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Evaluating the Phytoremediation Potential of *Lemna minor* for Iron-Contaminated Municipal Wastewater: A Case Study from Cuttack, India

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Abstract*:Municipal wastewater often contains high concentrations of contaminants, including heavy metals, which may cause serious adverse effects on aquatic ecosystems and human health. Phytoremediation using aquatic macrophytes has emerged as an environment friendly and cost-effective approach for removing metals from contaminated water. The present study investigated the phytoremediation potential of *Lemna minor* in municipal wastewater containing Iron (Fe) concentrations of 0.625, 0.937, and 1.25 mg L^{-1} over a period of 15 days. The physicochemical parameters of the wastewater were analysed, and the phytoremediation performance of *Lemna minor* was evaluated using relative growth rate (RGR), Fe accumulation (Fe Acc), bioconcentration factor (BCF), and Fe removal efficiency (RE). The results showed that *Lemna minor* effectively reduced Fe concentrations across all treatments, with the highest removal efficiency of 70.31% observed at 1.25 mg L^{-1} after 15 days of exposure. Fe accumulation in plant tissues increased with both wastewater concentration and exposure duration, reaching a maximum value of 1.462 mg g^{-1}. High BCF values >1 were recorded throughout the experiment, demonstrating the strong Fe accumulation capacity of *Lemna minor*, while positive relative growth rates indicated that *Lemna minor* was able to maintain growth under the Fe concentrations examined during the 15-day exposure period. Statistical analyses, including two-way ANOVA, Pearson correlation, and simple linear regression, demonstrated that Fe concentration significantly influenced Fe accumulation, RGR, and removal efficiency, highlighting the strong phytoremediation potential of *Lemna minor.* The findings suggest that *Lemna minor* can efficiently remove and accumulate Fe while maintaining healthy growth, highlighting its potential as a sustainable and low-cost phytoremediation species for the treatment of Fe-contaminated municipal wastewater.

Keywords: Wastewater, *Lemna minor,* Heavy Metal, Phytoremediation, Metal accumulation

I. INTRODUCTION

Advancing technology, industrialization, urbanization, and population growth are some of the main factors that contribute to the generation of toxic contaminants in the biosphere. These contaminants may comprise various toxic metals, hazardous chemicals, non-degradable substances and other hazardous materials, which may cause severe contamination of water bodies (Shrestha et al., 2021; Verma et al.,2022). Among the micropollutants detected in wastewater, including microplastics, heavy metals, pharmaceuticals, endocrine-disrupting chemicals (EDCs), and related contaminants, these pollutants are major contributors to the cumulative genotoxic burden on ecosystems (Gupta et al., 2025; Verma et al, 2022). These contaminants may be released from several sources, such as household sewage, industrial discharges, agricultural waste, plastics, and inadequate waste management practices (Chai et al., 2021; Mustafa & Hassan, 2024). Water pollution caused by untreated municipal wastewater has become a significant environmental issue in rapidly urbanizing areas with rapid population growth. According to the European Investment Bank, approximately 380 billion cubic meters of municipal wastewater are generated each year globally, and this volume is predicted to

increase by 24% by 2030 and 51% by 2050 (Qadir et al., 2020). In developing countries like India, wastewater treatment facilities and reuse practices are very limited, resulting in a large volume of untreated wastewater being discharged into nearby water bodies (Breitenmoseret al., 2022). The Central Pollution Control Board states that urban areas in India generated approximately 72,368 MLD (Million Litres per Day) of wastewater in year 2020-2021, of which only 31,841 MLD was treated. These untreated effluents often contain organic and inorganic pollutants, suspended solids, heavy metals, and nutrients (Miri et al., 2022). These contaminants can severely impact water quality because ecological disturbances contribute to eutrophication and algal blooms and increase the risk of contaminant bioaccumulation in food chains (Khan et al., 2022; AL-Huqail et al., 2022).

Cuttack has a population of 26,24,470 (Census of India, 2011), and most of its sewage is reportedly discharged untreated intolocal rivers. (Das & Acharya, 2003; Achary et al., 2016). The disposal of large amounts of wastewater into rivers not only degrades the water quality but also reduces their self-purification capacity (Achary et al. 2016). Hence, it is crucial to treat wastewater before it is released into rivers. Various conventional techniques are used for treating wastewater, such as precipitation, electrodialysis, reverse osmosis, solvent extraction, and adsorption (Altowayti et al., 2022; Gupta et al., 2012; De Gisi et al., 2016). However, these techniques can be expensive, may not remove all contaminants completely, require energy and maintenance, and can produce secondary pollutants, thereby limiting their use in various urban and semi-urban areas (Saha et al., 2016). In contrast to these methods, phytoremediation has emerged as an eco-friendly, cost-effective, and efficient method for removing toxic contaminants from wastewater (Kristanti&Hadibarata, 2023; Jeevananthan et al., 2019; Bayuo et al., 2024). It utilizes green plants that can absorb, degrade, and neutralize harmful contaminants in wastewater, thus making it a sustainable strategy for remediating contaminated water bodies (Kafle et al., 2022; Khan et al., 2022). Researchers have been using this technique for many years, and it continues to gain attention due to its easy maintenance, low operational costs, sustainability, and reduced sludge production (Kristanti&Hadibarata, 2023). Several species of aquatic macrophytes, including *Eichhornia crassipes*, *Pistia stratiotes*, *Nelumbo nucifera*, *Azolla pinnata*, and *Lemna minor*, have been utilized for removing toxic pollutants from wastewater (Saha et al., 2016; Nahar & Hoque, 2021; Saad et al., 2022; Sabli et al., 2019).

Lemna minor (Duckweed) is an invasive, fast-growing aquatic macrophyte that forms a dense layer on nutrient-rich freshwater or brackish water (Ekperusi et al., 2019). There are 38 species of Duckweed categorized into five aquatic genera. They are small, flattened structures with one or a few leaves, called fronds, and a single root or rootlet, whichfloats on or slightly beneath the surface of water bodies (Ekperusi et al., 2019). It is known for its cost-effective wastewater treatment capabilities and efficient removal of nutrients and heavy metals from wastewater (Zhou et al. 2023). Almost all duckweed genera have been reported to have the potential to remove toxic contaminants from various metal-contaminated industrial and municipal wastewaters, thus making duckweed an efficient plant to be used in toxicity testing (Ali et al., 2015). Besides metal removal from wastewater, duckweed also has the potential to improve various water-quality parameters, such as BOD, COD, total nitrogen, total phosphorus, total dissolved solids, electrical conductivity, and total suspended solids (Ali et al. 2015). Due to its rapid growth rate, ability to tolerate a wide range of contaminants, as well as ease of cultivation, *Lemna minor*has been identified as an effective option for phytoremediation of different types of wastewaters (Ziegler et al., 2014; Ekperusi et al., 2019; Zhou et al., 2023). Iron-related contamination of water is a concern in many countries, and excessive iron exposure has been associated with various health concerns, including Parkinson's, cardiovascular disease, hyperkeratosis diabetes mellitus, pigmentation changes, Huntington's, Alzheimer's disease, and respiratory, renal, hepatic, and neurological disorders (Hossain et al., 2023). High iron content in waterbodies may also contribute to bad odour, unpleasant taste, red coloration, and stains in laundry and plumbing fixtures (Khatri et al., 2017). Iron is the fourth most abundant element on Earth and is naturally found in water in diverse forms. Iron may enter water bodies through geogenic sources or through the discharge of domestic waste and industrial effluents (Khatri et al., 2017). Due to the adverse effects of excess iron ions on aquatic ecosystems, the World Health Organization has recommended a maximum permissible limit of 0.3 mg L^{-1} of iron in drinking water (WHO, 2003; Khatri et al., 2017). Different methods have been developed for the removal of excess iron from contaminated water, where the phytoremediation technique is suggested as one of the simplest, cost-effective, and efficient methods to treat iron-contaminated wastewater (Hanafiah et al., 2020). In the biological filtration process, the use of aquatic micro and macrophytes to remove harmful metal ions from surface water has been found to be an effective approach for decontaminating aquatic environments (Vardanyan & Ingole, 2005; Zhou et al., 2023).

Although *Lemna minor* has been widely studied for the phytoremediation of various heavy metals, most studies have primarily focused on toxic metals in industrial effluents under controlled laboratory conditions using relatively high metal concentrations. Limited studies have investigated the phytoremediation potential of Fe in municipal wastewater discharged into river systems, particularly in Odisha. Therefore, the present study evaluates the performance of *Lemna minor* under actual municipal wastewater conditions, which is essential for understanding its practical applicability in sustainable wastewater remediation.

II. MATERIAL AND METHODS

Plant Collection

The study was carried out using *Lemna minor* for phytoremediation of contaminants in wastewater. The plant

was collected from a local pond in Balasore, Odisha (India, N 21.545811°, E 86.815261°). After collection, the plants were washed with tap water to remove adhering organisms and dirt and were acclimatized for 10 days in a tub with normal tap water to adapt to the experimental conditions. Following acclimatization, healthy plants were selected for the experiment (Figure 1).



Figure 1: Experimental states of *Lemna minor* used for phytoremediation studies: (A) collection from a natural pond habitat, (B) acclimatization prior to experimental exposure and (C) growth of *Lemna minor* after 15 days of exposure to iron-contaminated municipal wastewater

Sampling of Water

The municipal wastewater sample was collected from the discharge point where wastewater enters the Kathajodi River near Khannagar in Cuttack, Odisha (N 20.444392°, E 85.890722°). The collected water sample was transported to the laboratory for water-quality analysis and experimental setup.

The experiment was conducted using a series of plastic containers, each containing 2 litres of contaminated municipal wastewater. The wastewater had an iron (Fe) concentration of 1.25 mg L^{-1}. Healthy plants, approximately 10g each, were placed in three wastewater concentrations (100%, 75%, and 50%), which were prepared by diluting the wastewater with tap water. The initial iron concentrations in the three treatments corresponded to 1.25 mg L^{-1}, 0.937 mg L^{-1}, and 0.625 mg L^{-1}. All experimental setups were maintained in the culture laboratory at a constant room temperature of 24 °C, with fluorescent lamps providing a 12 h light/12 h dark photoperiod. *Lemna minor*plants were harvested separately on days 0, 5, 10, and 15. After harvesting, the plants were evaluated for their relative growth rate (RGR), Fe accumulation, Fe removal efficiency (RE), Fe uptake rate, and bioconcentration factor (BCF).

Sample Preparation and Heavy Metal Analysis

The accumulation of Fe in plant and water samples was measured. A 50 mL water sample was digested with 5 mL of concentrated nitric acid and heated until its volume was reduced to approximately 10-20 mL. The digested sample was then diluted with distilled water to a final volume of 50 mL. The plants were heated at 120 °C for 24 h in a hot-air oven to obtain dry biomass (Giri & Patel, 2011). Subsequently, 0.5 g of plant material was digested with 10 mL of concentrated nitric acid and 4 mL of perchloric acid. The digest was then diluted to 50 mL with distilled water and filtered through Whatman filter paper No. 44 into a volumetric flask. The Fe concentration was determined using atomic absorption spectroscopy (AAS). The Fe concentrations in the water and plant samples were expressed as mg L^{-1} and mg g^{-1} (dry weight basis), respectively.

Removal Efficiency

The Fe removal efficiency (RE) was calculated using the following equation (Giri & Patel, 2011):

$$\%\ \text{Fe removal efficiency} = \frac{C_i - C_f}{C_i} \times 100$$

Where, C_i represents the initial Fe concentration and C_f represents the final Fe concentration in the solution (mg L^{-1}).

Relative Growth Rate

The relative growth rate (RGR) of *Lemna minor* was calculated after the experiment using the following equation (Saha et al., 2016; Bianconi et al., 2013):

$$\text{Relative Growth Rate } (gg^{-1}d^{-1}) = \frac{\ln W_2 - \ln W_1}{t_2 - t_1}$$

Where W_1 represents theinitial dry weight, W_2 represents the final dry weight of *Lemna minor* biomass, and $(t_2 - t_1)$ represents the duration of the experiment (days).

Metal Accumulation

The Fe accumulation (Fe Acc) in *Lemna minor* was calculated using the following equation (Al-Baldawi et al., 2022; Ismail et al., 2019)

$$\text{Fe accumulation } (mgg^{-1}) = \frac{C_{Fe} \times V_E}{M_p}$$

Where C_{Fe}represents the total concentration of Fe in the plant, V_E represents the volume of the extracted water sample, and M_P represents the mass of the plant.

Bio-concentration Factor

The bio-concentration factor (BCF) indicates the capacity of plants to accumulate metals in relation to the concentration of metals in the external solution (Saha et al., 2016). The BCF of the plants is classified into four categories: no phytoaccumulation (BCF<0.01), low phytoaccumulation (0.01–0.1), moderate phytoaccumulation (0.1–1) and high phytoaccumulation (1–10) (Al-Baldawi et al., 2022).

$$\text{BCF} = \frac{\text{Metal concentration in plant tissue } (mgg^{-1})}{\text{Initial metal concentration in external solution } (mgL^{-1})}$$

Statistical Analysis

All experiments were conducted in triplicate, and results are presented as mean ± standard deviation (SD). Data tabulation and preliminary graphs were prepared using Microsoft Excel (Microsoft Corporation, USA). Statistical analyses were

performed in R version 4.6.0 (R Core Team, Vienna, Austria), including two-way analysis of variance (ANOVA), followed by Tukey's HSD test for pairwise mean comparisons and Pearson correlation analysis to assess relationships among phytoremediation parameters. Simple linear regression analysis was performed to evaluate the relationships between Fe concentration and phytoremediation parameters, including Fe accumulation, relative growth rate, and removal efficiency. Correlation matrices and heatmaps were generated using the ggplot2, corrplot, and Hmisc packages. Statistical significance was accepted at $p < 0.05$.

III. Results and Discussion

Evaluation of Physicochemical Parameters in the Study Area

From the experimental results, the moderate pH and elevated EC and TDS values of the wastewater indicate the presence of dissolved ions, inorganic salts, and minerals, which may be due to the discharge of residential and domestic sewage (Bhutiani et al., 2018). High BOD and COD values indicate substantial concentrations of biodegradable and non-biodegradable organic matter, suggesting a high organic load and a potential for oxygen depletion. Untreated waste discharged into the river may be responsible for the elevated nitrate levels, which can promote algal growth and increase BOD levels (Verma et al., 2023). Total alkalinity was attributed to the presence of bicarbonates, as carbonates were found in negligible or variable amounts (Bhattarai et al., 2010). The iron concentration in Khannagar municipal wastewater exceeded the maximum permissible limits recommended by the WHO, BIS, and US EPA. Agricultural practices, weathering of rocks, and anthropogenic inputs are the main factors contributing to elevated iron concentrations (Parwin et al., 2024). Overall, several parameters, including turbidity, EC, TDS, BOD, COD, and iron, were found to be elevated, indicating a relatively high level of contamination in the wastewater.

TABLE 1
Mean ± Standard Deviation values of Physicochemical parameters of Municipal wastewater

Parameters	Municipal Wastewater	Permissible limit (CPCB/BIS)	Permissible limit (WHO/US EPA)
pH	7.9 ± 0.1	5.5-9.0[a]	6.5-8.5[d]
EC (µs/cm)	786.3 ± 44.1	-	400[c]
Turbidity (NTU)	131.3 ±17.2	5[b]	5[c]
TDS (mgL^{-1})	592.3 ± 72.4	500[b]	500[d]
Alkalinity (mgL^{-1})	172 ±8.7	200[b]	200[c]
Hardness (mgL^{-1})	168 ±19.8	200[b]	500[c]
Calcium (mgL^{-1})	48.2 ± 5.9	75[b]	200[c]
Magnesium (mgL^{-1})	11.8 ±1.5	30[b]	50[c]
BOD_5 (mgL^{-1})	168.3 ±16.4	30[a]	20[c]
COD (mgL^{-1})	523.6 ± 43.6	250[a]	1000[c]
Iron (mgL^{-1})	1.25 ± 0.03	0.3[b]	0.3[d]

[#]Values in bold exceed the given permissible level; Maximum permissible limit: Source [a]CPCB; [b]BIS, WHO[c], US EPA[d]

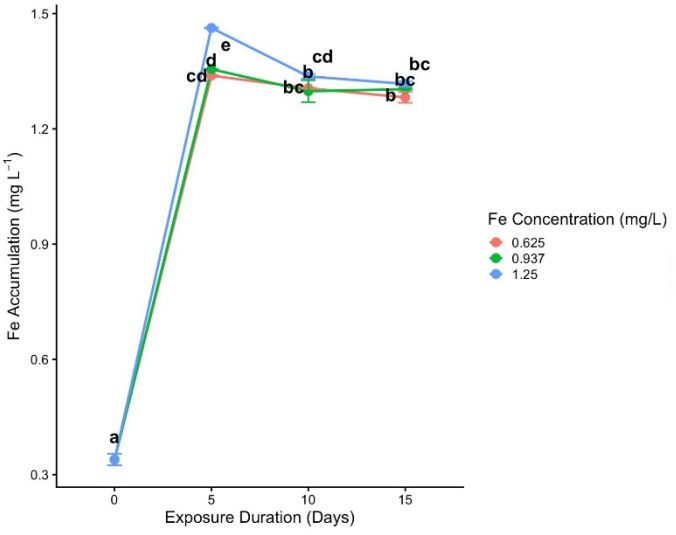


Figure 2.Interaction Plot showing the effect of Fe concentration and exposure duration on Fe accumulation in (*Lemna minor*). Values represent mean ± SD (n = 3). Different letters indicate significant differences among treatment combinations according to Tukey's HSD test ($p < 0.05$)

Figure 2 shows the rapid increase in Fe accumulation during the first 5 days, indicating efficient uptake and accumulation of Fe by *Lemna minor*. The maximum accumulation observed in the 100 % treatment (1.25 mg/L) suggests that increased Fe availability may have enhanced Fe uptake. However, the slight decline in accumulation observed after Day 5 may indicate saturation of uptake sites, physiological regulation of metal transport, or redistribution of accumulated Fe within plant tissues (Aryal, M, 2024; Rahman et al., 2024). Metal uptake was highest during the initial exposure phase and subsequently stabilized, potentially due to adaptive physiological responses. The ability of plants to accumulate and tolerate metals is attributed to several interconnected mechanisms, including adsorption at root and cell wall interfaces, chelation by

secreted organic ligands, uptake through membrane transport systems, and detoxification via vacuolar compartmentalization and binding to phytochelatins, metallothioneins, and organic acids (Zhang et al., 2025). The ability of *Lemna minor* to accumulate substantial amounts of Fe within a short period highlights its potential suitability for the phytoremediation of Fe-contaminated wastewater.

Pearson Correlation Analysis

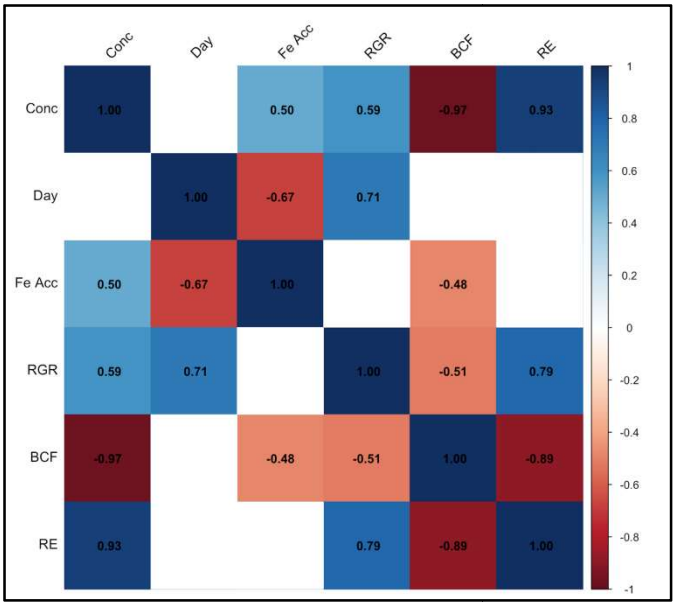


Figure 3. Pearson correlation heatmap showing the relationships among Fe concentration (Conc), exposure duration (Day), Fe accumulation (Fe Acc), relative growth rate (RGR), bioconcentration factor (BCF), and removal efficiency (RE) of *Lemna minor* during iron phytoremediation. Color intensity represents the strength of correlation, while blank cells indicate non-significant correlations ($p > 0.05$)

Pearson's correlation analysis revealed significant relationships among Fe concentration (Conc), exposure duration (Day), Fe accumulation (Fe Acc), relative growth rate (RGR), bioconcentration factor (BCF), and removal efficiency (RE) in *Lemna minor (Figure 3).* Fe concentration exhibited a strong positive correlation with RE ($r = 0.93$, $p < 0.001$), RGR ($r = 0.59$, $p < 0.01$), and Fe Acc ($r = 0.50$, $p < 0.01$), indicating that increasing iron concentration enhanced plant growth, metal uptake, and overall remediation performance. In contrast, BCF showed a very strong negative correlation with Fe concentration ($r = -0.97$, $p < 0.001$) and RE ($r = -0.89$, $p < 0.001$), suggesting a decline in accumulation efficiency at elevated iron levels despite improved removal efficiency. Exposure duration (Day) was positively correlated with RGR ($r = 0.71$, $p < 0.001$) but negatively correlated with Fe Acc ($r = -0.67$, $p < 0.001$), indicating that prolonged exposure promoted plant growth while reducing tissue iron concentration, possibly due to biomass dilution effects. Reduced biomass production under elevated metal exposure may be linked to impaired antioxidative enzyme activities, resulting in ROS accumulation and lipid peroxidation that adversely affect cellular metabolism and growth (Rahman et al., 2024). RGR was strongly positively associated with RE ($r = 0.79$, $p < 0.001$), highlighting the importance of plant growth in enhancing phytoremediation efficiency. However, Fe Acc showed no significant correlation with either RGR ($r = -0.09$, $p > 0.05$) or RE ($r = 0.26$, $p > 0.05$), suggesting that iron accumulation alone was not a reliable predictor of remediation performance. Overall, the results demonstrate that growth-related parameters and exposure conditions play a more critical role in determining phytoremediation efficiency than metal accumulation alone.

Relationship Between Fe Concentration and Relative Growth Rate of *Lemna minor*

Linear regression analysis (Figure 4) showed a significant positive relationship between Fe concentration and the relative growth rate (RGR) of *Lemna minor* ($R^2 = 0.35$, $p = 0.0012$), indicating that higher Fe concentrations were associated with higher RGR. The increase in RGR with increasing Fe concentration suggests that *Lemna minor* was able to maintain growth under the examined Fe concentrations, which may be due to the essential role of Fe in various physiological processes associated with plant growth. As an essential micronutrient, Fe plays a key role in photosynthesis and enzymatic processes, which may have contributed to the observed growth of *Lemna minor.* The moderate R^2 value indicates that factors other than Fe concentration, particularly exposure duration, nutrient status, metabolism, and stress responses, also influenced RGR. Gomes et al. (2026) reported that the associated microbial communities of *Lemna minor* enhance contaminant tolerance by reducing oxidative stress and stimulating detoxification pathways involved in xenobiotic metabolism.

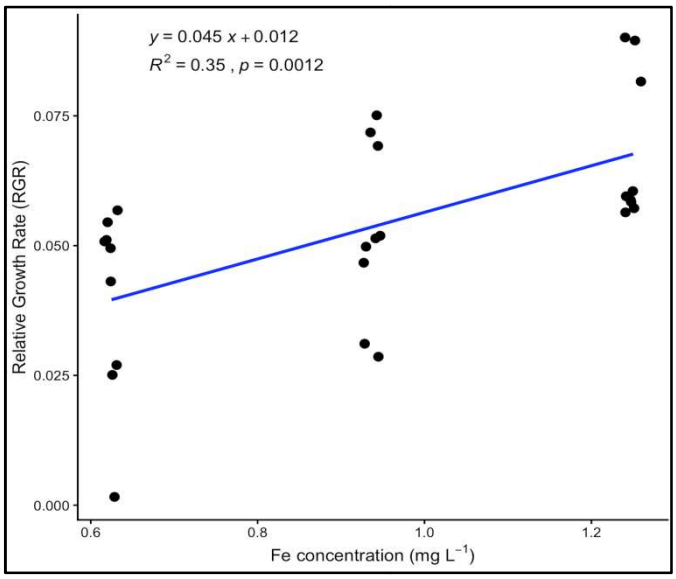


Figure 4. Linear regression curve between Fe concentration and relative growth rate (RGR) of *Lemna minor*

Relationship Between Fe Concentration and Fe Accumulation in *Lemna minor*

A significant positive relationship was observed between Fe concentration and Fe accumulation (R^2 = 0.253, p = 0.008) (Figure 5), indicating enhanced iron uptake with increasing Fe availability. Fe accumulation increased significantly with increasing Fe concentration (R^2 = 0.253, p = 0.008), which shows a strong capacity of *Lemna minor* to absorb and retain Fe from wastewater. The positive relationship suggests concentration-dependent metal uptake, likely driven by increased Fe availability in the wastewater. However, the moderate coefficient of determination (R^2)suggests that Fe accumulation was influenced not only by Fe concentration but also by factors such as exposure duration and physiological differences in metal uptake and sequestration (Bokhari et al., 2015). Nevertheless, the significant positive relationship confirms the ability of *Lemna minor* to accumulate Fe under increasing metal availability.

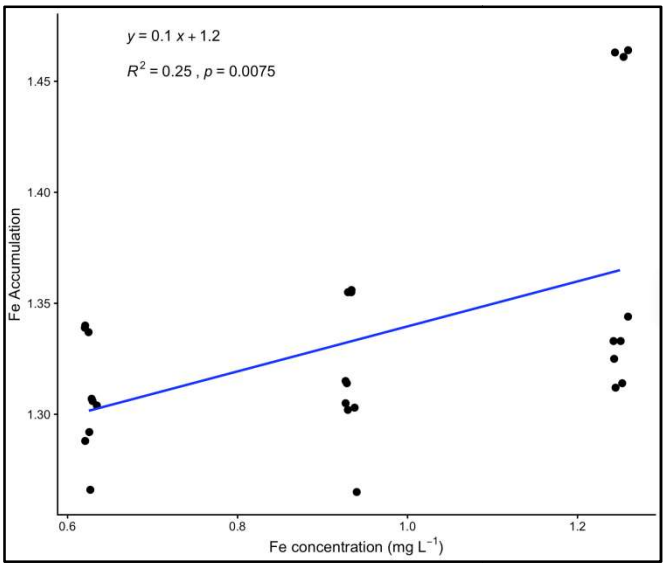


Figure 5. Linear regression curve between Fe concentration and Fe accumulation in *Lemna minor*

Relationship Between Fe Concentration and Removal Efficiency of *Lemna minor*

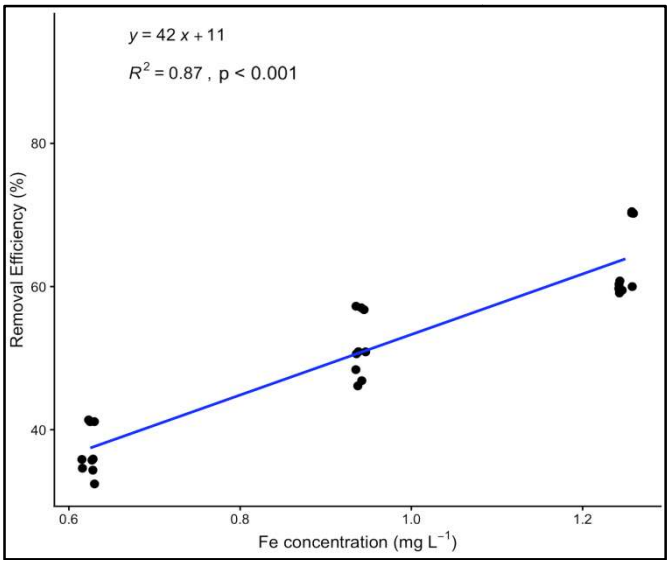


Figure 6. Linear regression curve between Fe concentration and removal efficiency of *Lemna minor*

Linear regression analysis (Figure 6) revealed a strong positive relationship between Fe concentration and removal efficiency (R^2 = 0.87, p < 0.001). Removal efficiency increased significantly with increasing Fe concentration, indicating the high phytoremediation potential of *Lemna minor*. The strong coefficient of determination suggests that Fe concentration was the primary factor influencing Fe removal from the wastewater. As Fe concentration increased, *Lemna minor* absorbed and accumulated more Fe, resulting in higher removal efficiency, making it an efficient phytoremediation plant species.

Relative Growth Rate

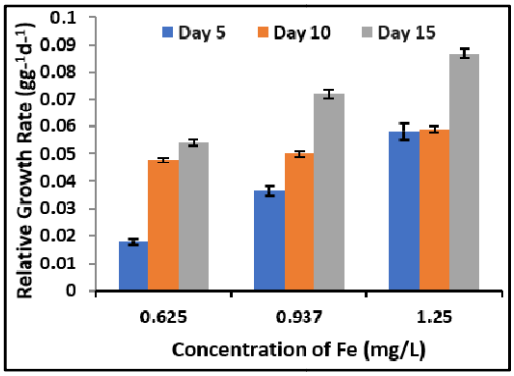


Figure 7. The Relative Growth Rate (RGR) of *Lemna minor* at different concentrations and exposure times

Relative Growth is regarded as the most widely used technique to evaluate plant growth (Saha et al., 2016). The relative growth of *Lemna minor* in the present study was studied after exposing it to different Fe concentrations at different intervals, and the results are presented in Figure 7. Lower relative growth rates were observed during the initial exposure period, while comparatively higher growth rates were observed after prolonged exposure. The highest value recorded in the study was 0.087 $gg^{-1}d^{-1}$ on day 15 at 1.25 mg L^{-1}, while the lowest value was observed at 0.625 mg L^{-1} on day 5, which is 0.0179 $gg^{-1}d^{-1}$. This indicates that the highest concentration of Fe supported active plant growth, which may be mainly due to the nutrient-rich municipal wastewater as well as the role of Fe as an essential micronutrient. Iron is required by plants for physiological processes like photosynthesis and respiration and is essential for growth and development (Chandwani&Amaresan, 2024; Ismail et al., 2019; Daud et al., 2018). Similar observations were also studied by Leblebici& Aksoy (2010), where nutrient-enriched municipal wastewater increases the growth rate of *Lemna minor* when exposed to heavy metals.

Removal Efficiency

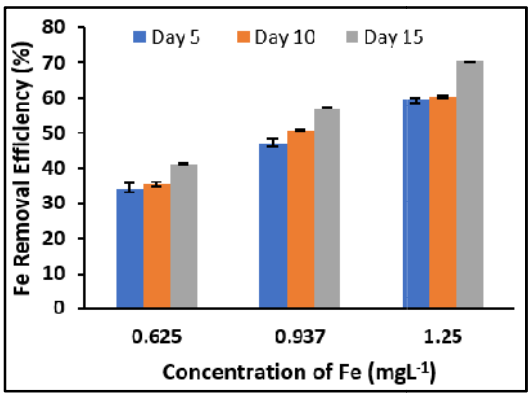


Figure 8. Removal efficiency of *Lemna minor* at different concentrations of Fe and exposure times

The efficiency of Fe removal (Removal Efficiency) for *Lemna minor* at different concentrations was determined (Figure 8). The Fe removal efficiency of *Lemna minor* increased over time across all wastewater concentrations. The highest removal efficiency was recorded at 1.25 mg L^{-1}, increasing from 59.43% to 70.31% on day 15. Similarly, Fe removal efficiency increased from 47.14% to 57.01% at a concentration of 0.937 mg L^{1} and from 34.22% to 41.20% at a concentration of 0.625 mg L^{-1} over the experimental period. The increase in iron removal efficiency over time suggests that *Lemna minor* continuously takes up and accumulates Fe, which may be attributed to efficient absorption of dissolved Fe ions by root cells as Fe concentrations in the wastewater increase. The uptake of Fe in *Lemna minor* is facilitated by transport systems and intercellular binding sites in the plant's roots, after which some of the metal accumulates in the roots, while the remainder is transported to the aerial parts of the plant (Aryal, 2024; Rahman et al., 2024). A similar observation was also reported by Bokhari et al. (2015), where *Lemna minor* showed enhanced removal at the highest exposure time.

Metal Accumulation in Plant Tissue

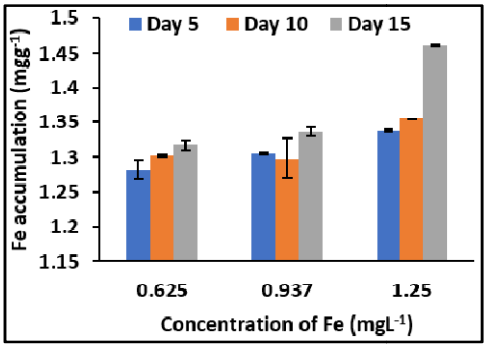


Figure 9. Metal accumulation in plant tissue at different concentration and exposure times

Aquatic plants are recognized for their ability to accumulate metals from contaminated water and substrates (Bokhari et al., 2015). Accumulation of Fe by *Lemna minor* at different concentrations with different time intervals is presented in Figure 9. The accumulation of Fe in *Lemna minor* increased with increasing Fe concentration at longer exposure times, which shows continuous absorption and accumulation of Fe in plant tissues. The highest Fe accumulation was observed at 1.25 mg L^{-1}. concentration after 15 days of exposure period (1.462 mg g^{-1}, whereas comparatively lower concentrations were seen at 0.937 mg L^{-1} and 0.625 mg L^{-1}. The increased Fe accumulation in *Lemna minor* is due to the transport of Fe into the plant root by diffusion due to higher external Fe concentrations (Ismail et al., 2019). After the Fe ion is transported inside the plant, it is then precipitated and becomes immobilized in the symplastic and apoplastic compartments (Aryal, 2024). Similar observations for the uptake of metals were also observed for different aquatic plants
like *Spirodelapolyrhiza* and *Scirpusgrossus* by Leblebici& Aksoy (2010) and Ismail et al. (2019), respectively.

Bioconcentration Factor

TheBioconcentration Factor (BCF) for *Lemna minor* under different concentrations is shown in Figure 10. BCF is an important parameter for assessing the capacity of aquatic plants to accumulate metals from contaminated water (Daud et al., 2018; Al-Baldawi et al., 2022). Metal concentration in water is one of the major factors determining the efficiency of metal uptake. In general, an increase in metal concentration in water may increase metal accumulation in plants while decreasing the BCF (Saha et al., 2016). In this study, the lowest BCF was observed at 1.25 mg L^{-1} on day 5, and the highest was observed at 0.625 mg L^{-1} on Day 15. BCF decreased with increasing Fe concentration at each exposure interval. This inverse relationship suggests that although Fe accumulation increased at higher Fe concentrations, the relative accumulation of Fe with respect to its external concentration declined, possibly due to saturation of metal transport sites and regulatory mechanisms controlling excess Fe uptake. The BCF of Fe increased over the exposure period, reaching its maximum at 15 days (Saha et al., 2016). All BCF values observed in this study were >1, indicating a high Fe accumulation capacity of *Lemna minor*(Ismail et al., 2019). The consistently high BCF values demonstrate the ability of *Lemna minor* to effectively concentrate Fe in its tissues while maintaining physiological activity, suggesting efficient Fe sequestration, storage, and homeostatic regulation.

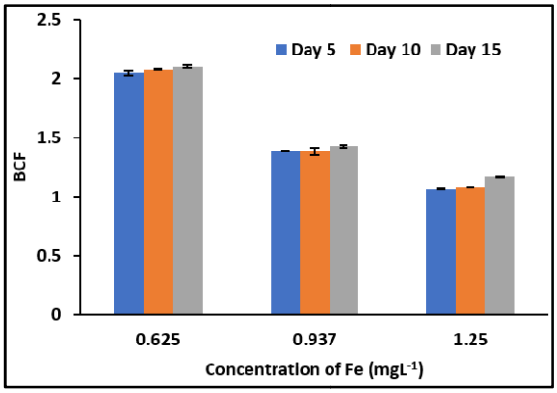


Figure 10. The effects of Fe on the Bioconcentration Factor (BCF) of *Lemna minor* at different concentrations and exposure times

IV. Conclusion

Lemna minor was found to be effective in reducing Fe concentration in Khannagar municipal wastewater over a treatment period of 15 days. The plant effectively reduced Fe concentration in all wastewater treatments, with the highest removal efficiency of 70.31% recorded at a concentration of 1.25 mg L^{-1} after 15 days of exposure. Fe accumulation in plant tissue increased progressively over time, reaching a maximum value of 1.462 mg g^{-1}, indicating the ability of *Lemna minor* to absorb and accumulate Fe from wastewater.*Lemna minor* maintained positive growth while effectively accumulating and removing Fe across the tested concentrations; however, the maximum Fe tolerance threshold could not be established. Future studies should evaluate longer exposure periods and a broader range of Fe concentrations to further assess its physiological responses and phytoremediation potential. A positive relative growth rate was also observed throughout the experiment, indicating that the wastewater conditions did not adversely affect plant growth**.** This may be attributed to the presence of Fe as an essential micronutrient required for various physiological processes in plants. However, the present study does not establish that Fe directly promoted the growth of *Lemna minor*.The significant relationships among Fe concentration, plant growth, and removal efficiency demonstrate the potential of *Lemna minor* as a cost-effective and environmentally sustainable phytoremediation species for Fe-contaminated aquatic environments.

The ability of *Lemna minor* to sustain growth while efficiently accumulating and removing Fe highlights its potential as an effective phytoremediation species for Fe-contaminated wastewater. Municipal authorities could consider integrating *Lemna minor*-based phytoremediation systems into existing wastewater management frameworks as a sustainable, low-cost, and environmentally friendly approach to reducing metal pollution and improving urban water quality. Future studies should investigate the long-term performance of Lemna minor under prolonged Fe exposure by evaluating temporal changes in Fe removal, Fe accumulation, plant growth, and physiological and biochemical responses, as well as assessing its feasibility for large-scale application in sustainable wastewater management.

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