.50, with a T value of -1.50, reaching 3.03 ± 0.32.

V. CONCLUSION

Investigating the impact of copper toxicity on Rohtee Ogilbii's blood parameters demonstrates substantial disturbances in hematological health, drawing attention to the negative consequences of copper pollution in the environment. Evidence from the drops in red blood cell (RBC), white blood cell (WBC), and hemoglobin levels shows that even at sublethal quantities, copper may hinder fish immunological responses and physiological processes. These results highlight the need to track the effects of heavy metal contaminants on aquatic ecosystems and the animals that call them home. To better understand the wider ecological effects of metal pollution, it is crucial to understand how Rohtee Ogilbii, an important indicator species, responds to copper poisoning. Mitigating these consequences and preserving the health and biodiversity of freshwater ecosystems requires effective environmental management and regulatory measures.

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