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Eichhornia crassipes Mediated Copper Removal Alleviates Bioaccumulation and Enzymological Alterations in the Major Carp *Catla catla* Exposed to a Sublethal Concentration of Copper Sulphate

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Abstract

Phytoremediation is an effective, cheap and eco-friendly method of heavy metal removal from polluted aquatic environments. To analyse the efficacy of water hyacinth in copper removal, and its ability to mitigate the copper-induced stress to fish, an Indian major carp, *Catla catla,* fingerlings were treated with a sub-lethal concentration of copper sulphate alone (0.09 ppm) (Treatment-1) and a sub-lethal concentration of copper sulphate treated along with water hyacinth (Treatment-2). Bioaccumulation in vital organs and transaminase activity in fish were analysed upon completion of the 5th, 10th, and 15th day from each treatments. The trend of copper accumulation was as follows: Treatment-1: gills > kidney > liver, and Treatment-2: kidney > gills > liver. In treatment-2, higher copper accumulation in kidney may be due to depuration process and translocation of metallothionein-bound copper from the liver. Likewise, lesser copper accumulation in gills up to the fifth day than in treatment-1 may be due to hyperaccumulation in plant roots via phytochelatin synthesis by glutathione synthase present in the root. Accumulation of copper in tissues disrupts the transaminase activity. Elevated GOT and decreased GPT activity in treatment-1 may be due to damage to the integrity of hepatic cells and cell organelles, leading to enzyme leakage into the blood. In contrast, in treatment-2, enzyme activity did not vary significantly relative to the control. The present study reveals that prudent use of *Eichhornia crassipes* can effectively remove copper and reduce its bioaccumulation in tissues, which in turn can mitigate copper-induced stress in fish.

Keywords

Copper sulphate, Bioaccumulation, Transaminase activity, *Eichhornia crassipes*, *Catla catla*

Introduction

Copper and zinc are often identified as the most common heavy metal contaminants in the water, mainly due to human activities (Li et al., 2015). Copper is a vital element necessary for

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photosynthesis and protein synthesis, yet its accumulation at high levels can trigger oxidative stress, leading to cellular damage (Chen et al., 2022). Copper sulphate was used as a source of copper (II) ions because it is one of the frequently employed agents for the management of phytoplankton growth in aquaculture ponds, reservoirs, and natural freshwater bodies (McDonald et al., 2025; Teodorescu et al., 2012).

Fish are typically regarded as the reliable bioindicator organisms of choice for assessing pollution in aquatic ecosystems, because they can be chemically exposed to various substances, such as heavy metals, and can bioaccumulate through direct exposure or through the food chain (Zahran et al., 2025). As the fish grows, high concentrations of metal can accumulate in their muscles, liver, and intestine, making the fish unsafe for human consumption (Gupta & Arunachalam, 2024). The metal contaminants in the sediment bioaccumulate in benthic organisms and are transferred to larger animals when they feed on these contaminated organisms. Increasing the concentrations of metals at successive trophic levels is known as biomagnification (Oros, 2025). The toxic impacts of any heavy metal have a potential target on all the tissues and organs in the body of an animal. Accumulation of copper in fish tissues can occur through gills, body surface and via ingestion (Ali et al., 2025). Accumulation patterns in fish rely balance between uptake and elimination rates, which can pose substantial health impacts influenced by the comparative levels of these processes. Metals are variably distributed across various tissues of the fish body (Páez-Osuna et al., 2023).

Effective removal of heavy metals via phytoremediation encompasses strategies such as phytodegradation, phytoextraction, phytostabilisation, and rhizofiltration (Tan et al., 2023). In phytoextraction, plants absorb heavy metals and transport them to their aboveground parts, which can later be collected to remove pollutants from the polluted site (Zhu et al., 2020). Water hyacinth, *Eichhornia crassipes*, is a rapidly growing aquatic weed plant that serves as a cost-effective, easily accessible, and sustainable biomass and biochar resource (Singh et al., 2023a). It is capable of growing in severely polluted waters and plays a crucial role in extracting and accumulating metals from the water. It is one of the most commonly used plants in constructed wetlands due to its fast growth rate and significant uptake of nutrients and contaminants. It is a cheap biosorbent material for metal ions (Hayyat et al., 2023; Sanmuga Priya & Senthamil Selvan, 2017). Although water hyacinth is an aquatic weed, it can indirectly protect aquatic organisms from exposure to heavy metals. Hence, this study aimed to analyze whether phytoremediation of copper by water hyacinth reduces its toxicity to aquatic organisms, particularly fish. If so, to what extent? To test the above hypothesis, the fish *Catla catla* was selected and exposed to sub-lethal concentrations of copper sulphate (with/without water hyacinth). To assess the ameliorating effects of *Eichhornia crassipes* on copper-induced stress in fish, bioaccumulation in soft tissues, and enzymological parameters were used.

Methodology

Healthy *Catla catla* fingerlings were procured from Tamil Nadu Fisheries Development Corporation Limited Fish Farm, Aliyar, Tamil Nadu, India, and acclimatized for 10 days in a concrete tank (1,500 L volume) under controlled laboratory conditions with a 12 hours light and 12 hours dark photoperiod. Fish were fed *ad libitum* once daily with a diet prepared from rice bran and groundnut oil cake in a 2:1 ratio, and feeding was stopped 24 h before experimentation. Three- to four-week-old *Eichhornia crassipes* plants collected from a local lake in Coimbatore were washed with distilled water and acclimatized for one week. Chlorine-free tap water was used throughout the experiment, and physicochemical parameters were monitored and maintained according to the standard procedures (APHA, 1998); temperature 25 ± 2 °C, pH 6.5 ± 0.02, salinity 0.650775 ± 0.01 ppt, dissolved oxygen 14.0996 ± 0.06 mg/L, total hardness 121.55 ± 0.17 mg/L, total alkalinity 128.25 ± 0.09 mg/L, calcium 80 ± 0.68 mg/L, and magnesium 41.55 ± 0.51 mg/L. Analytical-grade copper sulphate (HiMedia, India) was used without further purification, and a 1000-ppm stock solution was prepared using double-distilled water. Preliminary toxicity tests were conducted, and the 24-h LC_{50} was determined by probit analysis (Finney, 1978), one-tenth of the LC_{50} (0.09 ppm) selected as the sublethal concentration (Sprague, 1971). For sub-lethal studies, random selection of seventy fish was performed from the stock and transferred to each tank. Fish were exposed for 15 days to control and treatment groups; 1). Treatment-1 (T1) - *Catla catla* exposed to sub-lethal copper concentration (0.09 ppm) 2). Treatment-2 (T2) - *Catla catla* exposed to sub-lethal concentration of copper along with water hyacinth (0.09 ppm), four replicates per treatment were also maintained. The experimental workflow is illustrated in Figure 1. Blood, gill, liver, kidney, plant root and stem, and water samples were collected at 5-day intervals. Pooled blood sample (20 fish/treatment) was obtained by cardiac puncture with 26 gauge plastic syringe pre-coated with heparin (Biological limited, Hyderabad, India), and transferred into heparin-treated vials, and rapidly stored on ice. The copper content in the samples (gill, liver, kidney, plant root, stem, and water) from each treatment was assessed according to the double-acid digestion method (Topping, 1972). After completion of digestion, the solutions were analysed using an Atomic Absorption Spectrophotometer (Thermo Fisher Scientific iCE 3000 Series Atomic Absorption Spectrometer). Plasma GOT and GPT activities were estimated using SGOT (ASAT) and SGPT (ALAT) kits (Coral Clinical Systems, Goa, India) following the modified IFCC method (Bergmeyer, 1980) and analysed using an UV–VIS spectrophotometer (Agilent Technologies Cary 60). Data were analysed using two-way ANOVA followed by Duncan’s Multiple Range Test (DMRT) (Duncan, 1951) in SPSS Version 20, with significance at $p < 0.05$.

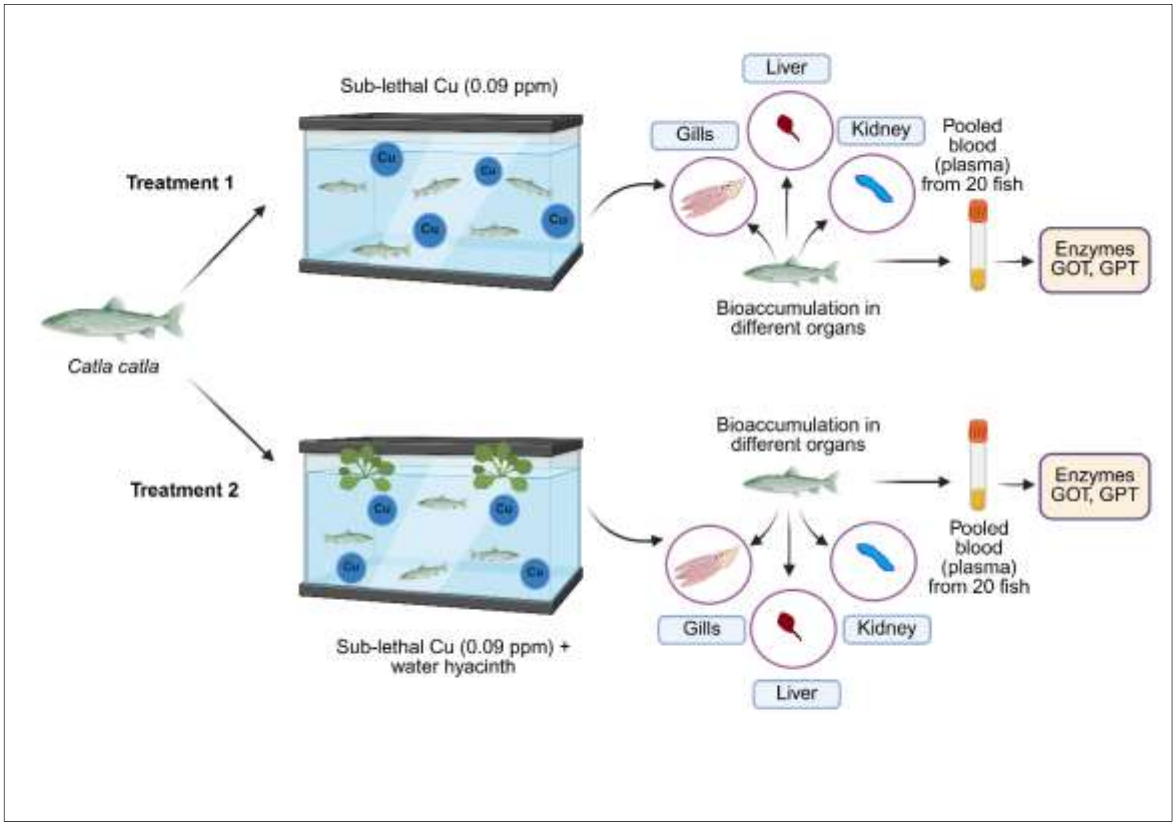

Figure 1. Schematic representation of sub-lethal copper exposure with and without water hyacinth; resulting bioaccumulation and enzyme activity in *Catla catla*

Results and Discussion

Probit analysis (Finney, 1978) revealed that the LC50 value of copper for the fish *Catla catla* after 24 h was 0.9 ppm. The chi-square test of the toxicity data used for probit analysis indicates that the fish populations used for the experiment were homogeneous (Busvine, 1971). Mortality was not found in treatment-2 during the sub-lethal experimental period. Over the past few decades, intensive industrial development has raised the copper concentration in aquatic ecosystems, impacting fish and deteriorating natural resources (Shahjahan et al., 2022). Aquatic animals may accumulate copper in their organs, which can lead to redox reactions that generate free radicals, thereby causing morphological alterations and changes in specific physiological processes (Zhang et al., 2022). During acute copper exposure, fish exhibited distinct behavioural changes, such as rapid movement around the tank surface, followed by periods of stillness. Later, they showed backward movement, spreading excess mucus around the gills. Then, towards the end of the experiments, rapid opercular movement was observed, followed by gulping air, erratic jumping movements, and finally, they floated upside down before dying. The marked behavioural changes observed during the determination of LC50 in the present investigation may be due to alterations in the membrane transport of the gills. The hyperexcitability of fish exposed to copper may be due to disturbances in acetylcholine esterase activity (AchE) in the nervous system, leading to the accumulation of acetylcholine and prolonged postsynaptic excitability (Haverroth et al., 2015). As an adaptive response, fish secreted excess mucus around the gills, even though it prevents the free entry of Cu^{2+}ions into the fish (Das & Mukherjee, 2003; Tavares-Dias, 2021; Vani et al., 2012), it blocks the diffusion of oxygen into the epithelial cells, which eventually causes rapid opercular movement and entry of excess Cu^{2+} ions, which inhibit the gill ATPase activity. As a result, the fish lost its balance and died.

During the study period, the copper level in water, as shown in Figure 2 is indirectly proportional to the exposure duration of each treatment. When experimental groups were compared alone, treatment-2 showed a higher copper content than the control and treatment-1. In the present study high copper content in the water from treatment-2 than samples without plant (Treatment-1) was registered and this depends on how copper and plants reacts with each other i.e., the root exudates from *Eichhornia crassipes* that could have kept copper soluble or suspended in water which caused copper to stay in aqueous solution for more extended period (He et al., 2024; Lu et al., 2018). The DMRT analysis indicates that a high amount of copper was present in the water in treatment-2, which differed significantly from treatment-1.

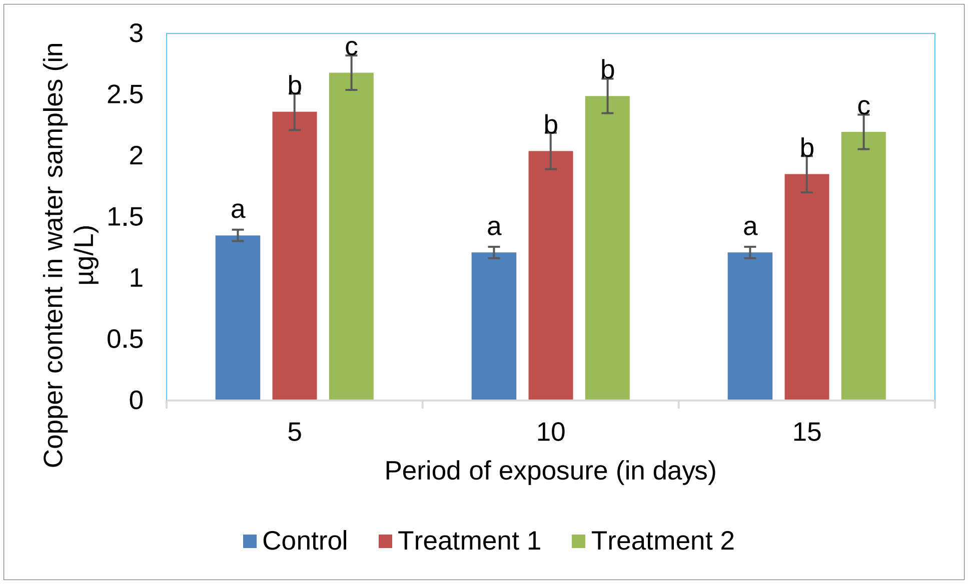

Figure 2. Copper level in the water samples in which *Catla catla* were subjected to different durations of exposure to sub-lethal concentration of copper sulphate alone (T1) and along with *Eichhornia crassipes* (T2)

Increased copper accumulation was observed in the plant parts (roots and stems), as shown in Figure 3 throughout the study period. The root accumulation was progressively reduced from the 5th day to the 15th day, with the least accumulation observed at the completion of the 15th day. However, the copper content in the stem increased gradually, and the percentage of increase was directly proportional to the duration of exposure. This indicates that *Eichhornia crassipes* can accumulate heavy metals in its roots and can also successfully translocate them to the stem. Several studies have shown that plant roots accumulate higher metal concentrations when exposed to contaminated media (He et al., 2024; Lu et al., 2018; Tőzsér et al., 2023). Carboxyl groups in the root system induce a significant cation exchange through the cell membrane, a mechanism by which heavy metals are transported in the root system during active absorption (He et al., 2024; Pang et al., 2023). The findings of the above authors may be the possible reason for the higher copper concentration in the plant root of this study. Accumulation of heavy metals in *Eichhornia crassipes*, accompanied by a rapid induction of phytochelatin biosynthesis, and the phytochelatin content was theoretically sufficient to chelate all heavy metals taken up; this protects photosynthesis as it does not inhibit chlorophyll and carotenoids function (Hall, 2002; Narayanan & Ma, 2023). The highest metal accumulation in plant roots was observed at the completion of the 5th day, followed by a slower rate of metal uptake during the subsequent period. Living plant roots absorb metals quickly; increased plant root surface area exposed to water volume results in higher efficiency of metal removal from the water (Li et al., 2015; Smolyakov, 2012). The statistical analysis also indicates the highest accumulation at the completion of the 5th day, followed by a progressive decrease at the completion of the 10th and 15th days, correspondingly. This result suggests metal accumulation in the root may be higher during the first few days of exposure. The movement of metal-laden sap from the root system to the shoot is termed translocation, which is chiefly regulated by two processes: root pressure and leaf transpiration (Hammad, 2011; Tőzsér et al., 2023). Metal uptake by the roots and their gradual redistribution to other parts of the plant, defines the dynamics of the secondary phase in the elimination of metal from the water (Li et al., 2015; Smolyakov, 2012). This may be the possible mechanism by which the roots translocated the copper to the stem in treatment-2 of the present study.

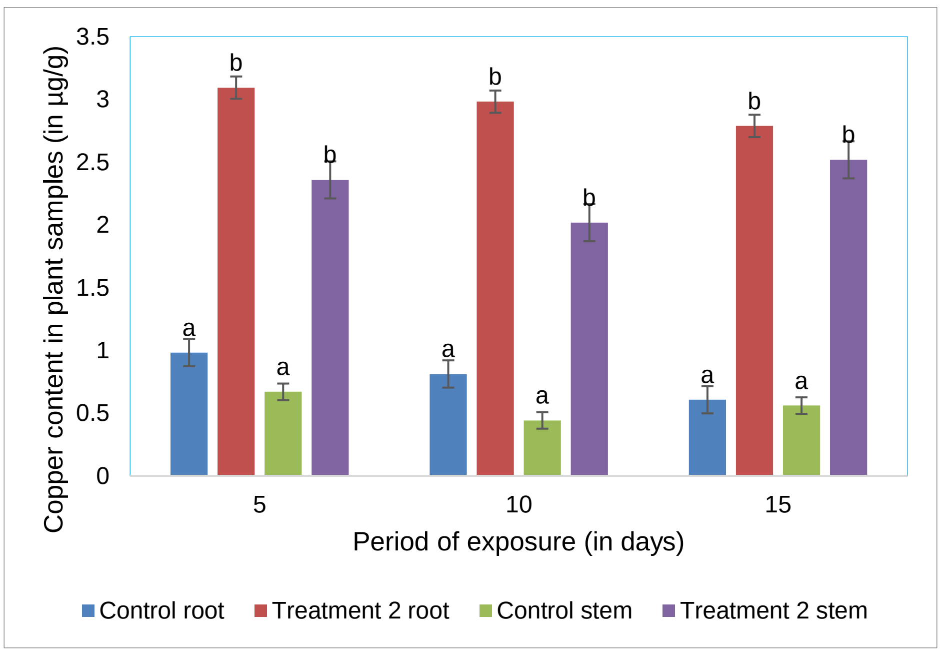

Figure 3. Copper level in the root and stem of *Eichhornia crassipes* subjected to different durations of exposure to sub-lethal concentration of copper sulphate along with *Catla catla* (T2)

Metal accumulation in living organisms relies on the (i) concentration of metal taken up by the organism from its surroundings, (ii) mechanism of metal's distribution in its body organs and (iii) the inherent ability of the fish to concentrate metal (Oros, 2025; Zahran et al., 2025). The gills of *Catla catla* exhibited varied concentrations of copper, as shown in Figure 4 throughout the duration of exposure. Copper accumulation in the gills of fish from treatment-1 was maximum towards the completion of the 5th day and then gradually decreased as the exposure duration was extended. However, the treatment-2 groups showed a minimum accumulation of copper at the completion of the 5th day, and it increased progressively throughout the exposure period. This clearly indicates the significant impact of water hyacinth on the bioaccumulation of pollutants in fish. Later, either the tolerance limit of copper was lowered, or the rate of accumulation in the root was decreased; as a result, the copper content in the fish gills increased progressively. In treatment-1, upto the 10th day, the concentration of copper in the gills was higher than in treatment-2 and the control. This may be due to the gill, the primary target organ exposed to high free Cu^{2+} ions in the water. When compared to treatment-1, treatment-2 fish gills showed less copper accumulation, which may be attributed to the phytoremediation potential of *Eichhornia crassipes* in removing copper from polluted water. In the presence of metal ions, the roots of the plant synthesis phytochelatins by using glutathione as a substrate through phytochelatin synthase. As a result, the free Cu^{2+} ions will be bound to phytochelatin, which might reduce the level of free Cu^{2+} ions in water (Hall, 2002). The gill is a key point for heavy metals entry which induce tissue lesions and gill injury (Pragnya et al., 2020; Vinodhini & Narayanan, 2008; Zahran et al., 2025). A high metal content in the gill tissues could be a consequence of metal complexing with mucus (Aldoghachi et al., 2016). This may be the possible factor for the high accumulation of copper in the gills of treatment-1 in the present study. As the days of exposure increased, the tolerance limit of *Eichhornia crassipes* was lowered, and the copper concentration was gradually reduced in the plant parts. This may be the reason for the increase in copper concentration in the gills of treatment-2 after the 5th day of exposure. This clearly indicates that the hyperaccumulation of copper in water hyacinth is a time-limited process. In both treatments, the copper level in the fish liver, as shown in Figure 5 was progressively increased throughout the study period. The least accumulation was registered at the completion of the 5th day. Treatment-2 showed higher copper accumulation in the liver than Treatment-1. This reveals that accumulated copper ions from the gills were rapidly redistributed to the liver for detoxification, which caused increased copper content in the hepatocytes.

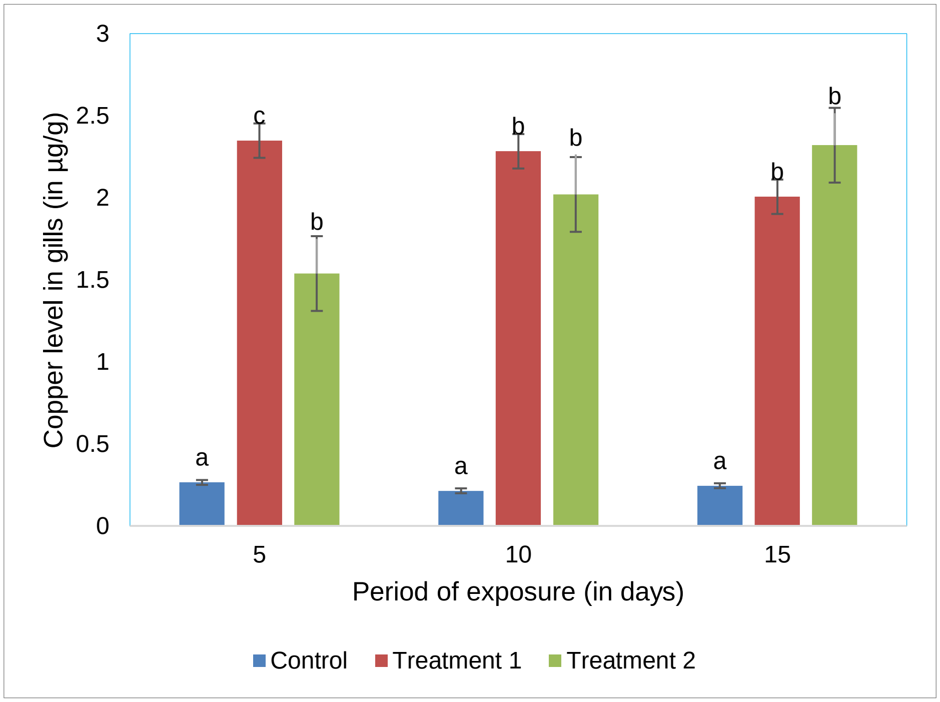

Figure 4**.** Changes in the copper level in the gills of *Catla catla* from sub-lethal concentration of copper sulphate alone (T1) and along with *Eichhornia crassipes* (T2)

Liver is the primary organ for copper storage in fish (Padrilah et al., 2017). Antioxidant defence system in liver and kidney shields them from metal-induced oxidative stress (Atli & Canli, 2010; Kumar et al., 2024). Copper storage in liver is typically associated with the induction of metallothioneins (Gora, 2025; Romeo et al., 2000). Liver accumulation of vital metals is often attributed to its metabolic role, where the presence of elevated concentrations of zinc and copper in liver tissues is generally linked to metallothioneins, like binding proteins (Bervoets et al., 2013), which the liver serves as a reservoir to store essential metals to support enzymatic activity and other metabolic functions (Amiard et al., 2006; Kaddour et al., 2022). The copper concentration in the liver increased with the duration of exposure in both treatments. The level of copper in the liver was higher in treatment-2 than in treatment-1. It may be due to the redistribution of copper from the gills to the liver as the gills reach their maximum tolerance limit, which induces a faster translocation process of copper from the gills to the liver. This critical analysis reveals that accumulated copper ions from the gills are redistributed to the liver, resulting in increased copper content in the hepatocytes.

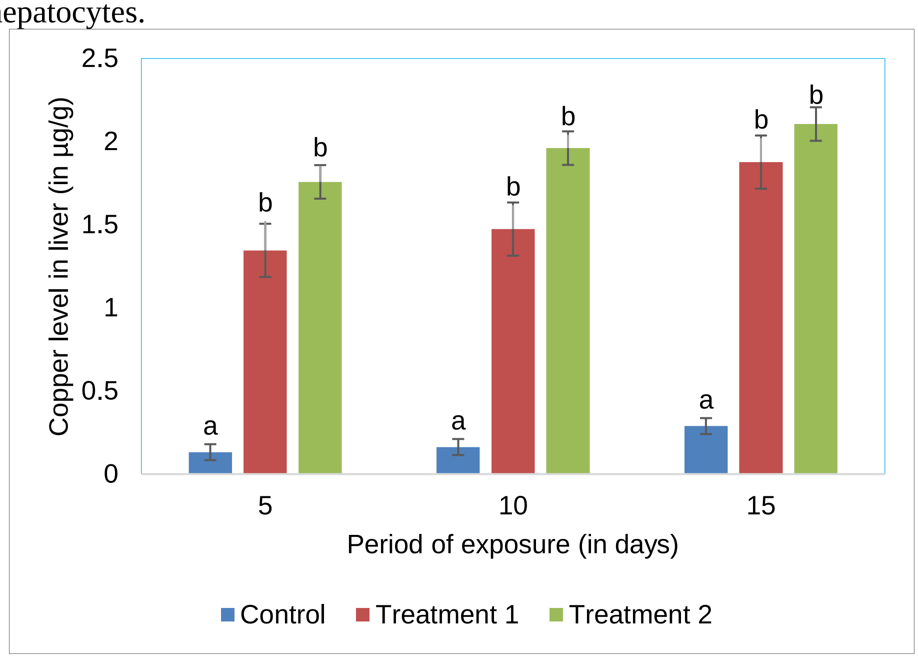

Figure 5. Changes in the copper level in the liver of *Catla catla* from sub-lethal concentration of copper sulphate alone (T1) and along with *Eichhornia crassipes* (T2)

Copper level in the kidney of *Catla catla* as shown in Figure 6 from both treatments was increased, and the percentage of increase was directly proportional to the duration of exposure. Treatment-2 showed a greater accumulation of copper in the kidneys than Treatment-1. However, experimental groups varied significantly from those of the control groups. The kidney’s main roles include eliminating excess water, maintaining acid-base equilibrium, regulating specific ions, and retaining urea (Holzer et al., 2004; Takvam et al., 2023). Kidney-mediated copper removal may occur partly due to lysosomal membrane damage, where lysosomal membrane disruption releases copper from lysosomes for excretion (Romeo et al., 2000). The kidney showed the highest pattern of accumulation in treatment-2. Heavy metals are first transported to the liver through the bloodstream, where they bind to metallothionein proteins to form complexes that are then transported to the kidneys. The metal then accumulates in the kidneys, which damages filtering mechanisms (Squadrone et al., 2013). This may be the cause of the higher accumulation of copper in the kidney. The increased copper concentration in the kidneys of both treatments is due to the selective reabsorption of essential electrolytes, glucose, and essential metals from the urine at the proximal tubules (Subathra & Karuppasamy, 2008). DMRT analysis illustrates that the level of copper accumulation in the kidney of *Catla catla* exposed to both treatments varied significantly.

The higher accumulation of copper in fish organs treated with sub-lethal copper sulphate in treatment-1 showed the following order: gills > kidney > liver. This finding aligns with the results of Kondera et al. (2014). The copper accumulation pattern in the tissues of *Ctenopharyngodon idella* showed high levels of copper in the following order: kidney > gills > liver (Malik et al., 2010). A similar trend of accumulation pattern (kidney > gills > liver) was observed in treatment-2.

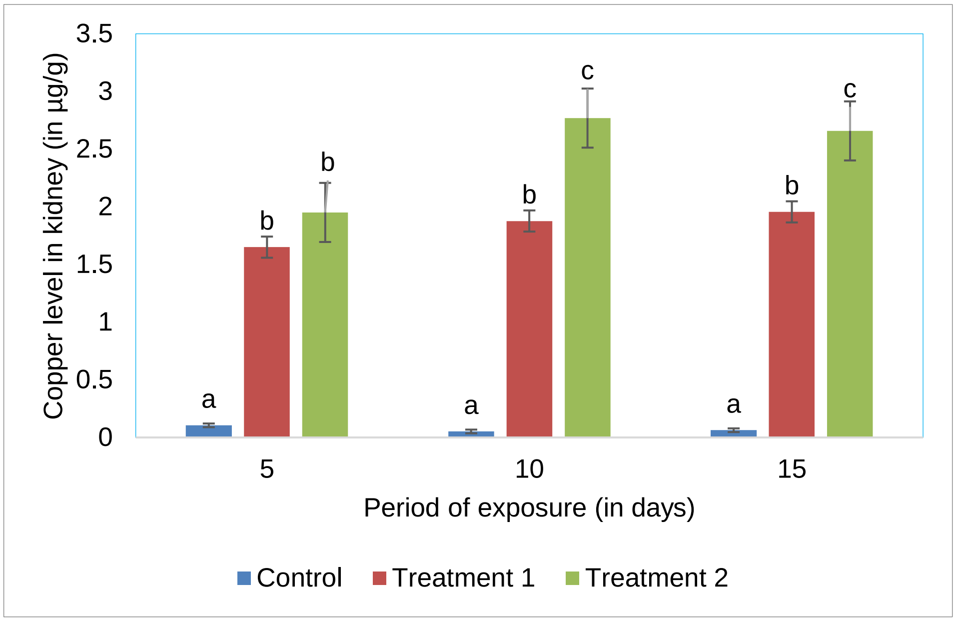

Figure 6. Deviations in the copper level in the kidney of *Catla catla* from sub-lethal concentration of copper sulphate alone (T1) and along with *Eichhornia crassipes* (T2)

Accumulation of copper in tissues/organs may have a significant impact on enzyme activity. The two key enzymes SGOT and SGPT are considered sensitive measures for evaluating hepatocellular damage and certain hepatic diseases (Javed et al., 2016). Glutamate Oxaloacetate Transaminase (GOT) activity in the plasma (Geetha, 2021) of *Catla catla* exposed to sub-lethal concentrations of copper and treated for copper toxicity, along with *Eichhornia crassipes* as shown in Figure 7, showed elevated GOT activity levels in treatment-1 throughout the exposure period. However, in treatment 2, the fish attempted to cope with the stressor, and as an adaptive response, they tried to maintain the enzyme activity at the same level as the control. A considerable increase in fish GOT levels indicates hepatic injury resulting from metal accumulation, thereby releasing the enzyme into the blood. The severity and damage to the organ depend on the toxicant type and the exposure duration (Lavanya et al., 2011; Öner et al., 2008). In this study, elevated GOT levels in treatment-1 may be a consequence of hepatic damage resulting from copper accumulation; however, the other organs (kidney/gill) may also be affected. Treatment-2 fish showed a reduction in GOT activity compared to treatment-1, but this variation was slight compared to the control. This may be due to the phytoextraction of Cu2+ ions by water hyacinth and the rapid redistribution of Cu2+ ions from the liver to the kidney. However, in treatment 2, the fish attempted to cope with the stressor, and as an adaptive response, they sought to maintain enzyme activity at a level close to the control. Slower accumulation of copper in the gills of fish in treatment-2 may provide ample time for the production of metallothionein protein; as a result, more copper will be bound to the metallothionein, which causes increased accumulation but minimises the organ damage.

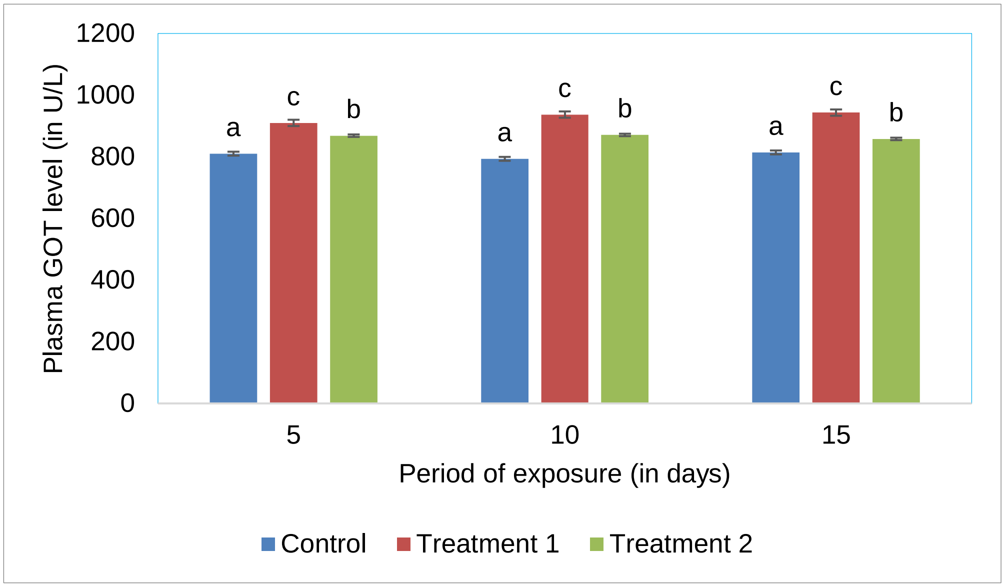

Figure 7. Fluctuations in the Glutamate Oxaloacetate Transaminase (GOT) activity in the plasma of *Catla catla* from sub-lethal concentration of copper sulphate alone (T1) and along with *Eichhornia crassipes* (T2)

Glutamate Pyruvate Transaminase (GPT) activity in the plasma of *Catla catla* subjected to sub-lethal concentrations of copper and treated for copper toxicity, along with *Eichhornia crassipes* as shown in Figure 8, showed decreased GPT activity in treatment-1. Treatment 2 showed a minimal change compared to the control group. This clearly indicates *Eichhornia crassipes* mitigates the toxicity-induced organ damage in fish. GPT activity was reduced in the liver of common carp (*Cyprinus carpio*) when subjected to heavy metals like cadmium, lead, nickel and chromium. This decreased activity of GPT indicates a disturbance in cell organelles. The reduction in GPT activity observed in treatment-1 may be due to copper buildup in the liver, which subsequently leads to the death of hepatocytes. A reduction in GPT level under chronic exposure suggests that the detoxification process might prove ineffective in preventing the copper sulphate action on the system. This decreased activity indicates an alteration in the structural integrity of cell organelles. Similar decrease in the activity of GPT in *Tilapia zilli* and *Boleopthalmus dussumier*, is reported respectively, contaminated with lead and toxicants (Lavanya et al., 2011). In this study, treatment-1 showed decreased GPT activity, which may be due to structural disturbances and the integrity of cell organelles, as suggested by the other studies. GPT activity in treatment-2 was almost close to that of control fish, and this may be due to minimal organ damage because of phytoextraction of Cu^{2+} ions by water hyacinth. Rapid translocation and redistribution of Cu^{2+}ions from liver to kidney causes minimal damage to the hepatocytes. As a result, fish can maintain regular GPT activity, similar to that of control fish.

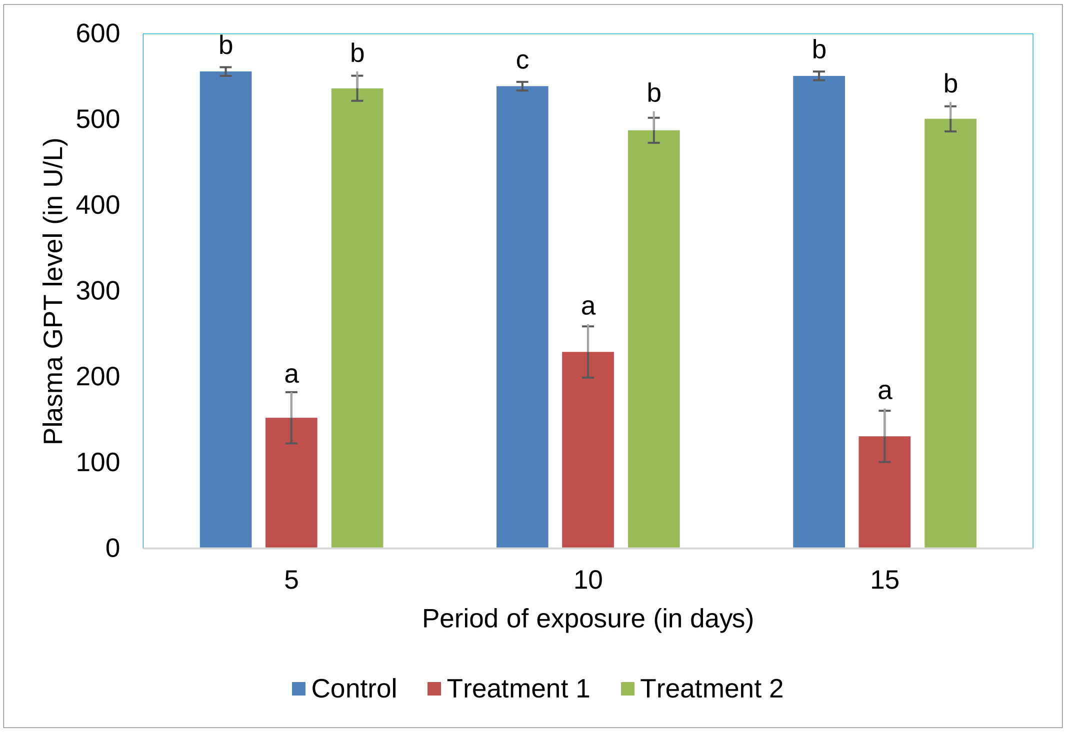

Figure 8. Distortion in the Glutamate Pyruvate Transaminase (GPT) activity in the plasma of *Catla catla* from sub-lethal concentration of copper sulphate alone (T1) and along with *Eichhornia crassipes* (T2)

Conclusion

The findings of this investigation indicated that the eco-friendly and economic removal of copper by water hyacinth resulted in lesser bioaccumulation of copper and mitigated the copper-induced stress response in fish. Water hyacinth (*Eichhornia crassipes*) can be efficiently used as a biosorbent for the removal of copper. Periodic removal of water hyacinth after a week of exposure, combined with the use of new plants, can effectively remove a significant amount of copper from industrial effluents. This eco-friendly phytoremediation technique of heavy metal removal from industrial effluents before they are released into the environment helps protect the environment and aquatic organisms. The study recommends incorporating water hyacinth-based phytoremediation, which can serve as an environmentally sustainable and preliminary treatment for copper-laden effluents. To ensure optimal copper removal efficiency, the water hyacinth plant must be harvested and replaced regularly, typically on a weekly basis. Safe management or proper disposal of biomass enriched with copper is also important to prevent secondary contamination. Further research should focus on large-scale implementation, evaluating its sustained effectiveness, and exploring its applicability to other heavy metals. In constructed wetland systems, integration of water hyacinth could enhance metal removal efficiency and facilitate wider utilisation in industrial settings. Additionally, continuous monitoring of water quality and aquatic biota is necessary to assess the ecological benefits resulting from treatment.

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