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The Phytoremediation Potential of *Lemna minor* L. on Heavy Metal Accumulation, Biochemical Responses, and Growth Tolerance

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ABSTRACT

Duckweed (*Lemna minor* L.) is an efficient aquatic plant that accumulates heavy metals. This study aimed to investigate the ability of duckweed. To accumulate selected heavy metals (Ni, Cu, Zn, Fe, Ba, Mn, and Co) at 5 mg/L concentrations of each metal, and to study the effects of metals on plant growth and biochemical changes in the plant. This study was conducted for 14 days to control the environmental conditions. The accumulation of metal inside the plant tissue, bioconcentration factor (BCF), and relative growth rate (RGR) were measured at the end of the experiments, while plant fresh and dry weight, total chlorophyll, and protein and proline contents were measured at the beginning and end of the experiments. The results showed that the highest metal accumulation in plant tissue and BCF were 4.112 and 0.822, respectively, in the iron treatment, while the lowest values were 2.334 and 0.466, respectively, in the Ba treatment. The highest reduction in fresh weight and growth percentage was in the Ba treatment, the highest reduction in chlorophyll content was in the Ba treatment, and the lowest protein content was in the Cu treatment. Therefore, the results indicate that Duckweed can tolerate selected heavy metals and can be used in phytoremediation technology.

HIGHLIGHTS

- *Lemna minor* demonstrates strong potential for phytoremediation of metal-contaminated water
- Duckweed efficiently removed iron while maintaining high growth performance
- Physiological stress indicators guide metal-specific remediation strategies

1. INTRODUCTION

Recently, rapid industrialization, population growth, and careless use of natural resources have produced wastewater with various organic and inorganic pollutants that find their way to natural surface water, which can reduce the quality of the natural water discharged into it (Al-Nabhan and Al-Abbawy, 2021; Fei et al., 2022; Garai et al., 2021).

Although some of these metals have beneficial effects as micro- and macronutrients, approximately 20 kinds of these metals have no beneficial effect on all organisms, and they are toxic at any concentration for both humans and the environment (Vardhan et al., 2019). The dangerous effects of heavy metals are not biologically degradable and accumulate in food, water, plants, and sediments before making their way up the food chain to humans (Fei et al., 2022; Goncharuk and Zagoskina, 2023). Before entering the receiving environment, large amounts of these components must be removed (Tatar et al., 2019).

Traditional heavy metal treatments include chemical precipitation, electrochemical processes, coagulants, flocculation, and ion exchange (Ubuza et al., 2020). A biological technology called Phytoremediation uses plants to remove pollutants and heavy metals from wastewater. It is an inexpensive and environmentally responsible way to dispose of trash while simultaneously saving money, protecting the environment, and improving aesthetics (Awa and Hadibarata, 2020; Jagetiya and Kumar, 2020).

Among aquatic plants, *L. minor* L., commonly known as duckweed, can remove pollutants from wastewater because of its high passive uptake through the root cell wall micropores (Ubuza et al., 2020).

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Deposited heavy metals increase efficiency, and pollutants are transported to plants via water. The potential of duckweed is constrained in biotic and immobile environments such as wetlands.

Despite such limitations, duckweeds are efficient phytoremediators and purifiers of contaminated water bodies because of their rapid reproduction and sensitivity to pollution (Zhou et al., 2023). Their accumulation enables the absorption of metals and metalloids into the cellular biomass. The monocotyledonous aquatic plant *L. minor* grows in both tropical and temperate climates. In marshes, slow-moving and nutrient-rich water grow well and form a mat on its surface. It grows more slowly at temperatures below 10°C and above 40°C than at the ideal growth temperature of 20-30°C (Al-Nabhan, 2022).

Several studies have confirmed duckweeds capacity for heavy metals accumulation. Thayaparan et al. (2015) studied the phytoremediation capacity of *L. minor* for chromium absorption in soil and water. The plant demonstrated a notable ability to absorb Cr at varying doses. Growth increased until the third day; however, the bioconcentration factor declined as the chromium in the solution grew. According to these findings, *L. minor* is an exceptional accumulator of chromium and may be used to remove chromium from streams, as traditional remediation techniques are insufficient.

Similarly, Neidoni et al. (2018) studied the ability of plants to accumulate and tolerate zinc, copper, and nickel; the quality of wastewater both before and after treatment; and the capability of plants to accumulate heavy metals from wastewater. According to Azeez (2021), *Utricularia vulgaris* and *Lemna minor* can significantly remove manganese (Mn), copper (Cu), and zinc (Zn) from wastewater with removal efficiencies over 68%. Thus, these plants demonstrate great potential for eco-friendly wastewater treatment and can be used as bioindicators of water quality.

Further studies have demonstrated the ability of some plant species to reduce pollutants from soil-contaminated variations. For example, *Polypogon monspeliensis* can reduce total petroleum hydrocarbon levels by up to 64.85% in soils contaminated with 20 g/kg over 30 d. The high translocation factor (TF>1) indicates good growth at lower contamination levels (Azeez et al., 2023).

Additionally, the bioaccumulation of heavy metals (Fe, Ni, Cr, Zn) in six species of aquatic plants from Al-Hawizeh Marsh was investigated by Al-Abbawy et al. (2021). According to this study, submerged plants, such as *Ceratophyllum demersum* and *Najas marina*, had the highest bioaccumulation capacities, particularly for Cr, which are above the limits allowed by the WHO. This suggests their potential role in the bioindication and remediation of water bodies polluted by heavy metals.

Research on *Lemna minor* phytoremediation of heavy metals has grown substantially, driven by increasing aquatic contamination from industrial, agricultural, and urban sources, and reinforced by its rapid growth, low cost, and demonstrated ability to accumulate metals such as cadmium, lead, chromium, copper, and iron (Ali et al., 2020; Islam et al., 2024). Recent studies have shown that uptake can be enhanced and that removal efficiency is coupled with complex physiological responses, including oxidative stress markers such as proline and chlorophyll degradation (Aslanzadeh et al., 2024; Mobin et al., 2025). Despite these advances, inconsistent findings regarding *L. minor's* accumulation capacity and tolerance affected by factors such as pH, metal speciation, and exposure regime reflect heterogeneous methodologies and incomplete integration of uptake with stress signaling (Shaari et al., 2022).

The present study attempted to fill this gap by exploring the joint accumulation of multiple Heavy Metals (Zn and Cu) in *L. minor*, as well as essential biochemical responses (antioxidant enzyme activities) and growth behavior under different contamination circumstances. Incidentally, the inclusion of growth parameters, metal-uptake efficiency, and physiological markers allows a better appreciation of this feature to compete amongst plants.

To our knowledge, this is one of the few studies that conducted research on the relations between accumulation as bioaccumulation indicators and biochemical responses in *L. minor* under the complex contamination environment of wastewater sludge leachate enriched with heavy metals in a long-term response.

These findings are anticipated to give a substantial contribution to the use of *L. minor* as a phytoremediator and even develop an actual solution for eco-friendly wastewater treatment systems in developing countries.

2. METHODOLOGY

2.1 Aquatic plant collection and acclimation

Lemna minor L. plants were collected from the Al-Hammar marsh in Basrah Province, south of Iraq.

The plants were transported to the laboratory in labeled plastic bags.

After washing several times with tap water to remove dust and dirt, it was placed in a 20-liter basin with tap water that was free of chlorine with the aerator provided and left to acclimatize to laboratory conditions for a week.

2.2 Heavy metals concentration preparation

Each metal was prepared at a concentration of 5 mg/L using 1,000 mg/L of a metal standard salt that had already been prepared. $FeSO_4{\cdot}7H_2O$; $CuSO_4{\cdot}5H_2O$; $ZnSO_4$; $BaCl_2$; $NiCl_2$; $CoSO_4$; $MnSO_4$ salt were used to prepare 1,000 mg/L of each metals according to equation bellow and the diluted to prepare desire concentration 5 mg/L of each metal)

$$\text{Weight (g)} = \frac{\frac{\text{mg}}{\text{L}}}{1{,}000} \times \frac{\text{molecular weight of salts}}{\text{atomic weight of metal} \times \text{no.of atoms}} \times \frac{\text{volume (mL)}}{1{,}000}$$

and

$$C_1 V_1 = C_2 V_2$$

Where; C_1: the concentration (1,000 mg/L prepared by salt); V_1: the volume (mL) needed to prepare 5 mg/L; C_2: desire concentration (5 mg/L) of each metal; V_2: volume of metal in each treatment (1 liter).

2.3 Experimental setup

Three duplicates of each metal were placed in 1.6-liter plastic aquariums, which were then filled with one liter of the previously prepared solution at a concentration of 5 mg/L. Hoagland nutrient solution (1%) was used. Three aquariums containing metal-free water were used as controls. About four grams of healthy plants were placed in each aquarium after washing with tap water and distilled water. The experiments were conducted for 14 days.

2.4 Parameters measurement

2.4.1 Plant fresh weight, dry weight and growth percentage

After washing the plant with tap water and distilled water to remove debris and get rid of excess water using filter papers, the fresh weight of the plant was measured using a scale and recorded in grams. The weight was measured in grams, as well as the dry weight, which was determined by drying the fresh plant at 70°C in an oven until the weight was constant. Growth percentage was calculated according to the equation described by Ubuza et al. (2020):

$$\text{Growth\%} = \frac{\text{final weight after exposureduration} - \text{Initial weight at 0 time}}{\text{Initial weight at 0 time}} \times 100$$

2.4.2 Heavy metal concentration in plant tissue and bioconcentration factor (BCF)

The ROPME (1982) method was used to evaluate the concentration of heavy metals in plant tissue using a Flame atomic absorption spectrophotometer (Buck 210BGP, Germany), and the value was recorded in mg/kg. BCF was calculated according to the equation described by Neidoni et al. (2018).

$$\text{BCF} = \frac{\text{metal concentrations in plant tissue (mg/kg)}}{\text{intital metal concentration in water (mg/L)}}$$

2.4.3 Total chlorophyll content

Total chlorophyll content was determined by the method described by Arnon (1949). Using 20 mL of 80% acetone, about 0.2 grams of fresh weight was extracted. A centrifuge (3,000 rpm) set at 5,000 rpm for ten minutes was used to separate the clear solution. Total chlorophyll content was measured at optical densities of 645 and 663 nm using a spectrophotometric technique (T80 UV/VIS, Instrument Ltd PG, UK). The concentrations are given in units of µg/g.

$$\text{Total chlorophyll } (\mu\text{g/g}) = (12.7 \times \text{OD } 663) + (16.8 \times \text{OD } 645)$$

Where; OD: optical density at 645 and 663 nm.

2.4.4 Protein content

The methods described by Bremner and Mulvaney (1982), Okwu et al. (2006), and AOAC (2000) were used to determine protein content. Multiplying the total nitrogen by 6.25 gives its representation.

2.4.5 Proline content

The proline content was determined using the procedure outlined by Troll and Lindsley (1955). Five milliliters of 95% ethanol were combined with 0.2 grams of dry, powdered plant for an hour, and then the mixture was centrifuged for five minutes at 5,000 revolutions per minute. Near drying, the translucent portion of the extract had disappeared. The mixture was supplemented with 2 mL of distilled water. Using spectroscopy at 520 nm, the proline content of 1 mL extract solution was determined in units using the proline standard curve.

2.4.6 Tolerance index rate percentage (TIR)

The tolerance index rate was calculated according to the equation mentioned in Wilkins (1978).

$$TIR\% = \frac{\text{dry weight of plant in metals treatment}}{\text{dry weight in control treatment}} \times 100$$

2.5 Statistical analysis

Data were analyzed using Statistical Package for Social Science (SPSS) version 23. Statistical analyses were conducted using one-way analysis of variance (ANOVA) to determine differences in plant fresh weight among treatments. Post hoc comparisons were performed using Tukey's honestly significant difference (HSD) test at a significance level of $p<0.05$.

3. RESULTS

3.1 Accumulation of heavy metals by plant tissues and bioconcentration factor (BCF)

At the end of the experiment, *L. minor* tissues exhibited varying capacities to accumulate the tested heavy metals (Figures 1 and 2), with statistically significant differences among treatments ($p<0.05$). Of the metals tested, the greatest levels of absorption were with Iron (Fe), averaging 4.112 mg/g, whereas the least was observed in Barium (Ba) 2.334 mg/g).

Correspondingly, the bioconcentration factor (BCF) values also differed widely among the treatments. The highest BCF was obtained from iron (Fe) at 0.822, indicating maximum uptake by the plant, whereas Barium (Ba) had a BCF value of 0.466, representing the least.

This demonstrates the capacity of duckweed to accumulate heavy metals in a specific manner, depending on the specific metal type and its interaction with the plant tissues.

3.2 Fresh weight and growth percentage

The results (Figures 3 and 4) showed that every metallic treatment lowered the fresh weight of *L. minor*, except for the Fe treatment, which promoted growth. The greatest reduction was observed in the fresh weight of plants treated with Barium (Ba), with an average fresh weight of 2.090 g and the lowest relative growth percentage at 0.505 g. Iron (Fe), in contrast, produced increased weight in plants, with values of fresh weight as high as 4.314 g and a relative growth percentage of 1.071, closely similar to the control values of 4.476 g and 1.104 g, respectively.

A statistically significant difference ($p\leq0.05$) was observed between the treatments and the control based on ANOVA, confirming the varied effects of metals on plant growth.

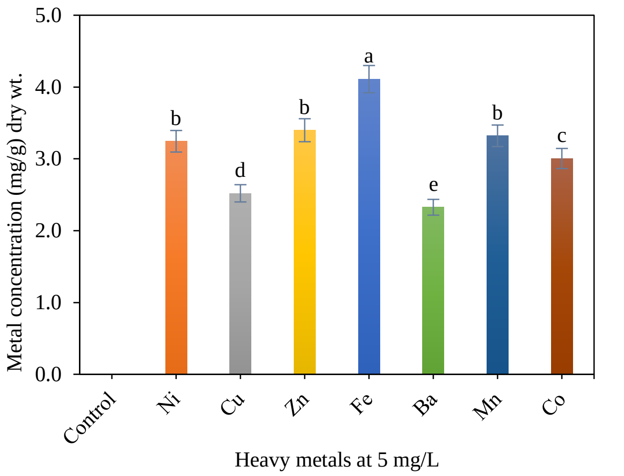

Figure 1. Metal concentration (mg/g dry weight) in plant tissues exposed to 5 mg/L of different heavy metals. Values represent mean±standard error (SE), n=5.

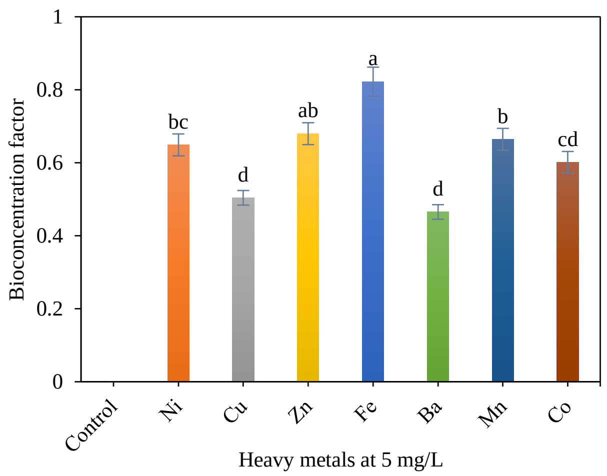

Figure 2. Bioconcentration factor (BCF) of metals in plant tissues exposed to 5 mg/L metal concentration. Values represent mean±standard error (SE), n=5.

3.3 Total chlorophyll content

As shown in Figure 5, the initial total chlorophyll content of *Lemna minor* was 4.552 µg/g. After 14 days of exposure, it was observed that the treatment influenced the chlorophyll content. For the iron (Fe) treatment, a slight increase was observed, with an average total chlorophyll content of 4.197 µg/g. In contrast, the control recorded the highest value of 4.941 µg/g. However, all other metal treatments reduced the total chlorophyll content compared with the control treatment. The most dramatic effect was observed in the Mn treatment (2.174 µg/g at 5 mg/L).

Statistical analysis (ANOVA) revealed significant differences between the treatments and their controls ($p \leq 0.05$). Therefore, heavy metals have different effects on chlorophyll biosynthesis and degradation.

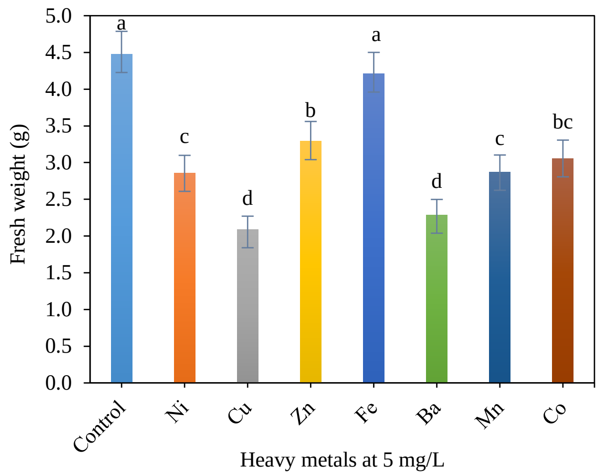

Figure 3. Effect of different heavy metals (5 mg/L) on plant fresh weight. Values represent mean±standard error (SE), n=5. Different letters above bars indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p<0.05$).

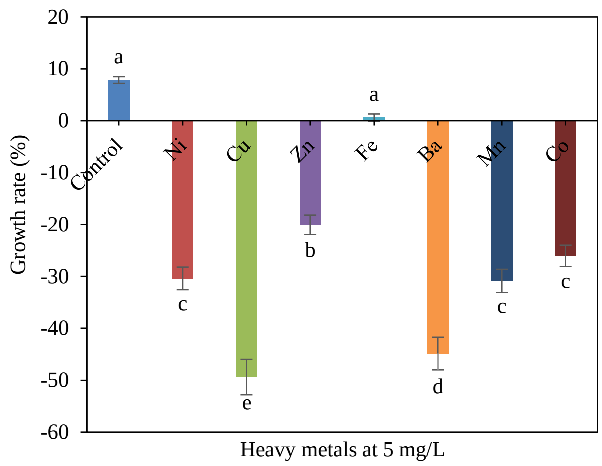

Figure 4. Percentage growth response of plants exposed to different heavy metals at 5 mg/L. Values represent mean±standard error (SE), calculated relative to the control (n=5).

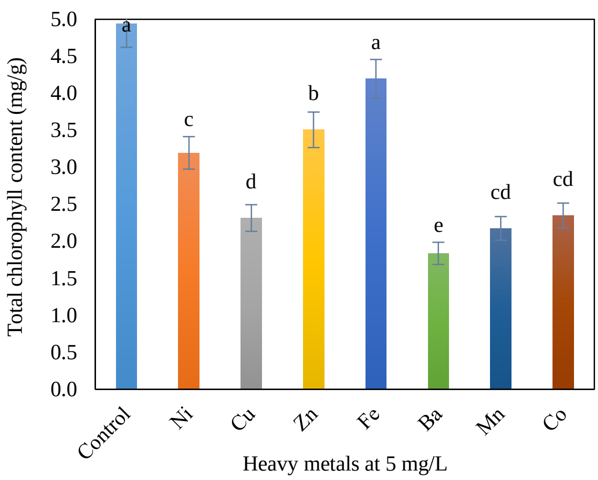

Figure 5. Effect of different heavy metals (5 mg/L) on total chlorophyll content of plants. Values represent mean±standard error (SE), n=5. Different letters above bars indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p<0.05$).

3.4 Protein content

The initial protein content of *L. minor* was 31.43%. After 14 days of exposure to heavy metals (Figure 6). All treatments showed a decline in the amount of protein with varying severities due to exposure to heavy metals after 14 days. The least reduced content was found in the iron (Fe) treatment, which had a protein content of 27.89%, indicating that Fe had only a minor negative effect on protein synthesis. About the copper (Cu) treatment, the most reduction occurred, and protein content decreased to 21.613%, indicating how toxic it was regarding copper's cellular activities. These data suggest that the level of protein reduction is directly related to metal toxicity. The differences between treatments and control with a significant p value ($p \leq 0.05$) in ANOVA statistical analysis confirmed significance in treatments and controls, emphasizing differences in the adverse effects of heavy metals on protein metabolism.

3.5 Proline content

Initially, *L. minor* had a proline concentration of 31.33 mg/g. After 14 d of exposure to heavy metals (Figure 7), all treatments increased the levels of proline, illustrating a physiological response to heavy metal-induced stress. The highest observed concentration of proline was found in copper (Cu) treatment, which had an average of 79.403 mg/g, corresponding to another strong evidence of physiological stress due to Cu toxicity. In contrast, the lowest proline concentration was recorded for the treatment control, with an average value of 34.25 mg/g since significant stressors were not available.

Heavy metals themselves substantially increase the concentration levels of proline, indicating that it may serve as a potential osmoprotectant as well as an antioxidant, thereby preventing oxidative stress from being imparted by exposure to metals. Statistically, ANOVA showed there are significant differences between treatments and control ($p \leq 0.05$), showing how heavy metals influence proline accumulation differently.

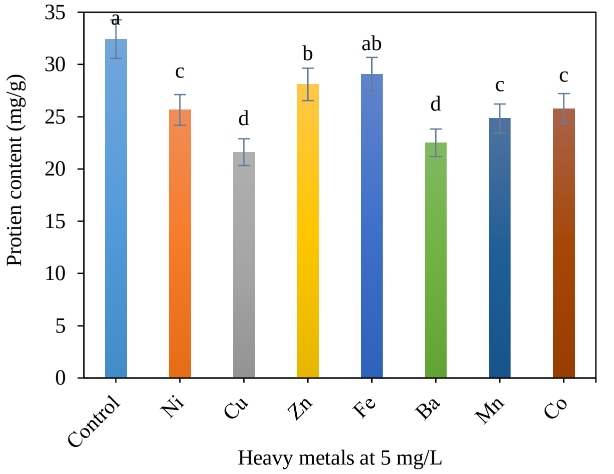

Figure 6. Effect of different heavy metals (5 mg/L) on protein content of plants. Values represent mean±standard error (SE), n=5. Different letters above bars indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p<0.05$).

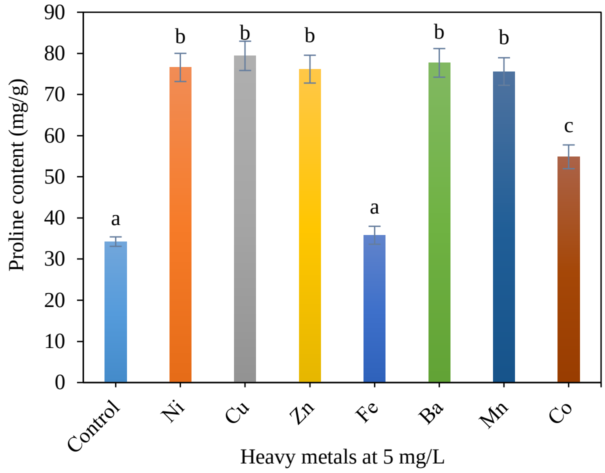

Figure 7. Effect of different heavy metals (5 mg/L) on proline content of plants. Values represent mean±standard error (SE), n=5. Different letters above bars indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p<0.05$).

3.6 Tolerance index rate (TIR %)

Figure 8 showed that at different heavy metal treatment concentrations of treatment concentration at 5 mg/L, after 14 days of exposure to the heavy metals, the tolerance index rate (TIR) of *L. minor* L. varied widely among metals. The highest value of TIR was obtained in treatment with iron (Fe), which was 97.57%, indicating a high tolerance of the plant against iron. The lowest TIR was recorded for the manganese (Mn) treatment, which indicated that the TIR value was 56.79%, showing a considerable reduction in plant tolerance under manganese exposure.

As featured by these results, the type of heavy metal affects the tolerance of *L. minor* L. Consistent with that, some metals (as Fe) were found to be less detrimental to the growth and physiology of plants than others (as Mn). ANOVA statistical analysis somehow proved the significant difference of treatments, because tolerances varied among metals ($p \leq 0.05$).

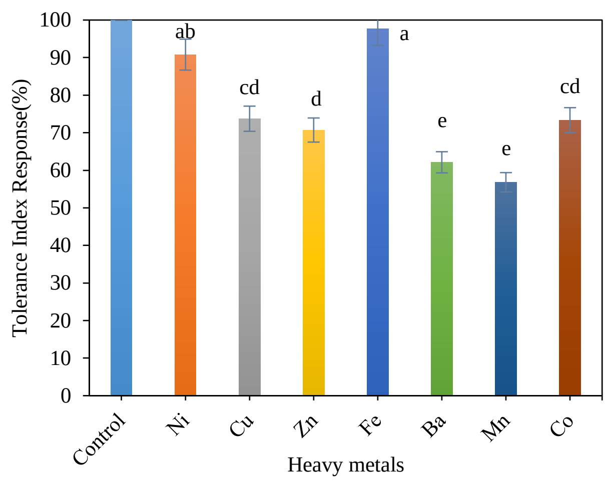

Figure 8. Tolerance index response (TIR %) of plants exposed to different heavy metals at 5 mg/L. Values represent mean±standard error (SE), n=5. Different letters above bars indicate significant differences according to one-way ANOVA followed by Tukey's HSD test ($p<0.05$).

4. DISCUSSION

The accumulation of metals inside the plant tissue is the process by which the plant absorbs the metal from the medium, such as water or soil. Metal accumulation in the plant increased with the duration of exposure. Moreover, the Bioconcentration factor (BCF) is an effective tool for assessing the plant's ability to accumulate heavy metals (Aziz et al., 2023). The BCF value depends on the initial metal concentration. Lower metal concentrations exert reduced toxicological effects on plants, thereby enhancing their capacity for metal uptake; however, plant growth responses vary according to metal type, varying between metals at the same concentration. Lower metal concentrations exert reduced toxicological effects on plants, thereby enhancing their capacity to absorb heavy metals from the surrounding environment. This effect may either stimulate or inhibit plant growth, depending on the metal involved. This effect may either enhance or inhibit plant growth, depending on the metal involved, even at identical concentrations. In this study, the accumulation of Iron slightly enhanced plant growth as a micronutrient, and other metals accumulated with different values depending on their toxicity on plants, such as Cu, Ba, and Mn. For instance, Du et al. (2020) reported a BCF of 0.75 for iron in *Brassica juncea*, which aligns with our findings that iron promotes growth. The first sign of plant toxicity is a reduction in plant growth. This may be caused by changes in the structure and function of the cell membrane brought on by ROS, which can disrupt the movement of nutrients and water between the cell and its surroundings, disrupt the balance of nutrients, and inhibit the activity of enzymes, all of which can impair physiological processes. Additionally, the efficiency of photosynthesis is decreased when the chlorophyll content is damaged.

Plant development is reduced when exposed to heavy metals (HM), mostly because the photosynthetic system is harmed (Hu et al., 2023). For example, Du et al. (2020) found that varying concentrations of Zn significantly impacted plant growth, biomass output, and root length. According to recent studies, *Lemna minor* L. plants are under stress since their biomass production is slightly decreased or increased depending on metal, and growth decrease is a general response to heavy metal stress. In the present study, *Lemna minor* exhibited enhanced growth under iron exposure, likely because iron functions as an essential micronutrient. In contrast, exposure to other metals resulted in growth inhibition to varying degrees. Bortoloti and Baron (2022) also noted significant reductions in biomass in plants exposed to heavy metals, with reductions of up to 40% in some species.

Plant leaves suffer chlorosis because of heavy metal stress, which lowers the amount of photosynthetic pigment in the thylakoid membrane. This is caused by three primary factors inhibiting the anabolism of chlorophyll, lowering the activities of antioxidant enzymes, and competing for active sites with Mg^{2+} in chlorophyll molecules (Sperdouli et al., 2022). Kapoor et al. (2019) reported a reduction in total chlorophyll content under Cd stress, with values dropping from 5.0 µg/g to 3.0 µg/g at 6.0 mmol/L, which is consistent with our findings where chlorophyll content decreased significantly in treatments with metals like manganese. According to An et al. (2019), Cd stress inhibits the synthesis of chlorophyll in cotton leaves, and when the concentration of metal increases, the amounts of total chlorophyll, chlorophyll a, and chlorophyll b drop dramatically. This outcome concurred with Khan et al. (2024) and Al-Nabhan (2022).

The reduction in protein content varied among treatments and is likely attributable to ribosomal interference, which disrupts protein synthesis. Additionally, metal-induced reactive oxygen species (ROS) generation can modify sulfhydryl (–SH) groups in proteins, altering their structure and reducing protein synthesis (Khan et al., 2024). Proline is an essential amino acid involved in plant stress responses, particularly under heavy metal toxicity. The concentration of proline frequently rises as a defense mechanism in plants exposed to heavy metals. Under stress, proline is produced from glutamate. When the proline is bound by metal, its free concentration and toxicity are reduced during the recovery from metal stress. This happens because osmoregulation keeps the cellular osmotic balance under severe metal stress. Proline breaks down to produce energy and the carbon skeleton needed for metabolic processes (Khan et al., 2024). These results consist with Al-Nabhan (2023), who reported proline levels increasing from 30 mg/g to 75 mg/g under heavy metal stress. This suggests that proline plays a crucial role in osmoregulation and oxidative stress mitigation.

An indicator of a plant's capacity to thrive in the presence of a specific metal concentration is the tolerance index (Bianconi et al., 2013). In this study, the plant tolerates Ni better than others. The decrease in tolerance index rate was attributed to a decrease in dry weight in the studies compared to the control. This is in agreement with Umebese and Motajo (2008), who found that TIR values for various metals varied significantly, with nickel showing better tolerance than lead.

5. CONCLUSION

The present results show that *Lemna minor* selectively accumulates and tolerates at least seven heavy metals, indicating its potential for phytoextraction. Interestingly, the roots accumulated iron preferentially compared to plants with a bio-concentration factor and exhibited minimal growth inhibition. This indicates that *L. minor* can assimilate iron while maintaining its vitality.

However, manganese tended to be less tolerant, probably inferring great physiological distress, while copper led to proline, showing osmotic and oxidative stress in plants. These results indicate that *L. minor* can be used for the phytoremediation of iron-laden waters. Nevertheless, in environments with high levels of phytotoxic metals, such as manganese or copper, supplementation or complex treatments may be needed to attenuate potential biomass decrease.

The physiological and biochemical indicators used in this study offer useful insights into the evaluation of plant stress during phytoremediation. Further studies are necessary to confirm these trends under field conditions, and considerations should also be made for the safe disposal and treatment of metal-loaded biomass. Overall, our results highlight the potential use of *L. minor* in environmental remediation applications, with a special emphasis on heavy metal pollution as an important ecological burden.

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AUTHOR CONTRIBUTIONS

Conceptualization, D.A., E.M., and N.M.; Visualization, Z.F.

DECLARATION OF CONFLICT OF INTEREST

The authors declare no conflict of interest.

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