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Phytoremediation of iron-contaminated soils using humic acid and hyperaccumulator grasses

Fitoremediasi tanah terkontaminasi besi menggunakan asam humat dan rumput hiperakumulator

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ABSTRACT

The Lapindo mud disaster in Porong, Sidoarjo, Indonesia, resulted from mining activities, leading to severe iron (Fe) contamination in the soil. This contamination has adversely impacted agricultural productivity in the affected areas. Phytoremediation, utilizing humic acid as a chelating agent and hyperaccumulator grasses, is a potential solution to mitigate this pollution. This study employed a factorial completely randomized design (CRD) to evaluate this approach with two factors: the application of 600 mg/kg humic acid and the use of different grasses (vetiver, elephant grass, nutgrass, and gotu kola). Observed parameters included plant dry weight and total iron content in soil and plant tissues. These data were used to determine the bioconcentration factor (BCF), translocation factor (TF), and absorption efficiency (%). The results indicated that humic acid significantly increased plant dry weight and iron uptake in plant tissues. The combination of gotu kola and humic acid showed the highest phytoremediation potential, with a BCF of 0.3121, TF of 1.4871, and an absorption efficiency of 55.7538%. This study highlights the effectiveness of humic acid and hyperaccumulator grasses in phytoremediation of iron-contaminated soils, offering a sustainable approach to improving soil health and agricultural productivity in polluted areas.

ABSTRAK

Bencana lumpur Lapindo di Porong, Sidoarjo, Indonesia, yang diakibatkan oleh aktivitas pertambangan, menyebabkan kontaminasi besi yang tergolong pencemaran berat di tanah. Kontaminasi ini berdampak buruk pada produktivitas pertanian di daerah terdampak. Fitoremediasi, yang memanfaatkan asam humat sebagai agen pengkhelat dan tanaman hiperakumulator, merupakan solusi potensial untuk mengatasi pencemaran ini. Penelitian ini menggunakan metode rancangan acak lengkap (RAL) faktorial dengan dua faktor dalam mengatasi permasalahan ini, yaitu aplikasi asam humat 600 mg/kg dan penggunaan berbagai jenis rumput (vetiver, rumput gajah, teki, dan pegagan). Parameter yang diamati meliputi berat kering tanaman dan kandungan total besi di tanah dan jaringan tanaman. Data ini digunakan untuk menentukan faktor biokonsentrasi (BCF), faktor translokasi (TF), dan efisiensi penyerapan (%). Hasil penelitian menunjukkan bahwa asam humat secara signifikan meningkatkan berat kering tanaman dan penyerapan besi dalam jaringan tanaman. Kombinasi pegagan dan asam humat menunjukkan potensi fitoremediasi tertinggi, dengan BCF sebesar 0.3121, TF sebesar 1.4871, dan efisiensi penyerapan sebesar 55.7538%. Studi ini menyoroti efektivitas asam humat dan tanaman hiperakumulator dalam fitoremediasi tanah yang terkontaminasi besi, menawarkan pendekatan berkelanjutan untuk meningkatkan kesehatan tanah dan produktivitas pertanian di daerah yang tercemar.

INTRODUCTION

The Lapindo mud disaster, caused by mining activities in the Porong area of Sidoarjo, Indonesia, has been an ongoing environmental issue since 2006. The continuous mudflow has led to several new environmental problems, particularly

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concerning pollution. This disaster has adversely impacted the quality of air, water, and soil. Several metals, including iron (Fe), aluminum (Al), copper (Cu), and phosphorus (P), have been detected in the contaminated areas (Mauliana & Suprayitno, 2017). The accumulation of Lapindo mud in soil and water bodies has increased the levels of heavy metals, thereby elevating toxicity and affecting various surrounding organisms (Armijn & Soegianto, 2020).

One of the areas affected by the Lapindo mud disaster is agricultural land. Iron (Fe) is the most commonly found metal in this region, with a concentration of 27.7%, which is considered high. This elevated iron content has contaminated the soil quality in several villages directly adjacent to the disaster area (Ulfindrayani et al., 2019). The abundance and availability of iron in the affected area pose a toxic threat to agricultural commodities. Although iron is abundant in the Earth's crust, plants require only a small amount. Therefore, excessive iron can lead to toxicity and act as a contaminant (Ferreira et al., 2022).

Phytoremediation presents an effective strategy for mitigating the extensive impact of heavy metal contamination resulting from the Lapindo mud disaster. This technique involves using plants to extract contaminants from soil or water at polluted sites without hindering their growth (Ariyachandra et al., 2023). The phytoremediation process begins with the absorption of heavy metals by plant roots, followed by the accumulation of these metals in various parts of the plant (Cahyati et al., 2022). Specific plant species are selected based on the type of heavy metal to be remediated. Grasses and weeds are particularly effective in areas affected by mining activities, where heavy metal concentrations are notably high (Shah & Daverey, 2020). Employing endemic grass species in phytoremediation is advantageous due to their rapid growth rate (Bonor et al., 2023). Grasses offer several benefits for phytoremediation, including extensive root systems that cover large areas, high biomass production, and greater tolerance to heavy metal contamination compared to dicotyledonous plants (Dzakwan & Ni'am, 2021).

Several types of grasses exhibit significant potential in absorbing iron, including vetiver (*Vetiver zizanioides*), elephant grass (*Pennisetum purpureum*), nutgrass (*Cyperus rotundus*), and gotu kola (*Centella asiatica*). Vetiver can absorb iron into its plant tissues up to 5000 mg/kg, with iron uptake in the roots reaching 1276 mg/kg in the leaves and 3852 mg/kg in the roots (Banerjee et al., 2019). Elephant grass can accumulate iron throughout its plant tissues, with concentrations of 418.01 mg/kg in the roots, 239.042 mg/kg in the stems, and 275 mg/kg in the leaves (Chandra et al., 2018). Nutgrass can accumulate iron within its tissues up to 42%, with a total accumulation of 1000 mg/kg (Syarifdan & Juhaeti, 2018). Gotu kola, meanwhile, can accumulate more iron than other grasses, with total iron accumulation reaching 1639 mg/kg and the highest concentration in the roots at 1043 mg/kg within 21 days (Bhat et al., 2016).

Humic acid is a commonly used soil amendment for enhancing heavy metal absorption. It functions by chemically reducing metal concentrations, particularly under alkaline conditions (pH > 6) with solubility exceeding 95%. Humic acid interacts with heavy metals through functional groups such as –COOH, phenolic –OH, and alcoholic –OH, acting as electron donors to bind metal cations (Maimunawaro et al., 2021). Research by Ruhaimah et al. (2009) found that a 600 mg/kg dose of humic acid effectively reduced Fe levels in rice paddies by up to 50%, approaching non-toxic levels for plants. Despite these findings, the combined use of grasses and humic acid for heavy metal absorption has not been extensively studied. There is a significant gap in understanding how these two methods work together, especially for Fe reduction in soils contaminated by the Lapindo mud disaster. Previous studies have focused primarily on either the use of specific plant species for phytoremediation or the application of humic acid independently.

This study addresses this gap by evaluating the combined effectiveness of humic acid and different grass species in reducing Fe content in soils affected by the Lapindo mud disaster. By investigating the synergistic effects of humic acid and hyperaccumulator grasses, this research aims to provide a comprehensive approach to phytoremediation, offering a sustainable solution to improve soil health and agricultural productivity in contaminated areas.

MATERIALS & METHODS

Soil sampling was conducted in the rice paddies on the eastern side of the Lapindo mud embankment in Glagaharum Village, Porong District, Sidoarjo Regency, Indonesia, at coordinates 7°31′36.1″S 112°43′51.6″E. The planting was carried out in Tanah Kali Kedinding, Kenjeran District, Surabaya City, Indonesia, at coordinates 7°13′27.0″S 112°46′32.3″E. The materials used in this study included soil samples, commercial humic acid (humic compounds > 65%, Huma TOP, Prima Agro Tech, Indonesia), and 3-month-old seedlings of vetiver grass, elephant grass, nutgrass, and gotu kola, purchased from local seed suppliers in Surabaya, Indonesia. The study employed a factorial design in completely randomized design (CRD). The first factor was the addition of humic acid at a concentration of 600 mg/kg, and the second factor was the type of grass, including vetiver, elephant grass, nutgrass, and gotu kola. The experiment consisted of 10 treatment combinations, each replicated 3 times, resulting in a total of 30 sample units.

Soil samples were collected from the rice paddies at a depth of 0-40 cm. The soil was sieved using a 20-mesh sieve to ensure uniformity and then placed into 25 cm polybags, each containing 3 kg of soil. Humic acid was applied at a dose of 600 mg/kg, equivalent to 100 ml per unit or 1200 kg/ha. The growing medium was incubated for 1 week before planting the prepared grass seedlings. Soil and plant samples were collected 28 days after planting (28 DAP). Plant samples were analyzed for dry weight using gravimetric methods and iron content in both plant tissues and soil. Soil iron content was determined using wet digestion with HNO_3 and $HClO_3$. Preliminary analyses included soil pH (potentiometric method), organic carbon (Walkley-Black method), cation exchange capacity (CEC) (NH_4OAc extraction procedure), available Fe (DTPA extraction method), and soil texture (pipette method).

The efficiency of metal accumulation in plants was evaluated using the bioconcentration factor (BCF) and translocation factor (TF). BCF was calculated as the ratio of heavy metal concentration in the roots to that in the soil. TF was determined as the ratio of heavy metal concentration in the shoots to that in the roots (Lestari & Aji, 2020). The effectiveness of metal absorption was assessed by comparing the increase in iron concentration in plants with the decrease in iron concentration in the soil (Wulan et al., 2020). Data were analyzed using analysis of variance (ANOVA) at a 5% significance level with SPSS version 23 (IBM Corporation, USA), followed by a post-hoc Tukey's honest significant difference (HSD) test at a 5% significance level.

RESULTS & DISCUSSIONS

Characteristics of contaminated soil

The initial identification of soil characteristics aims to establish a baseline for assessing the effectiveness of total iron absorption in contaminated soils. Contamination from the Lapindo mud disaster had significantly reduced soil productivity. The decline in soil quality is detailed in Table 1. The soil was characterized by a slightly alkaline pH. This alkalinity was attributed to the presence of basic metal contaminants reacting in the soil. Additionally, the organic carbon content was low, while the CEC was relatively high. The low organic carbon content affected the CEC, which was related to the soil particles dominating the soil.

According to Chandra et al. (2018), pH influences the availability of metals for plants. In alkaline pH, metals such as Mn are more available, affecting the mobility and absorption of available metals. Soil particles play a crucial role in the availability of metals during the absorption process, influencing the formation of organometallic compounds with strong bonds to various soil types. pH determines the solubility, absorption, and transport of heavy metals. Acidic pH in the rhizosphere helps the availability of iron, while alkaline pH enhances the mobility of available metals for plants. Although a slightly alkaline pH reduces the availability of iron, it can still be absorbed by plants up to a pH of 8.5 (Vimala et al., 2022). The available iron content of 14.08 mg/kg fell into the category of excessive metal availability, facilitating the absorption of iron by plants. The total iron content of 48729.83 mg/kg indicated a high level of iron contamination, categorizing the soil as heavily polluted and toxic to plants. The high level of contaminants and available iron was due to the high bioavailability and solubility of iron. Ferreira et al. (2022) explained that the high iron content in soil was due to its reductive nature, increasing the solubility and bioavailability of iron for plants.

Table 1. Characteristics of Lapindo mud-contaminated soil

Parameter	Result	Criteria	Standard criteria
pH	7.01	Slightly alkaline	Balittan (2009)
Organic carbon (%)	0.61	Low	
CEC (cmol/kg)	45.57	High	
Available Fe (mg/Kg)	14.08	Excessive	
Soil Texture			
Sand (%)	14.17		
Silt (%)	65.70	Silty loam	
Clay (%)	20.13		
Total Fe (mg/kg)	48729.83	Heavily contaminated	Komarawidjaja (2017)

Dry weight of roots and shoots

The dry weight of the plants serves as an indicator for assessing the metal absorption process into plant tissues. This parameter is critical for determining the values of BCF and TF. The combination of humic acid and grass species significantly affected the dry weight of the plants. However, the influence on dry weight varied among different grass species, influenced by their physiological forms and sizes. Serdani & Widiatmanta (2019) explained that humic acid helps neutralize contaminated soil, likely by increasing the nutrient content in the medium, which is then absorbed by the plants, thereby affecting their dry weight.

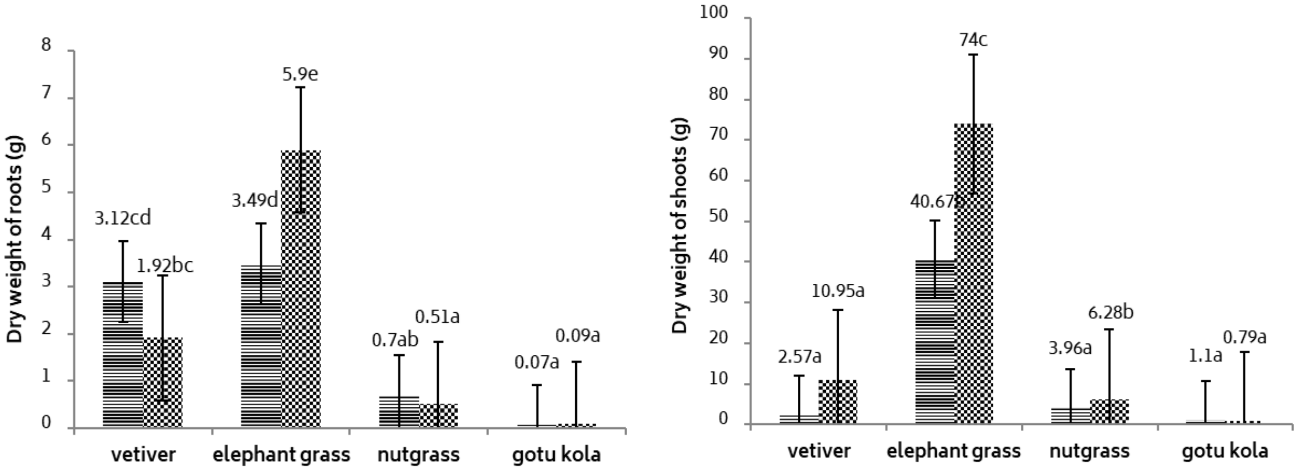

Figure 1. Dry weight of roots and shoots of grass species with (▩) and without (≡) humic acid

Figure 1 shows the dry weight of roots varied among the grass species. For elephant grass and gotu kola, the addition of humic acid resulted in an increase in root dry weight. Conversely, for vetiver grass and nutgrass, the addition of humic acid led to a decrease in root biomass. This can be attributed to the different physical sizes of the root systems of each grass species. The decrease in root dry weight is likely due to iron absorption, which inhibits root growth. Lower root biomass indicates higher toxicity levels from iron contamination. Root dry weight is greatly influenced by the iron content in the soil. Excessive iron content generally affects root growth and length. Iron concentrations exceeding the tolerance limit of roots lead to the formation of ferric oxide layers on the root surfaces, which hinder nutrient uptake, reduce root oxidation capacity, and limit iron prevention by the roots.

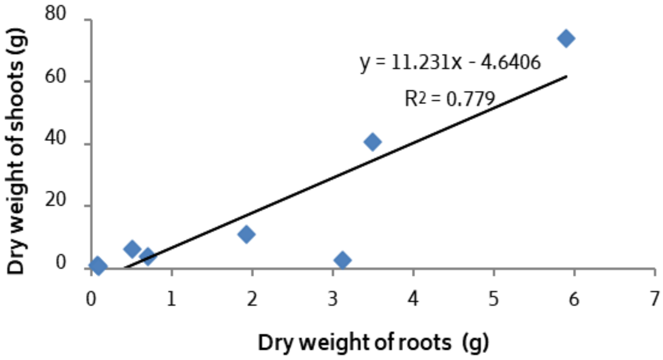

Figure 2. Interaction between root and shoot dry weight

The shoot dry weight also varied among the grass species, as shown in Figure 1. The addition of humic acid increased the shoot dry weight of elephant grass, nutgrass, and gotu kola, whereas gotu kola experienced a decrease in shoot dry weight. The highest shoot dry weight was observed in elephant grass with humic acid addition, reaching 74 grams. This increase in shoot dry weight indicates healthier plant growth, suggesting that there were no physiological disturbances in the roots' nutrient uptake. The increase in shoot dry weight is associated with the iron content in this part of the plant. The lower the iron content in the plant tissue, the more the iron toxicity has been translocated into other plant tissues. This finding aligns with Serdani & Widiatmanta (2019), who noted that the more nutrients absorbed by the plant, the higher the plant's dry weight, indicating sufficient water and nutrient availability.

The interaction between root dry weight and shoot dry weight is illustrated in Figure 2. There is a strong correlation between root dry weight and shoot dry weight; as root dry weight increased, shoot dry weight also increased. Root dry weight was generally lower than shoot dry weight. Comparing the humic acid treatments, grasses with lower root weights tended to have higher shoot dry weights. This correlation is related to the metal content absorbed and translocated into the plant tissues. The lower the dry weight in one part of the plant, the higher it tends to be in the other part. The relationship between roots and shoots is linked by their morphological characteristics in transporting water and nutrients or contaminants. Shoot dry weight is influenced by root activity in transporting substances to other parts of the plant (Chen et al., 2021).

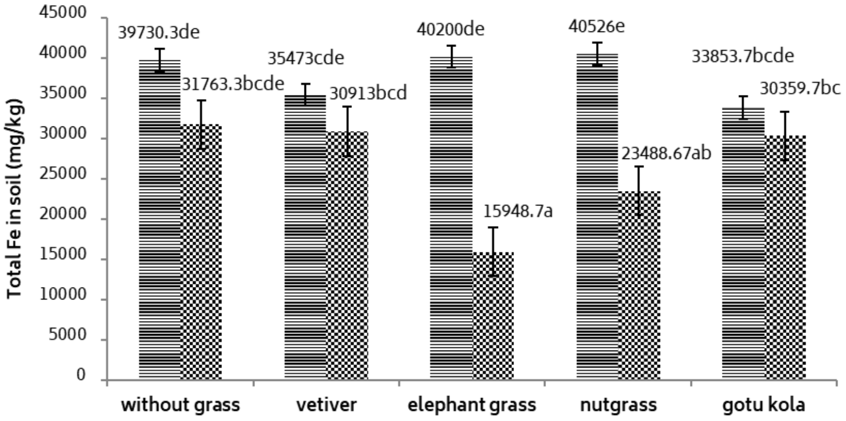

Figure 3. Soil iron content in various grass cultivation sites with (▩) and without (≡) humic acid

Total iron content in soil

The total Fe content in the soil significantly affects agricultural productivity and cultivation practices. The treatment with humic acid combined with various grass species influences the total Fe content. A decrease in the total Fe content was observed due to the addition of humic acid and the absorption of Fe by the different grass species. Figure 3 illustrates that the addition of humic acid significantly reduced the total iron content in the soil compared to treatments without humic acid. Humic acid contains functional groups such as –OH and –COOH that play a role in redox reactions, binding Fe^{3+} ions (Wang et al., 2023). The combination of humic acid with elephant grass notably reduced the total iron content to 15948.67 mg/kg. This reduction in soil iron content can transform the soil into a non-contaminated class suitable for plant growth. The lower the contaminant level in the soil, the higher the potential for iron absorption by plant tissues. This finding is consistent with Dzakwan & Ni'am (2021), who reported that elephant grass can absorb up to 2050 mg/kg of iron, with potential increases as the plant matures. Wulan et al. (2020) explained that iron concentration can vary due to factors such as water pH, minerals, sulfide oxidation from sediments, and organic matter. Mineral sulfides and iron oxides release metals into the water, resulting in leaching processes. Additionally, microorganisms contribute to metal absorption through organic matter and the plant's maximum absorption capacity.

Total iron content in plant tissues

The absorption process occurs in the roots and is then translocated to the upper plant tissues (shoots). Iron absorption predominantly occurs in the roots. According to Chandra et al. (2018), iron accumulation is mostly found in the roots because iron is a micronutrient that influences the plant's capacity to withstand metal stress from a toxicological perspective. Excess iron often deposits in the roots, disrupting other plant processes.

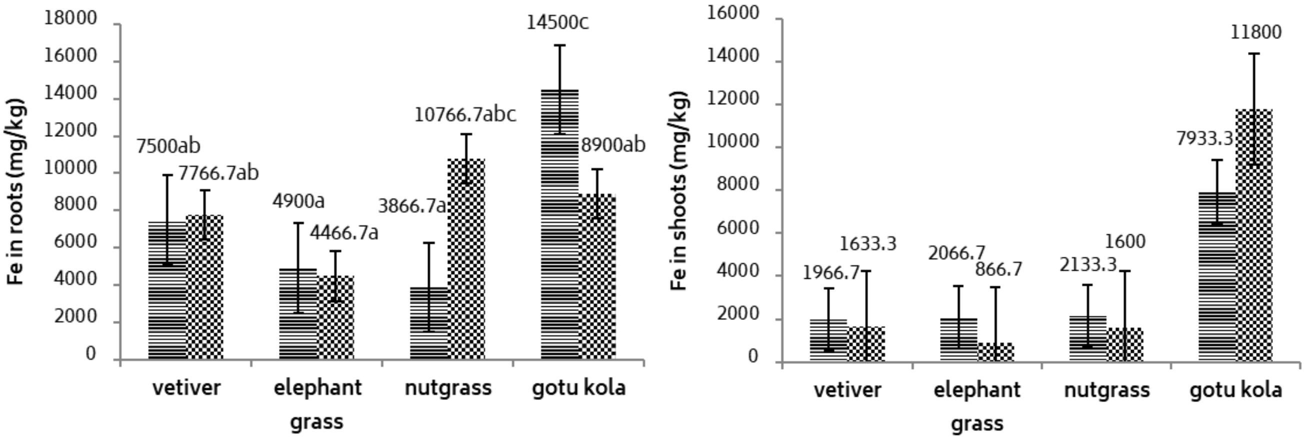

Figure 4. Iron content in roots and shoots of grass species with (▩) and without (≡) humic acid

Figure 4 shows that iron absorption by the roots is notable in gotu kola without humic acid and nutgrass with humic acid treatments. Both plants were able to absorb iron in the roots up to more than 10,000 mg/kg. Gotu kola in the treatment without humic acid absorbed the most iron compared to other grass species. Meanwhile, the highest iron translocation to the shoots was observed in gotu kola with humic acid, reaching 11,800 mg/kg. Gotu kola's ability to absorb iron effectively is related to the optimal biomass formation of its roots. Across all treatments, the highest iron content was found in the roots, indicating a rhizofiltration process. According to Mazumdar & Das (2015), gotu kola can absorb up to 2,100 mg/kg of iron. Grzegórska et al. (2020) explained that rhizofiltration utilizes the roots' ability to absorb metals into root tissues, concentrating and precipitating them. At pH 7, this process involves absorption and surface precipitation of iron in an insoluble form.

Figure 5 compares humic acid treatment with non-humic acid treatment in terms of iron absorption in roots and shoots. The difference between these treatments was not significant; however, the addition of humic acid increased the iron content absorbed by plant tissues. Both roots and shoots exhibited high iron levels due to the influence of humic acid. Humic acid acts as a biosurfactant that can react with heavy metals through adsorption and complexation reactions. It serves as an electron carrier, promoting the biogeochemical redox reactions of heavy metals (Wang et al., 2021). High root absorption is attributed to the plant's rhizofiltration capability, characterized by contaminant deposition predominantly in the root area. The high iron absorption in roots is due to their biomass formation capacity, allowing them to accumulate large amounts of heavy metals. Plants release organic compounds and enzymes through their roots, enabling soil microbes to adapt (Handayani & Winara, 2020). Phytoremediator plants have chelating compounds, especially in the roots. Photosynthetic activity produces dissolved oxygen for microorganisms, aiding in the breakdown of contaminants into nutrients absorbed by the plants (Wulan et al., 2020).

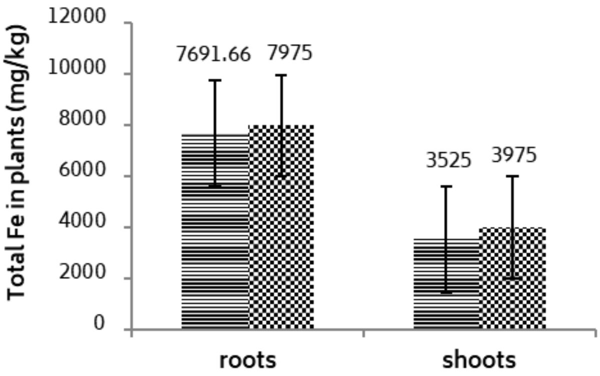

Figure 5. Comparison of iron absorption in roots and shoots with (▩) and without (≡) humic acid

BCF and TF values for total iron

The BCF and TF are influenced by several factors, including plant performance in contaminant uptake, the addition of chelating agents, soil properties, root zone characteristics, environmental conditions, and metal bioavailability (Bhat et al., 2016). A key characteristic of accumulator plants is having a TF value greater than 1, which indicates successful metal translocation from roots to shoots (Mazumdar & Das, 2021).

Table 2. BCF and TF values for all treatments (n = 3). Data are means ± SD

Treatment	BCF	TF
Without humic acid + vetiver grass	0.2113 ± 0.015	0.2553 ± 0.163
Without humic acid + elephant grass	0.1214 ± 0.016	0.4220 ± 0.053
Without humic acid + nutgrass	0.0949 ± 0.030	0.5894 ± 0.177
Without humic acid + gotu kola	0.4397 ± 0.142	0.4376 ± 0.068
With humic acid + vetiver grass	0.2450 ± 0.154	0.2442 ± 0.066
With humic acid + elephant grass	0.3310 ± 0.182	0.1921 ± 0.024
With humic acid + nutgrass	0.5797 ± 0.305	0.1422 ± 0.059
With humic acid + gotu kola	0.3121 ± 0.062	1.4871 ± 0.142

The data in Table 2 reveal considerable variation in BCF and TF values. The treatment with nutgrass in the absence of humic acid was classified as a non-accumulator, while other treatments fell into the category of moderate hyperaccumulators. Nutgrass with humic acid addition recorded the highest BCF value of 0.5797, indicating its enhanced ability to absorb iron due to the presence of humic acid. However, nutgrass in the absence of humic acid exhibited a higher TF compared to when it was grown with humic acid. This suggests a greater potential for iron absorption by nutgrass without humic acid, consistent with Jahan-Nejati et al. (2021), who noted that nutgrass tends to retain iron in the roots rather than in other plant parts.

For TF values, all treatments, except gotu kola with humic acid, exhibited TF values below 1, indicating a lower ability to translocate iron to the shoots. Gotu kola with humic acid showed the highest TF value of 1.4871. This high TF is attributed to the combined effect of humic acid and gotu kola's capacity to absorb iron. The concentration of iron in plant tissues depends on plant species, developmental stage, and environmental factors. Iron that can ionize into complexes with organic and inorganic materials in water affects its bioavailability to plants (Coimbra & Borges, 2023).

Effectiveness of absorption

The effectiveness of absorption, expressed as a percentage, is calculated by comparing the concentration of iron in plants with that in the planting medium. The calculation of absorption effectiveness can serve as a reference for identifying plant species effective in absorbing soil contaminants (Wulan et al., 2020).

Table 3. Effectiveness of total iron absorption (n = 3). Data are means ± SD

Treatment	Total efficiency (%)
Without humic acid + vetiver grass	19.427 ± 3.74
Without humic acid + elephant grass	14.297 ± 2.49
Without humic acid + nutgrass	12.313 ± 4.22
Without humic acid + gotu kola	46.036 ± 2.14
With humic acid + vetiver grass	25.318 ± 4.65
With humic acid + elephant grass	14.365 ± 4.34
With humic acid + nutgrass	22.209 ± 4.24
With humic acid + gotu kola	55.754 ± 3.67

The data in Table 3 show varying total efficiency values across different treatment combinations. In general, all grass species treated with humic acid exhibit higher efficiency compared to those without humic acid. This is due to the role of humic acid in the iron absorption process. Humic acid can absorb heavy metals in alkaline conditions as it is more soluble at pH ≥6, with solubility reaching ≥95% (Maimunawaro et al., 2021). The dynamics of iron in soil relate to changes in soil reactions, especially under alkaline pH conditions. Increased pH is marked by high hydroxyl groups in the soil solution, leading to the formation of iron hydroxide compounds. Differences in the number of absorbed ions affect the ionic potential value. The higher the ionic potential value, the more strongly hydrated the cation is. This causes the hydration energy of Fe^{3+} to release more H_2O molecules compared to the hydration energy of other metals (Oksana et al., 2024).

The highest total efficiency, with or without the addition of humic acid, was observed in gotu kola. The best treatment for reducing iron content was gotu kola with added humic acid. Economically, the treatment of gotu kola without humic acid is more efficient. Thus, gotu kola alone has significant potential in reducing iron content. This finding is consistent with Bhat et al. (2016), who explained that gotu kola is capable of absorbing and storing more iron in its tissues than other plants. Gotu kola stores iron in the form of Fe^{2+} and Fe^{3+} using various absorption mechanisms. These mechanisms involve the release of protons (H^+) and root exudates to lower the pH around the root zone, which enhances iron solubility and chelation for efficient absorption.

The phytoremediation process in all treatments falls under rhizofiltration. Rhizofiltration maximizes the ability of roots to absorb, precipitate, and accumulate heavy metals from contaminants. Ecophysiologically, rhizofiltration plants possess the ability to exclude or prevent metal translocation from roots to other plant parts. Rhizofiltration occurs when the pH conditions of the root zone are conducive to microbes that can extract contaminants into the roots. The formation of organo-metal complexes due to reactions between microbes and plants makes metals available to plants. Iron is absorbed by the roots through mass transfer via water. The concentration retained by each grass species is due to the different capacities of root cell vacuoles to hold metal ions (Jahan-Nejati et al., 2021).

CONCLUSION

This study focused on the phytoremediation of iron-contaminated soils using humic acid and hyperaccumulator grasses. The results showed that humic acid significantly improved iron absorption in plants, especially in gotu kola, which demonstrated the highest absorption efficiency. Humic acid also enhanced iron translocation from roots to shoots, promoting overall plant health. The significance of these findings lies in the potential application of humic acid-treated hyperaccumulator grasses for sustainable soil remediation. This approach not only reduces iron contamination but also supports healthier plant growth, essential for agriculture. In conclusion, humic acid and hyperaccumulator grasses effectively remediate iron-contaminated soils. Gotu kola, particularly when treated with humic acid, is a promising solution for soil remediation. Future research should investigate the long-term impacts of humic acid and its application to other contaminants.

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