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Hybrid phytoremediation-coagulation system for efficient removal of iron and manganese from groundwater

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ABSTRACT

Groundwater contamination by iron (Fe) and manganese (Mn) remains a persistent challenge in rural areas, where access to advanced treatment technologies is limited. Existing phytoremediation or natural coagulant methods are typically applied separately, resulting in slow removal rates and inconsistent efficiency. This study introduces a hybrid phyto-coagulation system that integrates *Eichhornia crassipes* with *Moringa oleifera* seed extract to accelerate metal removal through simultaneous biosorption, bio-oxidation, and protein-induced flocculation. A factorial Completely Randomized Block Design was used to evaluate combinations of plant biomass (0–30 leaves), moringa doses (0–100 mg/L), and treatment duration (0–16 days). Groundwater from nine wells in Margorejo Village, Indonesia, showed Fe and Mn levels exceeding WHO and national standards. Unlike conventional single-method approaches, the hybrid system demonstrated a synergistic removal pathway, achieving near-complete metal elimination. The optimal configuration (30 leaves + 100 mg/L, 16 days) reduced Fe to 0.082 mg/L (97.77%) and Mn to undetectable levels (98.71%). This study provides the first experimental evidence that coupling phytoremediation with natural coagulation significantly enhances metal removal kinetics, offering a scalable, low-cost treatment model for decentralized groundwater purification.

Keywords: phytoremediation, coagulation, iron, manganese, well water.

INTRODUCTION

Water is a fundamental natural resource that supports human survival, domestic activities, and industrial development (Zikirov and Zikirov, 2022). Adequate and safe water availability is widely recognized as an indicator of community well-being and environmental sustainability. However, increasing anthropogenic pressures, particularly industrial expansion and improper waste disposal, have accelerated the degradation of water bodies across many regions. Major Indonesian rivers such as the Cisadane and Angke–Pesanggrahan have been reported to exhibit pollutant levels exceeding permissible limits for domestic and recreational use (Makarim, 2024; Prayoga et al., 2023).

In rural and peri-urban areas of Indonesia, groundwater obtained from dug wells remains the primary water source for drinking, cooking, and other household uses (Utami et al., 2024). Yet this reliance is often accompanied by water quality issues, particularly turbidity, metallic odor, and discoloration, common indicators of heavy metal contamination. Among the most persistent contaminants are iron (Fe) and manganese (Mn), which, despite being essential micronutrients, can pose serious chronic health risks when present at elevated concentrations.

Such conditions were identified in Margorejo village, Tempel district, Sleman, Yogyakarta, where EIA (AMDAL) assessments conducted in accordance with Ministry of Environment and Forestry Indonesia Regulation No. 38/2019 revealed continued community dependence on well water. Laboratory analyses showed Fe levels of 2.7548 mg/L and Mn levels of 0.2376 mg/L, both exceeding Indonesian clean water standards (SNI

6989.84-2019) of 0.2 mg/L for Fe and 0.1 mg/L for Mn. Beyond aesthetic concerns, prolonged exposure to elevated Fe and Mn has been associated with organ damage and neurological disorders (Aind and Mukherjee, 2024a; Ngibad, 2023).

Conventional treatment approaches, including chemical oxidation, ion exchange, filtration, and synthetic resin systems, are effective but generally costly, infrastructure-intensive, and dependent on chemical inputs that may generate secondary environmental impacts (Alghamdi et al., 2024). These limitations underscore the need for low-cost, accessible, and environmentally friendly alternatives suitable for rural settings.

Nature-based remediation offers a promising pathway. *Eichhornia crassipes* exhibits high biosorption capacity through functional hydroxyl, carboxyl, and amine groups capable of binding heavy metals (Wiryono et al., 2021). Meanwhile, *Moringa oleifera* seeds contain cationic proteins that function as natural coagulants, promoting the aggregation and sedimentation of metal ions (Stanikina, 2023). When applied together, these agents have the potential to generate a synergistic mechanism that integrates biosorption, flocculation, and biological oxidation, thereby enhancing Fe and Mn removal efficiency beyond what either method can achieve independently.

Based on this rationale, the present study investigates the effectiveness of a combined phytoremediation–natural coagulation approach using *Eichhornia crassipes* and *Moringa oleifera* seed extract for Fe and Mn removal in contaminated well water. The study evaluates the influence of biomass dosage, coagulant concentration, and treatment duration, while assessing the feasibility of this hybrid system as a low-cost, practical, and sustainable solution for groundwater treatment in communities lacking advanced water purification infrastructure. *Eichhornia crassipes* was selected due to its high metal accumulation capacity, rapid biomass production, and widespread availability in tropical regions, particularly Indonesia. Its extensive root system contains abundant hydroxyl and carboxyl functional groups that facilitate effective biosorption of dissolved metal ions. *Moringa oleifera* seeds were chosen as a natural coagulant because they contain cationic proteins capable of destabilizing negatively charged colloids and metal complexes, promoting rapid flocculation and sedimentation. Compared with other aquatic macrophytes such as *Pistia stratiotes* and *Lemna minor*, water hyacinth demonstrates superior biomass yield and adsorption capacity, while moringa seeds offer a low-cost, locally accessible alternative to synthetic coagulants, making both species suitable for decentralized water treatment systems.

METHODS

Research location

This study was conducted in Margorejo village, Tempel aubdistrict, Sleman regency, Special Region of Yogyakarta, comprising 14 hamlets. The community relies heavily on surface water and groundwater accessed through dug and bore wells. Two hamlets, Tegal Domban and Jlegongan, were selected due to their dense and evenly distributed groundwater use. These locations were also chosen because preliminary surveys indicated recurring aesthetic and chemical water quality issues reported by residents, suggesting potential heavy metal contamination linked to local geological conditions and nearby anthropogenic activities. Nine wells were sampled: two from Jlegongan 1, three from Jlegongan 2, two from Tegal Domban 1, and two from Tegal Domban 2. A composite sampling method was applied to obtain a representative assessment of local groundwater quality. The geographic coordinates of the study area are 7°39’29.53” S and 110°19’37.61” E.

Experimental design

A factorial experiment was conducted using a randomized complete block design (RCBD). The first factor involved the combination of *Eichhornia crassipes* biomass (stems and leaves) and *Moringa oleifera* seed extract dosage as a natural coagulant. The second factor was treatment duration. This design enabled the evaluation of single and interactive effects of both factors on the reduction of Fe and Mn concentrations in well water (Table 1).

In this study, *Eichhornia crassipes* and *Moringa oleifera* seed extract were deliberately applied simultaneously as an integrated hybrid treatment system, rather than as independent remediation methods. This experimental design was intended to evaluate the synergistic interaction between phytoremediation and natural coagulation mechanisms in reducing Fe and Mn concentrations in

Table 1. Treatment for experimental

Name	Treatment
K0	No water hyacinth (0 stems + leaves) and no moringa seed coagulant (0 mg/L)
K10	10 stems + leaves of water hyacinth with 25 mg/L moringa seed coagulant
K20	20 stems + leaves of water hyacinth with 50 mg/L moringa seed coagulant
K30	30 stems + leaves of water hyacinth with 100 mg/L moringa seed coagulant
W0	Treatment duration of 0 days
W4	Treatment duration of 4 days
W8	Treatment duration of 8 days
W12	Treatment duration of 12 days
W16	Treatment duration of 16 days

groundwater. Accordingly, treatment levels K10, K20, and K30 represent hybrid configurations, in which increasing plant biomass is coupled with proportional doses of moringa seed extract. This approach enables assessment of both individual factor effects and their interaction under varying treatment durations, providing a comprehensive evaluation of hybrid system performance.

Although the highest removal efficiencies were achieved at a 16-day treatment duration, substantial reductions in Fe and Mn (>80%) were also observed at shorter durations of 8–12 days. This indicates that the hybrid system remains practical for decentralized and household-scale applications, particularly in passive treatment settings where extended retention time is feasible. Importantly, the system operates without electricity or synthetic chemicals, which offsets the longer treatment duration and enhances its applicability in rural or resource-limited communities. In rural household contexts where water is stored prior to use, retention times of 8–16 days are operationally feasible without additional infrastructure.

Sample preparation

A total of 40 experimental units were prepared, consisting of 20 treatment combinations, each replicated twice. The full matrix of treatment interactions is presented in the experimental design Table 2.

Materials and equipment

The equipment used included reactor tanks, a blender, a 100-mesh sieve, an oven, a balance,

Table 2. Experimental design table

Treatment interaction variations (K)	Treatment time (W)	Test (R)	
		1	2
K0	W0	R1K0W0	R2K0W0
	W1	R1K0W1	R2K0W1
	W2	R1K0W2	R2K0W2
	W3	R1K0W3	R2K0W3
	W4	R1K0W4	R2K0W4
K1	W0	R1K1W0	R2K1W0
	W1	R1K1W1	R2K1W1
	W2	R1K1W2	R2K1W2
	W3	R1K1W3	R2K1W3
	W4	R1K1W4	R2K1W4
K2	W0	R1K2W0	R2K2W0
	W1	R1K2W1	R2K2W1
	W2	R1K2W2	R2K2W2
	W3	R1K2W3	R2K2W3
	W4	R1K2W4	R2K2W4
K3	W0	R1K3W0	R2K3W0
	W1	R1K3W1	R2K3W1
	W2	R1K3W2	R2K3W2
	W3	R1K3W3	R2K3W3
	W4	R1K3W4	R2K3W4

a DR200 HACH spectrophotometer, beakers, a jar-test apparatus, a magnetic stirrer, and analytical reagents (Ferrover and Sodium Periodate) for Fe and Mn testing. Additional instruments included a pH meter and thermometer for supporting measurements.

Data collection and analysis

After treatment, water samples were analyzed in the laboratory following SNI 6989.84-2019 for Fe and Mn concentrations, SNI 06-6989.11-2019 for pH, and SNI 06-6989.23-2005 for temperature. Statistical analyses were performed to assess the effects of treatment factors and their interactions using ANOVA, followed by Duncan's Multiple Range Test (DMRT) for mean comparison. All analyses were conducted using SPSS version 27.0 and Microsoft Excel 2019.

RESULT AND DISCUSSION

Water conditions before treatment

Baseline water quality analysis was conducted to determine the contamination level of well water in Margorejo Village, Tempel, Sleman, prior to treatment. Table 3 summarizes the composite values of key physical and chemical parameters compared with the requirements of the 2017 WHO Guidelines and the Regulation of the Minister of Health of the Republic of Indonesia No. 2/2023.

The analysis followed national testing standards (SNI 6989.84:2019), which require accredited laboratory procedures for assessing clean water quality. Atomic absorption spectrophotometry was used to quantify Fe and Mn levels. The results showed that Fe (2.7548 mg/L) and Mn (0.2376 mg/L) significantly exceeded the permissible limits of 0.2 mg/L and 0.1 mg/L, respectively, indicating that the groundwater did not meet clean water standards. Elevated Fe and Mn concentrations commonly manifest as discoloration, metallic taste, and sediment deposition on household appliances. Beyond aesthetic issues, excessive exposure, particularly to Mn, poses substantial health concerns. The WHO (2017) reports that chronic manganese ingestion can impair neurological development in children. Ziegler et al. (2022) further emphasize that exposure to Mn at concentrations as low as 100 ppb may result in cognitive deficits, reduced IQ, and symptoms resembling Parkinsonism.

Several natural and anthropogenic factors can contribute to high Fe and Mn levels in groundwater. Soil geochemistry, local lithology, and prolonged interaction between groundwater and mineral-rich rock formations are common pathways. In addition, well construction materials and corrosion of metal pipes may intensify metal release. Aind and Mukherjee (2024) noted that even under neutral pH conditions, such as the pH 7.1 recorded in this study, dissolved oxygen and oxidizing agents can mobilize Fe and Mn ions from corroded pipes. Temperature conditions also influence metal mobility and oxidation kinetics. Although the recorded temperature of 24 °C is typical for tropical regions, it may still enhance the conversion of Fe^{2+} to Fe^{3+}, forming iron oxide precipitates that affect water clarity. Al-Abadleh et al. (2022) observed that such oxidation pathways can significantly deteriorate visual water quality. Furthermore, well design and maintenance practices play a critical role; wells lacking filtration layers or those that are infrequently cleaned are more vulnerable to metal accumulation, particularly at the bottom layers where sediment interacts directly with incoming groundwater (Hemakumar et al., 2024). Taken together, these findings establish a clear need for an effective, low-cost treatment approach capable of reducing Fe and Mn concentrations to safe levels before groundwater is used for household purposes.

Post-treatment water conditions

Initial post-treatment water samples (W0) were taken five hours after application of the

Table 3. Preliminary analysis results on water

No.	Parameter	Unit	Test Result	Parameter Value*
1.	Temperature	°C	24.0	Air Temperature ± 3
2.	pH	-	7.1	6.5–8.5
3.	Iron (Fe)	mg/L	2.7548	0.2
4.	Manganese (Mn)	mg/L	0.2376	0.1

Note: Laboratory analysis (primary data), valuation according to SNI 6989.84:2019.

bioremediation agent, when the initial interaction with the metal content begins. This phase affected the observed parameters, namely temperature, pH, Fe, and Mn, as seen in the differences between the control (K0W0) and treatments with varying doses (K10W0, K20W0, K30W0). Further analysis was conducted to assess the dynamics of water quality changes based on the combination of bioremediation agent dosage and treatment duration.

pH level

ANOVA results showed that water pH was not significantly influenced by the individual effects of the bioremediation agents or treatment duration ($p > 0.05$). However, the interaction between both factors exhibited a highly significant effect ($p = 0.002$; $p < 0.01$), indicating that pH variation was primarily governed by the combined influence of phytoremediation and natural coagulation (Table 4).

DMRT analysis revealed that three treatments, K0W4, K0W12, and K20W8, differed significantly from the control. Among these, K0W12 produced the highest pH value (8.463), approximately 2.58% higher than the control (8.25), although not significantly different from several other treatments (K0W16, K10W16, K20W4, K30W4, K30W16, K10W0, and K10W12). Conversely, K20W0 recorded the lowest pH value (8.150), about 1.21% lower than the control, but remained statistically similar to K0W4 and K20W8. These results suggest that K0W12 had a relatively greater ability to increase pH compared to treatments involving only one active component (Figure 1).

Table 4. pH level test

Indicator	Result
Combination of bioremediation agents	0.061
Treatment time	0.071
Combination of bioremediation agents and treatment time	0.002

Interestingly, the highest pH value in K0W12 occurred without the addition of either water hyacinth or moringa extract. This effect may be associated with natural processes occurring in static water systems, where microbial decomposition generates carbonate (CO_3^{2-}) and bicarbonate (HCO_3^-), which neutralize H^+ ions and increase pH. Ambaye et al. (2022) noted that organic matter breakdown in aquatic environments can produce alkaline substances, while Tampubolon et al. (2023) observed that amino acids and humic acids released from planktonic organisms may modify pH depending on concentration and chemical composition.

pH conditions play an important role in heavy metal removal. Nonh et al. (2023) demonstrated that adsorption efficiency of Pb using *Corbula trigona* shell powder exceeded 99% at pH 4.5, highlighting how slight shifts in pH can enhance or reduce metal uptake. Biological processes may further influence pH, particularly through plant root activity. Shi et al. (2024) reported that root exudates regulate nutrient uptake and shape microbial communities around the rhizosphere,

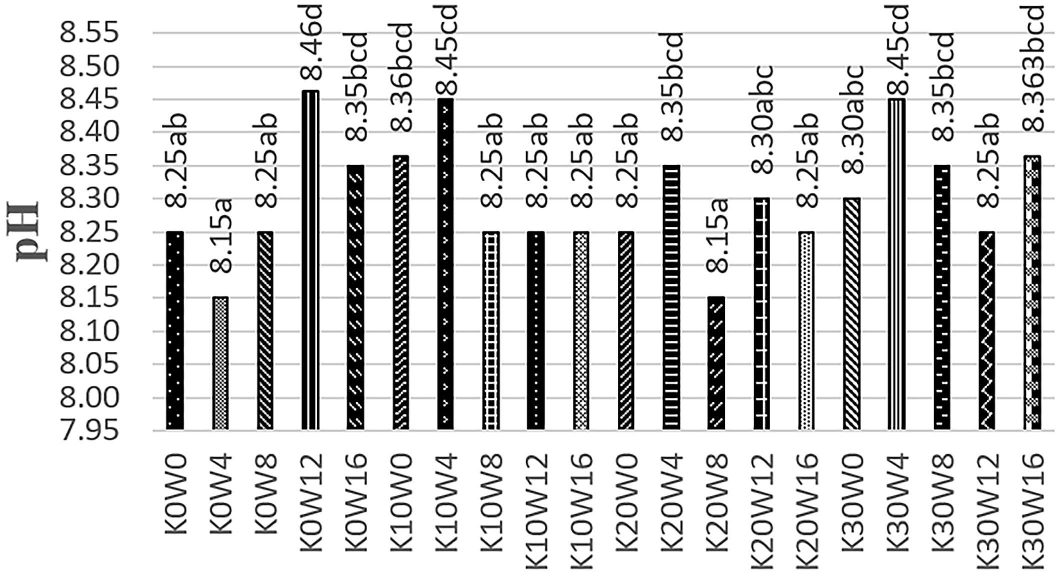

Figure 1. Effect of treatment on pH

thereby supporting pollutant degradation. Similarly, Syamlal and Sayantan (2024) emphasized that these exudates help maintain ecological balance while facilitating contaminant removal.

The reductions in pH observed in K0W4 and K20W8 likely stem from different mechanisms. The short treatment duration in K0W4 may not have allowed biological equilibrium to form, while initial oxidation of Fe^{2+} and Mn^{2+} likely released H^+ ions, lowering pH. In K20W8, the combination of water hyacinth and moringa appeared to stimulate microbial metabolism, producing organic acids through fermentation pathways, consistent with mechanisms described by Rao et al. (2022).

Throughout the experiment, pH values remained within the permissible range (6.5–8.5) specified by the Regulation of the Minister of Health No. 2/2023. Maintaining this range is essential to prevent adverse effects such as pipe corrosion, skin irritation, and gastrointestinal issues (Cameselle, 2021). However, prolonged or unbalanced application of bioremediation agents must be carefully managed. Ogundele et al. (2024) and Chaki and Flomo (2024) highlighted that disruptions in autotrophic–heterotrophic balance may reduce aquatic productivity, while Karsih et al. (2025) warned that excessive biomass accumulation can lead to the formation of phenolic compounds, ammonia, methane, and sulfide, substances harmful to aquatic ecosystems and human health. These considerations underscore that the synergistic use of phytoremediation and natural coagulation is most effective and sustainable when conducted under controlled water quality conditions.

Table 5. Temperature level test

Indicator	Result
Combination of bioremediation agents	0.002
Treatment time	0.001
Combination of bioremediation agents and treatment time	0.000

Temperature levels

ANOVA results indicated that water temperature was significantly influenced by both treatment factors and, most notably, by their interaction ($p < 0.05$) (Table 5). DMRT analysis identified three treatments, K20W0, K20W4, and K20W12, that differed significantly from the control. Among these, K20W12 exhibited the highest temperature (27.73 °C), reflecting a 2.51% increase relative to the control (27.05 °C), while the lowest temperature (26.95 °C) occurred in K20W0 and K20W4, approximately 0.37% below the control.

These variations suggest that the combined action of *Eichhornia crassipes* and *Moringa oleifera* seeds can influence thermal conditions within the treatment system. Longer treatment durations tended to produce increased water temperatures, likely due to enhanced oxidation of dissolved metals, accelerated degradation of organic matter, and elevated microbial activity, processes known to release heat through metabolic pathways (Anekwe and Isa, 2023). In contrast, shorter treatment periods were associated with slightly lower temperatures. This may be linked to the cooling effect generated by water hyacinth through transpiration during early growth phases, a phenomenon previously described by Trisnawati and Damajanti (2023).

As shown in Figure 2, post-treatment temperatures ranged from 26.95 °C to 27.73 °C. Although

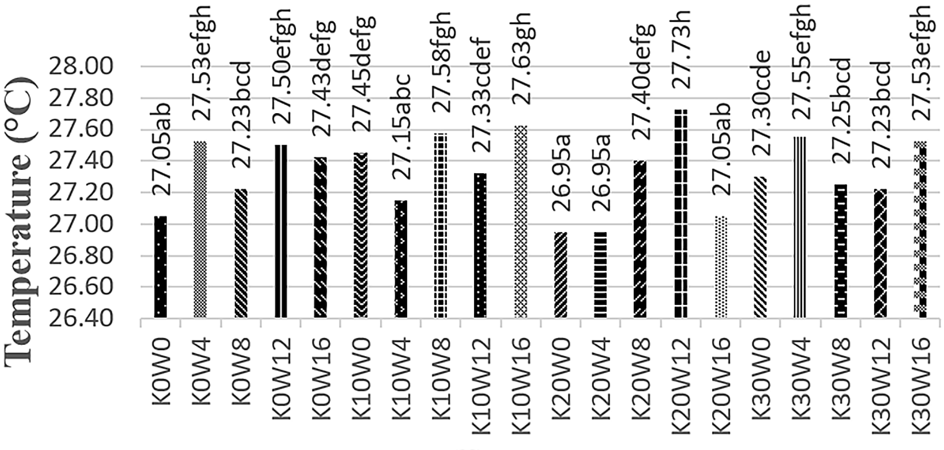

Figure 2. Effect of treatment on temperature

statistically significant differences were observed among several treatments, the overall temperature fluctuation remained narrow and within the typical range for shallow groundwater in tropical environments. The treatments K20W12, K0W0, K10W8, and K20W16, for example, did not differ significantly from one another, despite K20W12 recording the highest temperature. Likewise, the lower-temperature treatments (K20W0 and K20W4) showed no significant deviation from several other treatment groups.

Overall, the magnitude of temperature change was minor, indicating that the bioremediation agents exerted only a limited influence on this physical parameter. Since the observed temperatures remained within normal well-water thresholds, the stability of thermal conditions suggests that phytoremediation–coagulation processes did not compromise water quality in terms of temperature.

In this study, the optimal treatment condition was defined based on three criteria: (1) achievement of Fe and Mn concentrations below national and WHO drinking water standards, (2) maximum and consistent removal efficiency, and (3) system stability under prolonged contact time. These criteria were applied to identify the most effective hybrid configuration among all treatment combination.

Table 6. Iron level test

Indicator	Result
Combination of bioremediation agents	0.000
Treatment time	0.000
Combination of bioremediation agents and treatment time	0.000

Iron

ANOVA results showed that reductions in Fe concentration were significantly influenced by the type of bioremediation agent and treatment duration ($p < 0.001$), with their interaction also exerting a highly significant effect ($p < 0.01$) (Table 6). These findings indicate that the effectiveness of Fe removal is strongly dependent on both biomass dosage and adequate contact time (Figure 3).

The low Fe removal observed in treatment K20W0 can be attributed to insufficient contact time, which limited the occurrence of key remediation mechanisms. At this early stage, iron predominantly remained in its soluble Fe^{2+} form, with minimal oxidation, biosorption, or flocculation. As a result, interactions between dissolved iron ions, plant root surfaces, and moringa-derived coagulating proteins were not fully established. In contrast, treatment K30W16 exhibited near-complete Fe removal due to prolonged exposure that enabled progressive oxidation of Fe^{2+} to insoluble Fe^{3+}, extensive biosorption by water hyacinth roots, enhanced microbial activity, and stable floc formation induced by cationic proteins from *Moringa oleifera*. The combination of high biomass density and extended retention time allowed these processes to operate synergistically, resulting in a substantial reduction of dissolved iron.

DMRT analysis demonstrated clear differences across treatments. K30W16 achieved the highest reduction, lowering Fe concentration from 3.674 mg/L to 0.082 mg/L with a removal efficiency of 97.77%. This treatment was statistically comparable to K10W12, K10W16, K20W12, K30W8, and K30W12, suggesting that

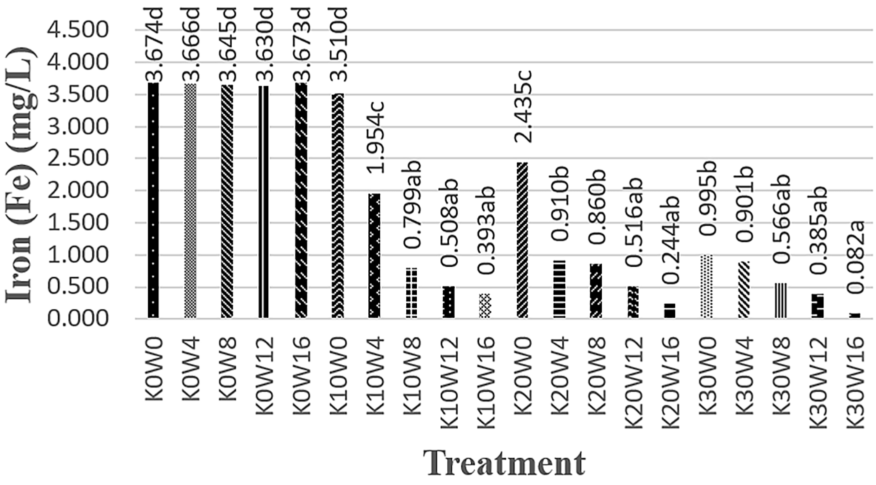

Figure 3. Effect of treatments on iron

higher biomass combined with longer retention time consistently improved removal outcomes. In contrast, K20W0 produced the lowest reduction efficiency (33.71%), with a final concentration of 2.435 mg/L, illustrating that short exposure times and insufficient biomass limit the interaction between Fe ions and the bioremediation components. The lack of notable differences between K10W4 and K10W0 further supports this trend.

Overall, the K30 group reached an average Fe reduction of 73.53%, outperforming the K20 group, which averaged 50–60%. This pattern aligns with findings by Singh and Pant (2023), who reported Fe reductions up to 69.99% using *Moringa oleifera* press cake, and by Trisnawati and Damajanti (2023), who observed substantial increases in Fe removal with higher moringa biosorbent dosages. These similarities reinforce the importance of optimizing biomass loading in treatment systems.

The Fe removal observed in this study is influenced by interconnected biochemical and physicochemical mechanisms. Microbial activity likely promoted biomineral precipitation through extracellular polymeric substances (EPS), facilitating the formation of Fe-rich minerals such as jarosite and ferrihydrite, as described by Ding et al. (2024). At the same time, the roots of *Eichhornia crassipes* functioned as effective biosorbents, utilizing carboxyl and hydroxyl functional groups, shown by Rani et al. (2024) to reduce Fe concentrations by up to 89.33%, to bind metal ions from the aqueous phase. These processes were further enhanced by the natural coagulating properties of *Moringa oleifera* seeds. The protein–polysaccharide complexes in moringa can immobilize Fe through neutral magnetophoresis, as reported by Ruiz et al. (2024), increasing the likelihood of floc formation and subsequent sedimentation.

Additionally, the coordination behavior of Fe ions supports these removal pathways. In aqueous environments, Fe^{2+} and Fe^{3+} readily form stable complexes such as $[FeL_6]^{2+}$ and $[FeCl_4]^-$ (Inoue et al., 2022), which allows efficient interaction with both plant root tissues and moringa-based coagulant matrices. The integration of hyperaccumulator plant species with natural coagulants therefore provides multiple, reinforcing mechanisms that enhance Fe removal. In summary, the findings indicate that both sufficient biomass and longer treatment duration are crucial for effective Fe reduction in groundwater bioremediation systems. While treatments with minimal contact time, such as K20W0, yielded limited results, the superior performance of K30W16 highlights the potential of hybrid phytoremediation–coagulation as a sustainable and efficient method for treating Fe-contaminated groundwater.

Effectiveness of iron bioremediation

ANOVA results revealed that the bioremediation agent, treatment duration, and their interaction had a highly significant effect on Fe reduction ($p < 0.001$), confirming that the success of Fe remediation depends on the correct combination of biomass dosage and contact time. DMRT analysis further verified significant differences among treatments, with combined factors consistently enhancing removal efficiency. Treatments belonging

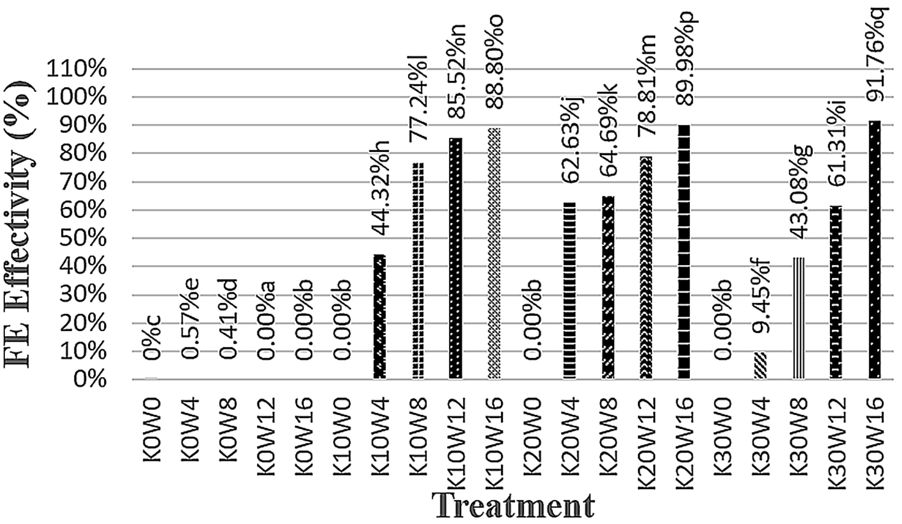

Figure 4. Effectiveness of reducing iron levels in well water

to the high-efficiency group, such as K30W16, K30W8, K10W12, and K10W16, did not differ significantly from one another, indicating that Fe removal reached an optimal range under conditions of higher biomass and extended exposure.

As shown in Figure 4, the effectiveness of Fe removal varied notably across treatments. The greatest reduction was obtained in K30W16, reaching 91.76%, followed closely by K30W12 (89.98%) and K20W16 (88.59%). These patterns clearly show that combining larger doses of bioremediation agents with longer treatment durations consistently improved the reduction of Fe concentrations. Conversely, treatments such as K0W8, K0W16, and K10W4 exhibited minimal or no Fe removal. For instance, K0W8 achieved only 0.41% effectiveness, while K0W12 and K0W16 showed no reduction at all. The stark contrast between these treatments and the higher-dosage groups demonstrates that passive oxidation alone is insufficient, and biological as well as physicochemical processes facilitated by biomass are critical for Fe attenuation.

The superior performance of K30W16 reflects the effective synergy between biological and chemical mechanisms within the system. Increased biomass contributes a larger adsorption surface and facilitates stable microenvironmental conditions that support biosorption, flocculation, and microbial oxidation. These findings are consistent with Mol and Murugesanb (2024), who emphasized the integration of phytoremediation and biosorption as key drivers in metal reduction, and Abdulkareem and Alaallah (2023), who reported that water lilies can remove up to 97% of metals depending on biomass density and contact duration. Without sufficient biomass, as seen in the control treatments, adsorption sites and bioactive compounds are absent, limiting Fe removal to weak natural processes. This aligns with Khan et al. (2024) and Haider et al. (2022), who demonstrated that plant biomass and prolonged retention time significantly improve bioremediation performance. Hasani et al. (2021) also noted that water hyacinth reduced Fe concentrations by as much as 97.49% when coverage reached 50% over a 21-day period, underscoring the importance of biomass dominance in facilitating metal uptake.

Mechanistically, the high efficiency achieved in K30W16 can be attributed to the combined action of root surface biosorption, natural coagulation, and microbially driven oxidation. The cationic proteins in *Moringa oleifera* seeds act as natural coagulants that bind and flocculate dissolved iron (Stanikina, 2023), while the carboxyl and hydroxyl functional groups in water hyacinth roots provide abundant binding sites for metal ion precipitation (Wiryono et al., 2021). Additionally, microbial species such as *Pseudomonas* and *Bacillus* accelerate Fe^{2+} oxidation to insoluble Fe^{3+} (Charron-Lamoureux et al., 2022; Rani et al., 2024), enhancing sedimentation and overall removal. This multifaceted synergy explains the exceptional remediation efficiency observed in K30W16.

These results demonstrate that integrating water hyacinth with moringa seed extract provides a highly effective, low-cost, and environmentally sustainable strategy for Fe removal. The consistent superiority of K30W16 highlights the importance of combining higher biomass loading with longer contact durations, offering a promising approach for communities lacking access to advanced water treatment infrastructure.

Manganese

ANOVA results showed that the bioremediation agent, treatment duration, and their interaction all had a highly significant effect on Mn reduction ($p < 0.001$), indicating that removal efficiency depends strongly on the synergy between biomass composition and adequate contact time (Table 7). DMRT analysis confirmed that treatments incorporating higher biomass with longer exposure durations yielded consistently superior results compared to those with limited biomass or shorter treatment times.

As shown in Figure 5, Mn concentrations varied widely across treatments. The control (K0) maintained high Mn levels (0.152–0.159 mg/L), while treatments integrating both water hyacinth and moringa seed extract effectively reduced Mn to near-zero levels. The best performance was obtained with K30W16, which lowered Mn concentrations from 0.155 mg/L to 0.002 mg/L, achieving a removal efficiency of 98.71%. Comparable performance was observed in K30W12 (0.019 mg/L) and K20W16 (0.022 mg/L). These outcomes clearly indicate that increasing biomass

Table 7. Manganese level test

Indicator	Result
Combination of bioremediation agents	0.000
Treatment time	0.000
Combination of bioremediation agents and treatment time	0.000

and extending contact time work synergistically to maximize Mn reduction. In contrast, treatments such as K30W4 and K10W0 demonstrated minimal effectiveness, each reducing Mn only to 0.115 mg/L (25.81%). This result underscores that biomass alone is insufficient; without adequate exposure duration, biosorption surfaces, microbial activity, and natural coagulation processes cannot operate optimally.

The high removal efficiency observed in treatments such as K30W16 reflects complementary interactions between biological and physicochemical processes. Water hyacinth roots contain carboxyl and hydroxyl functional groups capable of binding Mn ions, and their involvement in metal biosorption has been widely documented (Wiryono et al., 2021). Microbial oxidation also plays an important role; species such as *Lactobacillus fermentum* convert soluble Mn^{2+} into insoluble Mn^{4+} through enzymatic pathways (Nugraha et al., 2023). This process enhances the formation of particulate Mn suitable for sedimentation. Furthermore, the cationic proteins in moringa seeds promote flocculation by neutralizing metal charges and accelerating particle aggregation (Priya et al., 2023). Melaku (2023) and Alghamdi et al. (2024b) likewise reported that various parts of the moringa plant can achieve Mn removal efficiencies ranging from 79% to nearly 99%, depending on environmental conditions and biomass dosage.

While water hyacinth is known to effectively remove Mn, its use must be carefully controlled due to its invasive potential (Punitha et al., 2025). Optimal Mn adsorption also depends on environmental conditions, especially pH, which typically influences uptake efficiency and often aligns with pseudo-second-order kinetics dominated by chemisorption mechanisms (Hegazy et al., 2021; Alghamdi et al., 2024). The comparison between K30W16 and K30W4 highlights time as a critical determinant of bioremediation success; prolonged contact facilitates biosorption through plant tissues, microbial oxidation pathways, and the formation of stable flocs through moringa seed proteins.

A strong positive correlation between Fe and Mn (r = 0.821*) indicates that both metals tend to decrease simultaneously. This pattern reflects their similar geochemical behavior, including shared oxidation pathways, comparable valence transitions, and mutual sources such as rock weathering and anthropogenic inputs. Overall, the integration of *Eichhornia crassipes* and *Moringa oleifera* seeds proved highly effective for Mn removal, particularly when high biomass was maintained for longer durations. To avoid the risk of remobilizing accumulated metals, harvested plant biomass must be managed properly (Yadav et al., 2022). These results demonstrate that the hybrid phyto-coagulation approach offers a practical, low-cost, and environmentally sustainable method for treating Mn-contaminated groundwater.

Effectiveness of manganese (Mn) bioremediation

Figure 6 shows that the effectiveness of Mn reduction varied sharply among treatments. The K3W16 treatment produced the highest reduction efficiency at 98.84%, followed by K3W12 (98.19%) and K2W16 (87.41%). These values indicate that the K3 combination with longer

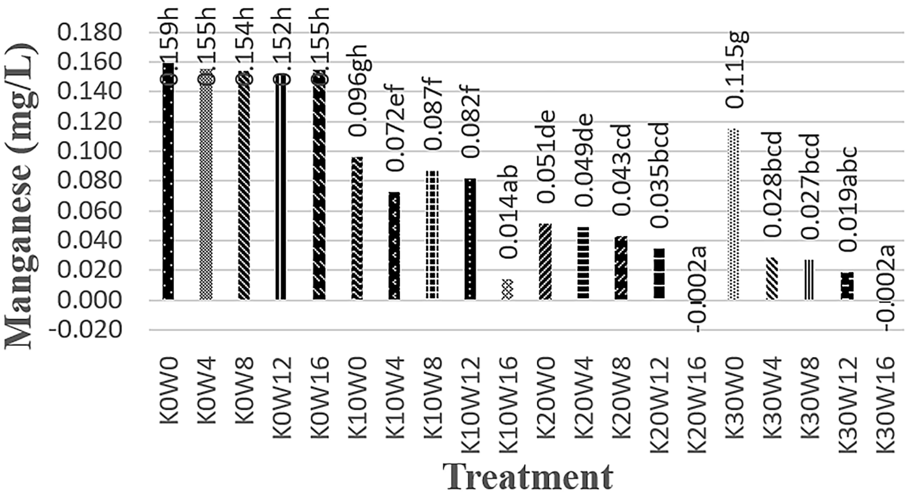

Figure 5. Effect of treatment on manganese

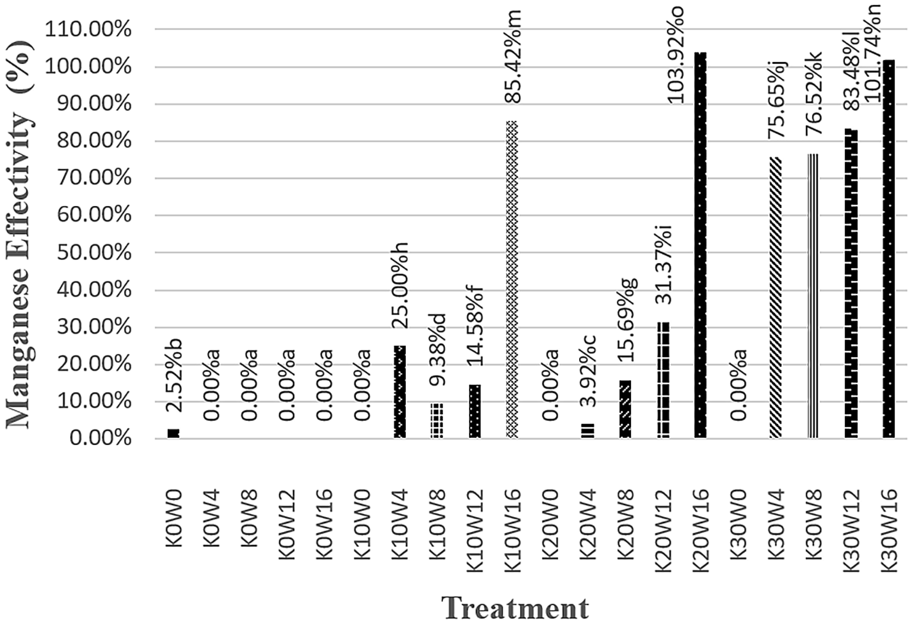

Figure 6. Manganese reduction effectiveness in well water

exposure times (12–16 hours) was able to reduce Mn concentrations to almost zero. In contrast, all control treatments (K0W0–K0W16) showed extremely low effectiveness (0–1.52%), confirming that treatment time alone does not significantly reduce Mn levels without active bioremediation agents.

DMRT analysis also identified significant differences among treatments. The K20W16 treatment reached the highest efficiency at 103.92%, which did not differ statistically from K30W8 (76.52%), K30W12 (83.48%), and K30W16 (87.40%). All four treatments fall within the high-efficiency category (≥80%), demonstrating that sufficient biomass density combined with appropriate contact duration significantly increases Mn removal. Conversely, all treatments without active agents (K0W0–K0W16) showed 0.00% efficiency, even at the longest contact time, reinforcing that passive processes such as natural oxidation or sedimentation are insufficient.

The superior performance of treatments such as K3W16, K3W12, and K20W16 is attributed to the synergy of three mechanisms: biosorption by water hyacinth roots containing carboxyl and hydroxyl functional groups, microbial oxidation of Mn^{2+} into insoluble Mn^{4+}, and flocculation driven by positively charged proteins from moringa seeds. These processes are consistent with previous findings that water hyacinth can remove Mn up to 99.7% within 14 days, while moringa seeds achieve efficiencies of 75–99% depending on dosage and pH conditions. However, not all treatments responded proportionally to biomass dosage. For example, K30W4 showed an effectiveness of only 25.81%, similar to K10W0, indicating that increasing biomass without sufficient contact time does not significantly enhance Mn removal. This is further emphasized by the comparison of K30W16, which reduced Mn from 0.155 mg/L to 0.002 mg/L (efficiency 98.71%), with K30W4, which produced only marginal reductions. Overall, the results confirm that the most effective configuration for Mn bioremediation was K20W16, combining 20 water lily leaves and 50 mg/L moringa seeds over 16 days, achieving an efficiency of 103.92%. Extended contact time enables optimal biosorption, microbial oxidation, and flocculation, providing a robust and low-cost strategy for reducing Mn contamination in groundwater. These findings reinforce that both sufficient biomass and longer treatment duration are essential for achieving consistent high-efficiency bioremediation.

Compared to previous studies employing single phytoremediation or single coagulation techniques, the hybrid system evaluated in this study demonstrated superior removal efficiency and process stability. Phytoremediation-only approaches often require longer treatment periods and exhibit variable performance depending on biomass density, while standalone coagulation relies on repeated chemical dosing. The hybrid configuration combines the strengths of both systems, achieving higher metal removal with reduced chemical input and improved consistency. These findings confirm that integrating phytoremediation with natural coagulation enhances treatment performance beyond what can be achieved by individual methods.

Synergistic mechanism of the hybrid phyto-coagulation system

The superior performance of the hybrid system is governed by the interaction of biological and physicochemical mechanisms. Water hyacinth roots provide extensive surface area and functional groups that bind Fe and Mn ions through biosorption and phytouptake processes. Simultaneously, microbial communities associated with the rhizosphere facilitate oxidation of Fe^{2+} and Mn^{2+} into less soluble forms, enhancing precipitation. The addition of *Moringa oleifera* seed extract further accelerates metal removal by introducing positively charged proteins that neutralize metal complexes and promote flocculation. This coagulation process reduces the metal load in the aqueous phase, thereby improving the efficiency of plant-based biological treatment. The integration of these mechanisms explains the consistently higher removal efficiencies observed in hybrid treatments compared to single-technology approaches.

CONCLUSIONS

This study demonstrates that integrating phytoremediation using *Eichhornia crassipes* with natural coagulation derived from *Moringa oleifera* seeds provides an effective and sustainable approach for removing iron and manganese from contaminated groundwater. The hybrid system outperformed passive and single-treatment configurations by leveraging synergistic mechanisms of biosorption, microbial oxidation, and protein-induced flocculation. The optimal configuration achieved regulatory compliance for both metals while maintaining system stability under extended contact time. These findings highlight the potential of hybrid nature-based solutions as low-cost, scalable alternatives for decentralized groundwater treatment. Future research should focus on system scaling, long-term operational performance, and environmentally safe management of harvested biomass to ensure sustainable implementation.

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