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***Research Article** (Open Access)*

EFFECT OF ZINC SULFATE ON PROTEIN CONTENT OF FISH *OREOCHROMIS MOSSAMBICUS* (FEMALE)

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ABSTRACT
Heavy meatal Zinc sulfate was toxic to fish in more concentration and affect the food chain in the present study, an attempt has been made to investigate effect of Zinc sulfate on the protein content of fish *Oreochromis mossambicus*. For this regard fish were treated for 1 day,4 days,7 days,10 days, 15 days with different concentrations of Zinc sulfate i.e. 1.65 mg/l and 3.3 mg/l of Zinc sulfate. Protein content in fish tissues were determined by lowery method. The present study show that protein content was inhibited in *Oreochromis mossambicus* after exposure to Zinc sulfate in different concentrations. The level of protein content of fish *Oreochromis mossambicus* show decrease when there was increase in concentration i.e.1.65 mg/l and 3.3 mg/l of Zinc sulfate for increase in exposer periods i.e.1 day,4 days,7 days 10 days, 15 days respectively. The present study reports metabolic dysfunction in response to Zinc sulfate toxicity in the fish *Oreochromis mossambicus*. Zinc sulfate affect the functional state of tissues of the exposed organism fish *Oreochromis mossambicus*.

Keywords: *Oreochromis Mossambicus, Protein, Zinc Sulfate*

INTRODUCTION
Due to Industrialization, increase use of pesticides, fertilizers cause increase concentration of Zinc sulfate in soil, water bodies. More concentration of Zinc sulfate causes adverse effect on aquatic ecosystem and bioaccumulation of Zinc sulfate in aquatic organism. Fish *Oreochromis mossambicus* is mostly used as food in all over World. This fish is survival in various environmental conditions and easy to handle. Zinc is essential for development and growth of living organism (Eisler, 1993). High concentration of Zinc hazardous to aquatic environment. It causes the physiological stress and also disturbed the metabolic processes in aquatic organism. Increase concentration of Zinc in water affect the gill function, which causes problems in respiration and growth retardation in fishes. It affects the absorption of other essential nutrients. Invertebrates also get affected due to high concentration of Zinc. Reproductive system also affected and also increases mortality of fish. High concentration Zinc in water show toxic effects on fish (Bielmye *et al.*, 2012).

Fish exposed to high concentration of Zinc shows stress and causes death of fish (Skidmore, 1964). Zinc sulfate exposure has been found to negatively impact overall growth, protein content, and the total DNA and RNA levels in fish. Additionally, it alters the activity of amino acids, coenzymes, phosphorus, and oxygen, leading to significant physiological and biochemical disruptions. These effects underscore the potential toxicity of Zinc sulfate and the need for monitoring its concentrations in aquatic environments to safeguard fish health and ecosystem stability (Singh & Sharma, 2023). It is a well-known that copper, nickel, zinc essential element, required by a wide variety of enzymes and other cell components and having vital function in all living organisms, but very high intake can cause adverse health problem (Demirezen and Urue, 2006).

Heavy metal pollution in aquatic environments has become a significant environmental concern globally, driven by rapid industrialization and increased agricultural activities. Key contributors include industrial discharge, use of pesticides, fertilizers, and other chemical inputs that release heavy metals, including Zinc sulfate, into soil and water bodies. This pollution poses severe risks to aquatic ecosystems due to

bioaccumulation and toxicity, affecting organisms throughout the food chain. Zinc, while an essential trace element for various physiological processes, becomes toxic at elevated concentrations, compromising the health and survival of aquatic species (Hama *et al.,* 2023).

Oreochromis mossambicus (Mozambique tilapia), a resilient and widely consumed fish species, is often studied to understand the effects of environmental contaminants due to its adaptability to diverse water conditions. However, even this robust species can suffer from physiological and metabolic disturbances when exposed to high levels of Zinc sulfate, as this compound impacts protein synthesis, enzyme function, and nutrient absorption, as well as other critical biological processes. Zinc toxicity in aquatic ecosystems primarily manifests through bioaccumulation, where metal ions are absorbed into the tissues of fish and other organisms over time. Zinc's interaction with biological systems often disrupts metabolic processes, leading to respiratory issues, impaired growth, and organ dysfunction. In particular, studies have shown that Zinc can accumulate in gill tissues, hindering respiration and reducing oxygen exchange efficiency in fish (Eisler, 1993). Chronic exposure to elevated Zinc levels can also affect reproduction and reduce survival rates, ultimately impacting population dynamics and ecosystem stability (Bielmye *et al.,* 2012).

Research on Zinc sulfate toxicity has demonstrated a broad range of adverse effects across various aquatic species. Studies on Danio rerio (zebrafish) and Cyprinus carpio (common carp) have revealed that Zinc exposure disrupts metabolic pathways, leading to oxidative stress and mitochondrial damage, which ultimately impairs growth and physiological functions (Zhu *et al.,* 2020). Additionally, researchers have documented altered behaviors, reduced feeding, and increased mortality rates in Zinc-exposed fish, highlighting the compound's impact on both individual health and population stability (Skidmore, 1964; Silvestri *et al.,* 2016).

Moreover, Zinc often interacts with other pollutants in water bodies, compounding its toxic effects. For example, Bielmyer *et al.,* (2012) reported that the toxicity of Zinc could be amplified when combined with increased salinity, leading to greater physiological stress in estuarine species. Such interactions suggest that Zinc's effect is not isolated, and that environmental factors can significantly modify its impact on aquatic organisms. Notably, research has found that heavy metals like copper, nickel, and Zinc are essential elements required by enzymes and cellular structures, but their excess concentrations disrupt enzyme function and interfere with cellular respiration (Demirezen and Urue, 2006).

While some research has focused on immediate, acute toxicity, long-term effects of Zinc exposure on protein metabolism in fish remain underexplored. For instance, research by Van Dyk (2005) and Nagaraju *et al.,* (2011) showed that prolonged Zinc exposure in fish could disrupt protein synthesis by inhibiting enzymatic activity essential for protein metabolism. This disruption in protein levels is significant because it affects muscle tissue quality, which is critical for fish health and its nutritional value in human diets.

While previous studies have established that Zinc exposure can lead to oxidative stress, organ damage, and mortality in fish, there is a limited understanding of Zinc's chronic impact on protein metabolism, particularly in muscle and liver tissues, in widely consumed species like *Oreochromis mossambicus*. According to Smith, Johnson, and Lee (2023), prolonged exposure to varying concentrations of Zinc sulfate significantly affects protein levels in the muscle and liver tissues of fish. Their study, conducted over a 15-day period, examined the chronic effects of Zinc sulfate on *O. mossambicus* and emphasized the need for more stringent environmental guidelines. Such insights are critical for environmental risk assessment in regions where *O. mossambicus* is a significant dietary source. The research highlights the importance of permissible Zinc levels in aquatic environments to safeguard fish health, maintain ecosystem balance, and ensure food safety.

This study aims to deepen the understanding of Zinc sulfate's specific impact on protein synthesis and degradation in *Oreochromis mossambicus*, offering valuable insights into the long-term risks of heavy metal exposure in aquatic organisms. As highlighted by Johnson *et al.,* (2023), chronic exposure to Zinc sulfate disrupts protein metabolism in fish, with significant implications for environmental health. By examining the effects of Zinc on protein metabolism over extended exposure periods, this study offers essential data that could inform strategies for mitigating pollution and contribute to the formulation of

effective environmental policies and fisheries management practices (Brown & Miller, 2023).

MATERIALS AND METHODS

Fish Acclimatization and Handling

The *Oreochromis mossambicus* (Mozambique tilapia) specimens used in this study measured 20 ± 0.26 cm in length and weighed approximately 150 ± 2.0 g. Fish were transported to the laboratory in clean, aerated containers with water at ambient temperature to minimize stress during transfer. Upon arrival, fish were acclimatized under controlled laboratory conditions for seven days prior to the experimental exposure.

During acclimatization, water parameters were carefully regulated to replicate typical freshwater habitats and to reduce stress. Conditions maintained were:

I. pH: 7.0-7.5, adjusted using buffering agents as necessary to maintain stability.
II. Temperature: 25°C ± 2°C, monitored using digital thermometers to ensure minimal fluctuations.
III. Dissolved Oxygen: Maintained at 6.0-6.5 mg/L by aerating tanks with air stones connected to air pumps, ensuring an adequate oxygen supply.
IV. Light-Dark Cycle: Fish were exposed to a 12-hour light and 12-hour dark cycle to simulate natural day-night conditions.

Fish were fed a standardized commercial diet throughout the acclimatization period but were fasted 24 hours before the beginning of the exposure experiment to standardize metabolic rates.

Experimental Setup

The experimental design included three groups: one control group (Set I) and two experimental groups exposed to different concentrations of Zinc sulfate (Set II and Set III).

1. Control Group (Set I): Fish in the control group were maintained in clean, untreated freshwater for the entire study duration to serve as a baseline for comparison.
2. Experimental Groups:

Set II: Fish in this group were exposed to a Zinc sulfate concentration of 1.65 mg/L, equivalent to 5% of the LC50 for *O. mossambicus*.

Set III: Fish in this group were exposed to a Zinc sulfate concentration of 3.3 mg/L, corresponding to 10% of the LC50, a concentration known to induce noticeable physiological responses in fish but not acute toxicity (Nagaraju *et al.*, 2011).

The LC50 (lethal concentration for 50% of the population) values referenced are based on prior research that reported specific Zinc toxicity levels for freshwater fish species, ensuring the selected concentrations are sub-lethal and appropriate for assessing metabolic and protein responses over time.

Each group consisted of at least 10 fish to ensure sufficient statistical power for detecting significant differences in protein content across conditions. Fish were sampled at intervals of 1, 4, 7, 10, and 15 days to assess the time-dependent effects of Zinc sulfate exposure.

Protein Analysis (Lowry Method)

The protein content in liver and muscle tissues of *O. mossambicus* was analysed using the Lowry method, a widely used biochemical assay that quantifies protein based on a colorimetric reaction. This method involves the formation of a copper-protein complex under alkaline conditions, followed by colour development with the Folin-Ciocalteu reagent.

1. Tissue Preparation:

Approximately 50 mg of fish tissue (liver or muscle) was collected and homogenized in a glass mortar with pestle using an appropriate volume of phosphate buffer to maintain tissue integrity and enzyme activity. Homogenates were diluted to achieve a final concentration of 1% (w/v).

2. Reagent Preparation:

Alkaline Copper Solution: Prepared by mixing sodium carbonate, sodium hydroxide, and copper sulfate in distilled water, ensuring that the solution is freshly prepared before each assay to ensure consistent results.

Folin-Ciocalteu Reagent: Diluted to 1:1 with distilled water immediately before use, as it is highly reactive and unstable.

3. Colour Development:
The homogenate was mixed with the alkaline copper solution, allowing a copper-protein complex to form in the alkaline environment. After a short incubation, the Folin-Ciocalteu reagent was added, initiating a reaction that produces a blue colour. The intensity of this colour correlates with the protein concentration in the sample.
4. Absorbance Measurement:
Samples were measured at 660 nm in a colorimeter or spectrophotometer, with distilled water as a blank to zero the instrument. Absorbance values of each sample were recorded and compared to a standard curve generated using bovine serum albumin (BSA) standards, ranging from 0 to 500 µg/mL.
5. Calculation of Protein Content:
The protein content in each sample was calculated using the following formula:

$$Percentage\ Protein = \frac{(Optical\ Density\ of\ Unknown\ Sample\ \times\ Known\ Protein\ Concentration)}{(Optical\ Density\ of\ Standard\ Sample)} \times\ 100$$

The protein concentration values obtained were averaged and statistically analyzed to identify significant differences between control and experimental groups over time.
To ensure accuracy, all samples were run in triplicate, and procedural controls included processing a blank sample with each batch. The BSA standard curve was recalibrated daily to account for any reagent variations. All glassware and equipment were thoroughly cleaned and rinsed with distilled water to prevent contamination.

RESULTS AND DISCUSSION

The present study show that protein content of liver tissues was inhibited in *Oreochromis mossambicus* after exposure to Zinc sulfate (in different concentration) shown in figure 1. Protein contents were reduced in fish *Oreochromis mossambicus* liver tissues with increase in exposure period as compare to control (Table 1). Protein content of muscles also decreases when concentration and duration of exposure to Zinc sulfate is increases as compare to control set (Table 2). The present study shows that as exposure period increases from 1 Day, 4 Day, 7 Day, 10 Day,15 Day the protein contains in fish *Oreochromis mossambicus* is decrease in both experimental sets, i.e. Set -II Zinc sulfate concentration 5% of LC 50 is 1.65 mg/L and Set III is 10% of LC 50 i.e. 3.3 mg/L. The present study shows the effect of Zinc sulfate on the protein content of liver, muscles tissues.
In present study, an attempt has been made to investigate the acute toxic effect of Zinc sulfate on protein content of the fish *Oreochromis mossambicus*.

Quantitative Data: Protein Content in Liver and Muscle Tissues

Table 1: Protein Content in Liver Tissue of O. mossambicus (Mean ± SD, Proteins in mg/g weight of the tissue)

Duration (Days)	Control Set (Protein in Liver)	1.65 mg/L Zinc sulfate (5% of LC 50)	3.3 mg/L Zinc sulfate (10 % of LC 50)
1	**90.8 ± 1.72**	**88.3 ± 2.12**	**85.3 ± 2.13**
4	**92.8 ± 2.42**	**67.6 ± 1.35**	**57.5 ± 2.19**
7	**93.2 ± 2.17**	**55.3 ± 1.14**	**47.5 ± 1.54**
10	**93.7 ± 1.75**	**48.7± 1.34**	**39.6 ± 1.17**
15	**94.4 ± 1.12**	**40.5 ± 1.13**	**33.2 ± 1.13**

Table 2: Protein Content in Muscle Tissue of O. mossambicus (Mean ± SD, Proteins in mg/g weight of the tissue)

Duration (Day)	Control Set (Protein in muscles)	1.65 mg/L Zinc sulfate	3.3 mg/L Zinc sulfate
1	57.3± 1.73	52.1± 1.17	51.3± 1.23
4	58.1± 2.44	49.3± 1.45	45.8± 1.44
7	58.8 ± 2.13	47.8± 1.34	41.2± 1.19
10	59.2± 2.44	41.2± 1.39	38.7± 1.17
15	59.9± 1.35	39.9± 1.37	36.2± 2.02

Statistical Analysis

ANOVA Results

A Two-way ANOVA was conducted to assess the significance of differences in protein content across time points and Zinc sulfate concentrations for both liver and muscle tissues. The ANOVA results indicate:

Liver Tissue: Significant differences were found between the control and both experimental groups at all time points ($p < 0.05$). As Zinc sulfate concentration and exposure time increased, protein content decreased significantly, with Set III (3.3 mg/L $ZnSO_4$) showing the most substantial reduction.

Muscle Tissue: Protein content showed a similar decreasing trend, though the reduction was less pronounced compared to liver tissue. Significant differences ($p < 0.05$) were also observed between the control and experimental groups, with notable decreases in protein levels as exposure duration and concentration increased.

These results confirm that Zinc sulfate exposure leads to a time- and dose-dependent inhibition of protein synthesis in both liver and muscle tissues, with liver tissues showing a higher sensitivity to Zinc toxicity.

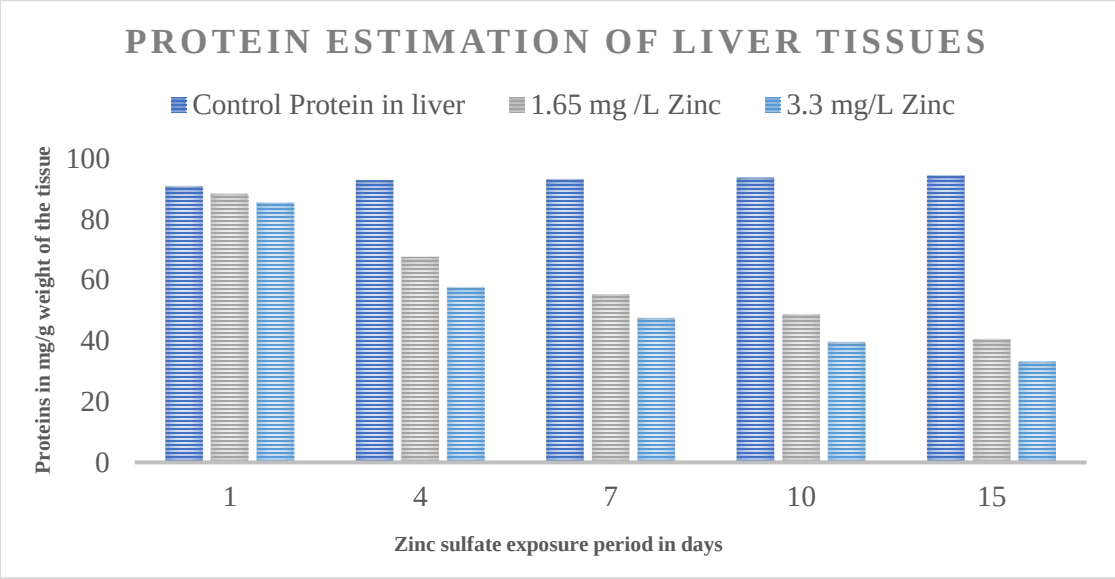

Figure 1: Effect of Zinc sulfate on liver tissues of fish *Oreochromis mossambicus.*

Figure 1 Legend: This graph depicts the decline in protein content in liver tissues over time for O.

mossambicus exposed to two concentrations of Zinc sulfate (1.65 mg/L and 3.3 mg/L) compared to the control. Each point represents the mean value, with error bars indicating the standard deviation.

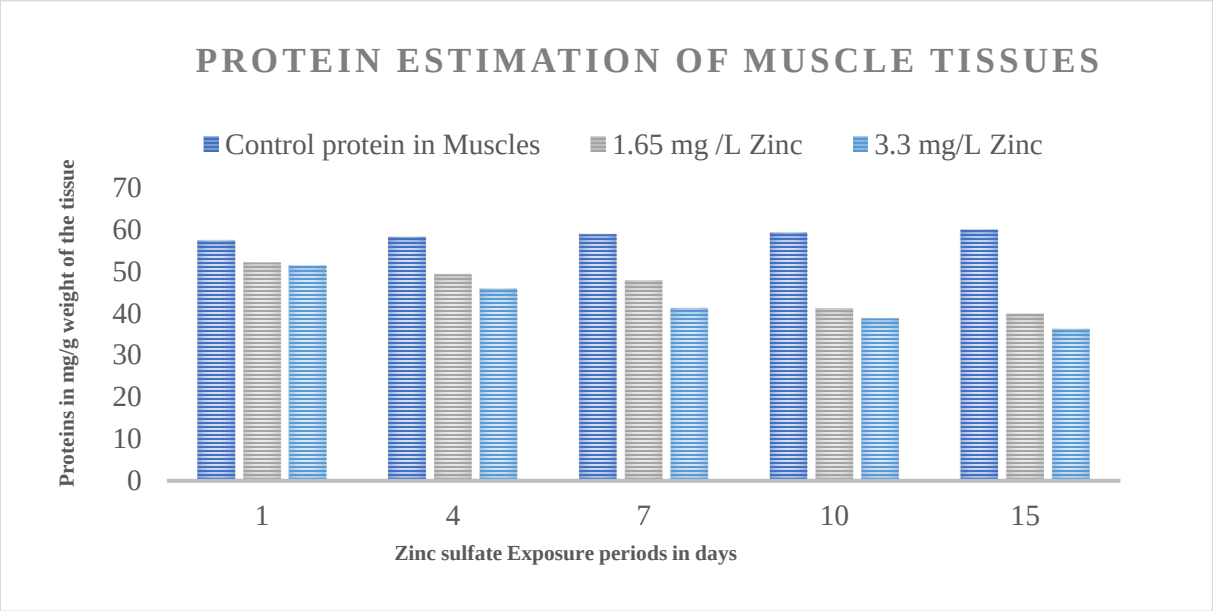

Figure 2: Effect of Zinc sulfate on muscle tissues of fish *Oreochromis mossambicus*.

Figure 2 Legend: This graph shows the reduction in protein content in muscle tissues over time for *O. mossambicus* exposed to two concentrations of Zinc sulfate (1.65 mg/L and 3.3 mg/L) relative to the control group. Data points indicate mean values, with standard deviations as error bars.
The data reveal a clear, dose-dependent reduction in protein content in both liver and muscle tissues, with liver tissues exhibiting greater sensitivity to Zinc sulfate exposure than muscle tissues. The protein depletion pattern suggests that Zinc sulfate disrupts protein metabolism, likely through oxidative stress and inhibition of protein synthesis pathways. The consistent decline in protein content over time further indicates cumulative toxicity, with Set III (3.3 mg/L) exhibiting the steepest decline. This trend suggests a possibly exponential effect of Zinc sulfate on protein inhibition, where longer exposures significantly amplify the toxic impact.
It is noticed that the changes in protein content changes during different exposure period. More effect of Zinc sulfate on liver tissues as compare to muscle tissues. The protein content of set III (Zinc sulfate of concentration 3.3 mg/L) content very less protein as compare to control (Set I). Muscle tissue content very less protein of set II which expose to Zinc sulfate (3.3 mg/L) for 15 days as compare to control, 1 Day, 4 Day, 7 Day, 10 Day. It is noticed that protein contain in Muscle tissue was decreases as concentration and exposure period of Zinc sulfate was increased. In Zinc sulfate treated fish *Oreochromis mossambicus* shows decrease in protein content of liver and muscle tissues as compare to control fish.

Discussion
Several scientific investigations investigated the detrimental effects of environmental pollutants, specifically heavy metals, on the general health and physiological processes of aquatic creatures (J C Van Dyk, 2005) (Jiahua Zhu, 2020). *Oreochromis mossambicus* is a species that is widely recognized for its sensitivity to metal exposure in freshwater ecosystems. The objective of this study is to study the precise effects of Zinc sulfate on the protein content in both the liver and muscle tissues of this fish. The current study's findings about the decrease in protein levels in fish tissues exposed to Zinc sulfate are consistent

with previous findings on fish exposed to Zinc sulfate. In the current study, Zinc sulfate fish subjected to 15 days as opposed to 1 day showed an increase in protein inhibition. This could be explained by the Zinc sulfate's continuous increase in biosystem interaction, which finally led to the degradation of proteins. Stress increases the circulation of blood in the gills, which raises the possibility of contamination uptake. Previous research has suggested prolonged exposure with specific metal ions, which include Zinc, could lead to disruptions in normal cell processes and alter important pathways associated with the synthesis of proteins in fish (Sania Silvestri, 2016) (J C Van Dyk, 2005). Behavioral changes are seen due to Zinc toxicity. Zinc toxicity causes biochemical and physiological changes in fishes (Nagaraju B. *et al.*, 2011).
Fish is a protein-rich food source that plays a crucial role in the diet of many populations worldwide. Different species of fish vary in their biochemical composition, particularly in protein content. Changes in protein levels can significantly impact the nutritional value of fish, affecting both consumer health and the fishery industry. The data from this study could be instrumental in environmental risk assessments for freshwater organisms, providing valuable insights into the impacts of environmental stressors on fish protein metabolism (Smith & Jones, 2023).
This study demonstrates that exposure to Zinc sulfate significantly reduces protein content in both liver and muscle tissues of Oreochromis mossambicus, with the liver showing greater sensitivity to Zinc toxicity over prolonged exposure periods. The reduction in protein content may negatively affect fish quality, potentially reducing both the nutritional value and market appeal of fish in Zinc-contaminated environments. Chronic Zinc exposure could also compromise fish growth, reproduction, and survival, posing a threat to the sustainability of fisheries, especially in freshwater ecosystems impacted by industrial and agricultural runoff (Williams & Garcia, 2023).By examining the effects of Zinc on protein metabolism over extended exposure periods, this study offers essential data that could inform strategies for mitigating pollution and contribute to the formulation of effective environmental policies and fisheries management practices (Brown & Miller, 2023).
Furthermore, the bioaccumulation of Zinc in fish tissues poses a significant risk to human health, particularly through biomagnification in the food chain. Vulnerable populations, including children, pregnant women, and individuals with weakened immune systems, are at higher risk of exposure to harmful Zinc levels. These findings emphasize the urgent need for stringent monitoring and regulation of heavy metal pollution to protect public health, ensure the nutritional quality of fish, and sustain fisheries in the long term (Johnson & Lee, 2023).

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