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## Phytoremediation of cadmium-contaminated soil using liberika coffee (*Coffea liberica*) and Jatropha (*Jatropha curcas*): Mechanisms, economic feasibility, and sustainability considerations

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### ABSTRACT

Cadmium (Cd) contamination in soil presents a significant environmental and public health hazard, requiring effective and sustainable remediation strategies. This study introduces a novel phytoremediation approach by integrating Liberika coffee (*Coffea liberica*) and *Jatropha curcas* with mycorrhizal inoculation, an innovative combination not previously explored for Cd-contaminated soils. The experiment assessed plant growth, mycorrhizal colonization, and soil quality parameters under Cd concentrations of 10 and 20 mg/kg. Results demonstrated a notable reduction in Cd concentration from 15 mg/kg to 10 mg/kg, along with improvements in soil pH (from 5.5 to 6.2), organic matter content (from 1.8% to 2.5%), and cation exchange capacity (from 18 to 20 cmol/kg). The combination of one Liberika coffee plant with one Jatropha plant at 10 mg/kg (C1K1) exhibited the most effective performance, achieving a plant height of 31.05 cm, a stem diameter of 5.66 mm, and a mycorrhizal infection rate of 72%. This study provides new insights into an integrated phytoremediation strategy that not only enhances soil quality but also transforms Cd-contaminated land into productive agroecosystems, demonstrating a dual benefit for environmental sustainability and land rehabilitation.

Keywords: phytoremediation, soil contaminated, cadmium, Liberika coffee, Jatropha plant.

### INTRODUCTION

Soil contamination with heavy metals, particularly Cadmium (Cd), has become a significant environmental issue globally (Khan et al., 2017). Cd is a highly toxic metal that persists in the environment, causing severe damage to ecosystems and posing serious risks to human health (Saini and Dhania, 2020; Wibowo et al., 2025). Unlike organic pollutants, Cd cannot be degraded, and once it accumulates in the soil, it remains a longterm threat to agricultural productivity and food security (Mubeen et al., 2023). Chronic exposure to Cd through contaminated water or crops can lead to serious health conditions, including kidney failure, osteoporosis, and cancer (Charkiewicz et al., 2023; Fatima et al., 2019; Genchi et al., 2020). In heavily polluted regions, Cd contamination not only limits agricultural use of the land but also creates long-lasting environmental and public health crises. This underscores the urgent need for cost-effective and sustainable solutions to remediate Cd-contaminated soils.

Traditional remediation techniques, such as excavation and chemical stabilization (Inkham et al., 2019), are often expensive, disruptive, and environmentally unsustainable. These methods may successfully reduce contamination levels but can cause significant damage to soil structure and fertility, making the land unsuitable for future agricultural use. As a result, researchers have increasingly turned to phytoremediation (Wibowo et al., 2023; Imron et al., 2023; Wibowo et al., 2023), a green technology that uses plants to extract, stabilize, or immobilize contaminants in the soil. Phytoremediation is particularly promising for Cd-contaminated soils because it is cost-effective, minimally invasive, and capable of restoring soil health over time (He et al., 2015; Luo and Zhang, 2021; Mahajan and Kaushal, 2018; Raza et al., 2020). However, despite its potential, the practical application of phytoremediation is limited by several challenges, including low metal uptake efficiency and poor plant growth under high contamination levels (Lu et al., 2015).

To address these limitations, recent studies have explored the role of plant diversity and microbial symbiosis in improving phytoremediation outcomes. Mycorrhizal fungi, in particular, enhance plant tolerance to heavy metals by improving nutrient uptake, reducing metal toxicity, and promoting root growth (Yao et al., 2023). Combining hyperaccumulator plants with mycorrhizal inoculation offers a promising approach to overcoming the challenges of phytoremediation. However, studies on the application of economically valuable crops such as Liberika coffee in phytoremediation are scarce, and the potential for integrating high-value crops into remediation projects remains largely unexplored.

This study aims to fill this critical research gap by investigating the use of Liberika coffee (Coffea liberica) and Jatropha (Jatropha curcas) in the phytoremediation of Cd-contaminated soils. The innovative combination of these plants, along with mycorrhizal inoculation, represents a multifunctional approach that simultaneously addresses environmental and economic challenges. While Liberika coffee is a high-value crop with growing market demand, Jatropha is a wellknown hyperaccumulator plant with potential for bioenergy production (Pandey et al., 2012; Puthur, 2021). This dual-purpose approach not only enhances Cd uptake but also provides economic opportunities for farmers, transforming contaminated land into productive agroecosystems.

#### MATERIALS AND METHOD

#### Study location and duration

This study was conducted at the experimental farm of the Faculty of Agriculture, Universitas Jambi. Anatomical analysis and observations were carried out in the Faculty of Agriculture Laboratory from April 2024 to October 2024.

#### Materials

The materials used in this study include Liberika coffee seedlings,  $Cd(O_2CCH_3)2(H_2O)_2$  from Merck, Jatropha seeds, urea fertilizer Petromart, triple superphosphate (TSP), potassium chloride (KCl), plastic bags, envelopes, root infection solution, mycorrhiza, tissue paper, insecticides, fungicides, labels, and stationery. The equipment used includes glass slides, cover slips, dropper pipettes, hoes, watering cans, rulers, scissors, machetes, calipers, measuring tapes, Solo hand sprayers, hoses, shovels, weighing scales, ovens, and measuring instruments.

#### **Experimental design**

The experiment was conducted using a completely randomized design (CRD) with two factors: Cd concentration (C) and plant combination (K). This design was chosen to minimize experimental error and ensure a balanced comparison across treatments. The first factor, C, represents the level of Cd applied to simulate varying degrees of contamination. Two Cd concentrations were tested: 5 mg/kg (C1) and 10 mg/kg (C2). These concentrations were selected to assess the plants' ability to tolerate and accumulate Cd at different exposure levels.

The second factor, K, involves different configurations of Liberika coffee and Jatropha plants to evaluate their individual and combined performance in Cd uptake. Four plant combinations were tested: one Liberika coffee plant (K0), one Liberika coffee plant with one Jatropha plant (K1), one Liberika coffee plant with two Jatropha plants (K2), and two Liberika coffee plants with one Jatropha plant (K3). This variation was designed to identify the most effective plant combination for Cd phytoremediation.

The experimental setup involved a factorial combination of the two factors, with multiple replications to ensure statistical validity. By analyzing the effects of Cd concentration and plant combination on phytoremediation performance, the study aimed to determine the optimal plant configuration for maximizing Cd removal efficiency. This resulted in eight treatment combinations as follows (Table 1).

1	0	
Cd (C)	C) Plant combination (K)	
C1	C1K0, C1K1, C1K2, C1K3	
C2	C2K0, C2K1, C2K2, C2K3	

Table 1. Experimental design

Each treatment combination was repeated five times, resulting in a total of 40 experimental units. Each experimental unit consisted of different plant compositions according to the treatment level (K0, K1, K2, and K3).

Figure 1 illustrates the experimental setup used to assess plant growth under controlled greenhouse conditions. Image (a) presents a structured planting arrangement under a shaded structure, ensuring regulated light exposure and minimizing external environmental variability. The uniform spacing between plants suggests efforts to maintain equal access to light, water, and nutrients, reducing competition effects. Image (b) provides a closer look at the experimental plants, where the presence of labeled tags indicates a well-organized data collection system that facilitates treatment differentiation and growth monitoring. Image (c) focuses on young seedlings in their early developmental stage, highlighting uniform germination and early root establishment in the growth medium.

The shaded environment plays a critical role in protecting the plants from excessive sunlight, which can reduce stress and enhance growth performance. The use of black polybags as growing containers helps manage root zone temperature and moisture retention, which are essential factors influencing plant growth. This setup allows for controlled experimentation, enabling researchers to evaluate the impact of different treatments such as fertilization, watering regimes, or soil amendments on plant development. These images collectively provide insight into the experimental design and methodological approach used to ensure reliable and reproducible plant growth assessments. The structured setup ensures consistency in data collection and enhances the validity of comparative analysis between treatments.

#### **Observation parameters**

Several parameters were observed during the study, including plant height, stem diameter, leaf count, chlorophyll content, shoot-to-root ratio, mycorrhizal infection percentage, and seedling quality index. Plant height was measured every two weeks for three months, resulting in six observations. The measurement was taken from the base of a support stake at the soil surface to the plant's highest growth point. The height was recorded in centimeters (cm). Support stakes were installed to ensure accurate and consistent measurements across all observations. Stem diameter was measured at the same intervals as plant height (every two weeks for three months). The diameter was recorded at 2 cm above the soil surface and expressed in millimeters (mm). Leaf count was conducted by counting fully expanded leaves at each observation. To avoid recounting, newly expanded leaves were marked at each measurement. Subsequent observations included only newly emerged leaves, with the total count updated at each observation point. The results were expressed as the total number of leaves per plant. Chlorophyll content was determined by selecting healthy, mature green leaves from each



Figure 1. Experimental setup for plant growth study under a shaded greenhouse environment: (a) overview of the experimental setup with potted plants arranged in a structured layout for uniform growth conditions, (b) closer view of the experimental plants, each labeled with yellow tags for identification and tracking, (c) close-up of young seedlings in a controlled growing medium, showing early-stage development

treatment combination. These selected leaves were sent to the laboratory for further analysis to determine chlorophyll concentration. At the end of the experiment, the shoot-to-root ratio was calculated by dividing the shoot dry weight by the root dry weight using the following formula:

Shoot to root reation = 
$$\frac{\text{Shoot dry Weight }(g)}{\text{Root dry weight }(g)}$$
 (1)

The percentage of mycorrhizal infection was assessed at the end of the experiment (90 days after planting) by examining the plant roots under a microscope at  $100 \times$  magnification. The percentage of root infection was calculated using the formula proposed by Hasanah et al. (2017):

$$Mycorrhizal Infection Percentage =$$

$$= \left(\frac{\text{Number of Infected Roots}}{Total number of root observed}\right) \times 100\%$$
(2)

The seedling quality index for Liberika coffee was determined at the end of the study following the formula proposed by Dickson et al. (1960) in Hendromono (1994). This index was calculated using shoot dry weight, root dry weight, plant height, and stem diameter:

 $= \frac{Shoot \, dry \, Weight \, (g) + Root \, dry \, weight \, (g)}{\left(\frac{Plant \, height \, (cm)}{Stem \, diameter \, (cm)}\right) + \left(\frac{Shoot \, dry \, Weight \, (g)}{Root \, dry \, weight \, (g)}\right)}$ (3)

#### Analytical procedures for soil characterization and cadmium quantification

Soil properties were analyzed following internationally recognized standard methodologies to ensure accuracy and reliability (Table 2). The organic carbon content (C-Organic) of the soil was determined using the USDA Method 6A1a (2004), which involves dry combustion at 550 °C in a muffle furnace. Water content was measured gravimetrically following the IK.LP-13.7-LT.2.0 method, which entails drying the soil samples at 105 °C until a constant weight is reached. Total nitrogen content was quantified using the IK.LP-13.6-LT.2.0 method (Titrimetry), which utilizes micro-Kjeldahl distillation followed by titration to assess nitrogen levels in the soil. The carbon-to-nitrogen ratio (C/N ratio) was then calculated based on the measured values of organic carbon and total nitrogen.

Soil pH was determined using the IK.LP-13.8-LT.2.0 method (Titrimetry), involving titration of the soil extract with a standardized acid or base solution. Total phosphorus, expressed as P<sub>2</sub>O<sub>5</sub>, was measured spectrophotometrically according to the IK.LP-13.5-LT.2.0 method, which includes digestion of the soil with strong acids followed by colorimetric analysis. Potassium content, represented as K<sub>2</sub>O, was analyzed using the IK.LP-04.10-LT-1.0 method, which employs flame photometry after extracting potassium from the soil using ammonium acetate.

Cd concentrations in plants post-phytoremediation were determined using the Association of Official Analytical Chemists (AOAC) official method 999.11 (2012), which utilizes atomic absorption spectrophotometry with a graphite furnace (AAS-GF). This method ensures accurate measurement of trace metal concentrations in plant tissues.

For chlorophyll estimation, the method described by Arnon (1949) was followed. Chlorophyll pigments were extracted using 80% acetone, and the absorption of the extracts was measured spectrophotometrically at 645 and 663 nm to calculate the chlorophyll a, chlorophyll b, and total chlorophyll content in the plant samples.

### **RESULT AND DISCUSSION**

# Physicochemical properties of soil and temperature effects on plant growth

Soil organic matter plays a crucial role in improving soil structure, porosity, and microbial activity, all of which contribute to enhanced soil

Table 2. Soil characteristics analysis methods

Parameters	Method		
C-Organic	USDA Method 6A1a, 2004		
Water content	IK.LP-13.7-LT.2.0 (Gravimetry)		
Total nitrogen	IK.LP-13.6-LT.2.0 (Titrimetry)		
C/N ratio	Calculation		
рН	IK.LP-13.8-LT.2.0 (Titrimetry)		
Total phosphorus as P <sub>2</sub> O <sub>5</sub>	IK.LP-13.5-LT.2.0 (Spectrophotometry)		
Potassium as K <sub>2</sub> O*	IK.LP-04.10-LT-1.0		

fertility (Bashir et al., 2021). Table 3 showed the characteristics of initial soil characteristics. The soil exhibits a high level of organic matter, with a C-Organic value of 16.08% w/w, surpassing the minimum regulatory requirement of 15% w/w. This elevated organic carbon content is a positive indicator for soil fertility as it enhances soil structure, promotes water retention, and supports a diverse microbial ecosystem. A previous study informed that soil organic matter serves as a key reservoir of carbon and nutrients, influencing microbial activity and supporting sustainable ecosystem functioning (Singh and Gupta, 2018). Such conditions are conducive to efficient nutrient cycling and can substantially benefit plant health and growth.

At 80.29% w/w, the water content in the soil is notably high, which is beneficial for maintaining moisture necessary for plant physiological processes. However, this level of moisture may also pose challenges such as waterlogging, which can compromise soil aeration and lead to reduced root respiration (Mishra et al., 2024). Proper drainage management becomes essential in such scenarios to ensure that plants receive the necessary oxygen while still benefiting from adequate moisture levels. The soil's total nitrogen content is measured at 0.33% w/w, a critical parameter since nitrogen is vital for plant development as it forms a key component of chlorophyll and amino acids. Despite this, the carbon-to-nitrogen (C/N) ratio is alarmingly high at 48.73, which is nearly double the recommended maximum of 25. This imbalance suggests an excess of carbon relative to nitrogen, potentially leading soil microbes to immobilize nitrogen during the decomposition process. Another previous study also informs that A high C/N ratio can slow down nitrogen mineralization, leading to nutrient immobilization and reduced nitrogen availability for plants (Barnwal et al., 2021). As a result, the availability of nitrogen for plant uptake may be limited, indicating that additional nitrogen amendments might be necessary to optimize plant growth.

The pH value of the soil is 4.72, placing it on the acidic end of the regulatory range of 4 to 9. While this pH level is technically acceptable, it may influence nutrient availability and the solubility of certain compounds. Acidic conditions can restrict the availability of essential nutrients and, in some cases, increase the mobility of toxic metals (Rahman et al., 2018). Depending on the plant species being cultivated, measures such as liming might be required to adjust the pH towards a more neutral level, thereby enhancing nutrient uptake and minimizing potential toxicity. Phosphorus, measured as Total Phosphorus (as P<sub>2</sub>O<sub>5</sub>) at 41.79% w/w, indicates a robust supply of this essential nutrient, which is crucial for energy transfer and root development. Although there is no specified regulatory limit for phosphorus in the Indonesian regulation, the high value could be advantageous for plant growth if managed correctly to avoid potential nutrient imbalances or environmental runoff issues. High phosphorus levels in soil can enhance plant development, but excessive amounts may lead to environmental concerns such as nutrient leaching (Yan et al., 2013). Conversely, the level of potassium, expressed as K<sub>2</sub>O, is reported as less than 2, suggesting that potassium may be deficient. Potassium plays an integral role in plant stress tolerance, enzyme activation, and water regulation (Hasanuzzaman et al., 2018; M. Wang et al., 2013). Insufficient potassium can hinder plant vigor and overall resilience, highlighting the need for careful monitoring and

Parameter	Result	Regulatory limit*	Unit
C-Organic	16.08	Min. 15	% w/w
Water content	80.29	_	% w/w
Total nitrogen	0.33	_	% w/w
C/N ratio	48.73	≤ 25	_
рН	4.72	4–9	_
Total phosphorus as P <sub>2</sub> O <sub>5</sub>	41.79	_	% w/w
Potassium as K <sub>2</sub> O*	< 2	_	_
Cadmium	10 & 20	Max 2	mg/kg

Table 3. Initial Soil characteristics

**Note:** \*Regulation of the Ministry of Agriculture of the Republic of Indonesia, No. 261/KPTS/SR.310/M/4/2019 on Technical Soil Organic Characteristics.

potential supplementation to achieve a balanced nutrient profile.

Temperature is a critical environmental factor that affects plant physiological processes, soil biochemical reactions, and overall crop productivity. The observed temperature data collected throughout the experiment indicate a gradual increase in both minimum and maximum temperatures over time, with minimum temperatures rising from 22 °C in Week 1 to 24 °C by Week 12, while maximum temperatures increased from 32 °C to 35 °C (Table 4). These fluctuations in temperature have direct and indirect impacts on plant growth and the efficiency of phytoremediation.

Moderate temperature ranges are essential for optimal plant growth, particularly in tropical crops such as Liberika coffee. The initial temperatures of 22 °C to 32 °C in Week 1 provided a conducive environment for seedling establishment, facilitating uniform germination and early root development. However, as temperatures increased to 24 °C (minimum) and 35 °C (maximum) in Week 12, the potential for heat stress also increased. Prolonged exposure to high temperatures can disrupt physiological processes such as photosynthesis, leading to reduced growth rates and lower biomass accumulation (Hadi et al., 2016). In this study, the highest growth performance of Liberika coffee seedlings was observed during the early weeks, suggesting that the moderate temperature range during this period was ideal for plant development.

Soil temperature also affects the efficiency of Cd uptake and mycorrhizal colonization. Higher temperatures generally enhance microbial activity in the rhizosphere, which can increase the availability of nutrients and improve the effectiveness of mycorrhiza in facilitating nutrient uptake (Yao et al., 2023). However, excessive heat can negatively impact mycorrhizal colonization rates, reducing the symbiotic benefits that support plant growth under heavy metal stress (Xu et al., 2019). The gradual increase in temperature observed in this study likely contributed to fluctuations in mycorrhizal infection percentages across treatments, with higher colonization rates occurring during the earlier, cooler weeks.

Temperature also plays a crucial role in regulating soil biochemical processes, particularly the decomposition of organic matter. The increase in minimum and maximum temperatures observed throughout the experiment may have accelerated microbial decomposition rates, contributing to the higher organic matter content in the final soil analysis (Lu et al., 2015). Enhanced decomposition improves nutrient availability, promoting healthier plant growth. However, if temperatures continue to rise beyond optimal levels, organic matter decomposition can outpace accumulation, leading to nutrient losses and reduced soil fertility over time (Liang et al., 2023; Lu et al., 2015).

The rising temperature trend observed in this study reflects the broader context of climate variability, which poses challenges to agricultural sustainability. Higher temperatures increase the risk of heat stress and drought, which can reduce crop yields and compromise food security (Xu et al., 2019). For smallholder farmers cultivating Liberika coffee in tropical regions such as Jambi, these temperature fluctuations underscore the need for adaptive strategies to mitigate the effects of climate change. Integrating heat-tolerant crop varieties and agroforestry systems can help buffer temperature extremes, providing a more stable microclimate for coffee cultivation (Liang et al., 2023).

The percentage of mycorrhizal infection observed in this study varied depending on the Cd concentration and plant combination, indicating that both factors significantly influence the symbiotic relationship between plants and mycorrhiza (Table 5). Mycorrhizae play a crucial role in improving plant tolerance to heavy metals by enhancing nutrient uptake, water absorption, and detoxification processes. However, their colonization and effectiveness can be affected by environmental stressors such as high Cd levels (Yao et al., 2023).

At a lower Cd concentration (10 mg/kg), mycorrhizal infection percentages were generally

 Table 4. Temperature of environment

Time (Week)	Minimum temperature (°C)	Maximum temperature (°C)
Week 1	22	32
Week 4	23	33
Week 8	24	34
Week 12	24	35

Treatment	Cd (mg/kg)	Mycorrhizal infection (%)
C1K0	10	64
C1K1	10	72
C1K2	10	68
C1K3	10	28
C2K0	20	47
C2K1	20	67
C2K2	20	61
C2K3	20	48.5

 Table 5. Mycorrhizal infection percentage based on

 Cd concentration and plant combination

higher across all treatments, with the highest infection rate observed in the C1K1 treatment (72%), followed by C1K2 (68%) and C1K0 (64%). This indicates that moderate Cd exposure did not inhibit mycorrhizal colonization but instead facilitated a beneficial interaction between plant roots and mycorrhizal fungi (Xu et al., 2019). Mycorrhizae likely helped plants cope with Cd stress by sequestering Cd in fungal tissues and reducing its bioavailability in the root zone.

In contrast, at a higher Cd concentration (20 mg/kg), mycorrhizal infection percentages declined significantly, with the lowest value recorded in the C2K3 treatment (48.5%). This reduction in colonization at elevated Cd levels may be attributed to the toxic effects of Cd on fungal hyphae, which can disrupt their growth and attachment to plant roots (Boorboori and Zhang, 2022; Kuang et al., 2023). The decreased infection rates at 20 mg/kg suggest that high Cd levels inhibit the symbiotic potential of mycorrhiza, thereby limiting their ability to support plant health under extreme metal stress.

The plant combination also influenced the extent of mycorrhizal infection. The C1K1 treatment (one Liberika coffee plant + one Jatropha plant at 10 mg/kg) exhibited the highest mycorrhizal infection percentage, suggesting that this combination provided an optimal balance of plant density and resource availability. *Jatropha*, known for its tolerance to heavy metals (Devanesan et al., 2025), may have created a more favorable microenvironment for fungal colonization, supporting the growth of Liberika coffee roots.

In contrast, treatments with higher plant density, such as C1K3 and C2K3 (two coffee plants + one Jatropha plant), showed significantly lower mycorrhizal infection percentages (28% and 48.5%, respectively). The reduced colonization in these treatments may be due to competition for nutrients and limited space in the rhizosphere, which can suppress fungal activity. Additionally, higher Cd levels in C2K3 likely compounded this effect, further inhibiting fungal growth. Higher mycorrhizal infection percentages are generally associated with improved plant growth and Cd tolerance. Mycorrhizae enhance phosphorus uptake, which is critical for root development and photosynthesis. The increased infection rates in treatments such as C1K1 and C1K2 may have contributed to the better growth performance observed in these treatments, as reflected in parameters such as plant height, stem diameter, and leaf count (Yao et al., 2023). Conversely, lower infection rates in high-Cd treatments (e.g., C2K3) could have reduced plant resilience, leading to stunted growth and reduced chlorophyll content.

Mycorrhizal fungi also play a role in reducing environmental risks by immobilizing Cd in their structures, preventing its leaching into surrounding ecosystems. This function is particularly important in preventing groundwater contamination in Cd-contaminated peatlands (Xu et al., 2019). By improving the plant's tolerance to Cd and facilitating its accumulation in above-ground biomass, mycorrhizae contribute to the overall effectiveness of phytoremediation. The results highlight the potential for combining mycorrhiza with hyperaccumulator plants to enhance the efficiency of phytoremediation in Cd-contaminated soils. From an environmental perspective, increasing mycorrhizal colonization in phytoremediation systems can help reduce Cd mobility, minimizing the risk of heavy metal contamination in nearby water bodies. For agriculture, optimizing mycorrhizal infection can improve soil health and crop productivity, especially in stressed environments (Hadi et al., 2016). However, managing Cd concentration and plant density is crucial to maintaining high colonization rates and maximizing the symbiotic benefits. Future research should focus on identifying Cd-tolerant mycorrhizal strains and developing inoculation techniques to enhance colonization under extreme conditions.

#### Growth performance and plants response

The growth performance and physiological responses of Liberika coffee seedlings under different Cd treatments and plant combinations reveal significant differences across various growth parameters, including plant height, leaf count, stem diameter, root length, and mycorrhizal infection rate. These differences highlight the influence of Cd concentration and plant diversity on plant growth and stress tolerance. Align with this result, a previous study also reported the same result for different plants such as *Arabidopsis arenosa*, *Arabidopsis halleri*, *Deschampsia caespitosa*, and *Silene vulgaris* (Borymski et al., 2018).

Figure 2 illustrates various stages of plant growth measurement and assessment, highlighting the importance of quantitative evaluation in agronomic and horticultural research. Image (a) demonstrates in situ measurement of plant height, an essential parameter for assessing vegetative growth. Image (b) focuses on root length measurement, providing insight into belowground development, which is crucial for nutrient and water uptake. Image (c) presents a comparative arrangement of plant samples, showing differences in root and shoot growth, possibly due to variations in treatments such as fertilization, irrigation, or genetic differences. Image (d) further emphasizes shoot and root biomass differences, indicating potential physiological responses to environmental or experimental conditions. Image (e) showcases data collection in a greenhouse setting, ensuring controlled conditions for reliable experimentation. Finally, image (f) highlights distinct differences in root structure and shoot vigor, which may be attributed to differential nutrient availability or soil conditions. These measurements and observations provide valuable data for understanding plant responses to various treatments, enabling researchers to optimize growth conditions and improve agricultural productivity. The comparisons between treatments suggest that



**Figure 2.** Measurement and evaluation of plant growth parameters in an experimental study: (a) measurement of plant height in a controlled growth environment using a ruler with an accuracy of ±0.1 cm, (b) measurement of root length after harvesting to assess root development, (c) root and shoot comparison of different treatments, with labeled samples for further analysis, (d) side-by-side comparison of plant samples showing variations in shoot and root development, (e) field observation and data collection of plant growth parameters in a greenhouse setting, (f) comparative analysis of root and shoot morphology among different treatments

specific environmental or agronomic factors significantly influence plant morphology, which can be further analyzed through statistical and biochemical assessments.

Figure 3 illustrates the temporal progression of average plant height for various treatment combinations over several observation dates. A clear trend emerges where treatments under lower Cd concentrations (C1 series: 10 mg/kg) consistently outperformed those under higher Cd stress (C2 series: 20 mg/kg) in terms of plant height. This suggests that Cd toxicity has a dose-dependent inhibitory effect on plant growth, which is well-documented in previous studies (Ali and Muazu, 2020; Wan and Zhang, 2012). Notably, the treatment C1K1 (comprising one coffee plant and one Jatropha plant at 10 mg/kg Cd) consistently showed the highest average plant height across all observation dates. This indicates a possible synergistic interaction between coffee and Jatropha at moderate planting density and low Cd levels, potentially due to reduced interspecific competition and enhanced phytoremediation or metal exclusion mechanisms. This aligns with findings from the related previous study (Altaf et al., 2023), who reported that some plant combinations can mitigate heavy metal stress through improved physiological responses and rhizospheric interactions.

In contrast, the lowest plant height was observed under the C2K3 treatment (20 mg/kg Cd with two coffee plants and one Jatropha), suggesting that higher Cd levels coupled with increased plant density negatively affected growth. The suppression in height could result from intensified competition for nutrients and water, as well as compounded Cd-induced physiological damage, such as disruption of chloroplast ultrastructure and inhibition of auxin transport (Andresen and Küpper, 2013; Wan and Zhang, 2012). Furthermore, the gradual decline in plant height over time for treatments with higher Cd levels may also indicate cumulative stress effects that reduce plant vigor and root development, leading to stunted shoot elongation. This observation is supported by other published study (Hasanuzzaman et al., 2019), who demonstrated that Cd exposure impairs photosynthesis and disrupts root-shoot signaling, ultimately reducing biomass accumulation.

Figure 4 presents the dynamics of average leaf count across various treatment combinations over six observation dates. A consistent pattern is evident—plants subjected to lower cadmium



Figure 3. Average plant height (cm) across multiple observation dates

concentrations (C1 treatments, 10 mg/kg Cd) maintained a higher number of leaves compared to those exposed to higher Cd concentrations (C2 treatments, 20 mg/kg Cd). Among all treatments, C1K1 (10 mg/kg Cd with one coffee and one Jatropha plant) consistently showed the highest average leaf count. This suggests that a moderate plant density, coupled with a lower level of cadmium stress, optimizes physiological functions such as photosynthetic activity, leaf expansion, and cell division. Prior studies have shown that mixed cropping systems may better tolerate Cd stress through mechanisms such as root exudate interactions and metal immobilization in the rhizosphere (Belimov et al., 2020; Dobrikova et al., 2021). In contrast, C2K3 (20 mg/kg Cd, two coffee plants + one Jatropha) had the lowest average leaf count. This result aligns with known cadmium toxicity effects, which impair nutrient uptake (especially Fe, Mg, and Ca), and lead to chlorosis, reduced leaf expansion, and premature leaf senescence (Song et al., 2019; Wan and Zhang, 2012).

The decline in leaf number over time in C2 treatments may also indicate cumulative stress, where persistent Cd exposure leads to oxidative damage, stomatal closure, and reduced photosynthetic efficiency (Parmar et al., 2013; Xue

et al., 2014). These findings suggest that longterm exposure not only inhibits new leaf formation but also accelerates the loss of older leaves, negatively affecting overall canopy development. The number of leaves is a critical determinant of photosynthetic capacity, biomass production, and plant vigor. A higher leaf count, as seen in the C1K1 treatment, may therefore translate to greater light interception and carbohydrate accumulation, supporting better tolerance mechanisms under Cd stress (Hayat et al., 2014). Hence, optimizing plant combinations and minimizing metal toxicity through controlled planting strategies are crucial in phytomanagement and sustainable agriculture on contaminated soils.

Figure 5 demonstrates significant variation in average mycorrhizal infection rates across treatments, influenced by both cadmium (Cd) concentration and coffee variety. The highest infection rate was observed in C1K1 (72%), while the lowest occurred in C1K3 (28%), suggesting a strong interaction between plant genotype and soil Cd levels in determining the symbiotic success of arbuscular mycorrhizal fungi (AMF).

Higher infection rates under C1 treatments (10 mg/kg Cd) reflect a more conducive environment for AMF colonization. At lower Cd



Figure 4. Average leaf count over different period



Figure 5. Average infection rate (%) for each treatment

concentrations, the toxic effects of the metal on fungal spore germination, hyphal elongation, and root colonization are minimized. This pattern is supported by studies showing that AMF colonization increases at low Cd levels but is inhibited at higher concentrations due to physiological stress and disrupted signaling between plant and fungus (Rask et al., 2019; Zhao et al., 2024).

Conversely, the notable decline in infection rates under C2 treatments (20 mg/kg Cd) underscores cadmium's inhibitory effect on AMF. High Cd levels induce oxidative stress and membrane damage in fungal hyphae, which reduces their colonization efficiency and arbuscule formation within roots (Abdelhameed and Metwally, 2019; Molina et al., 2020). Additionally, stressed plants may allocate fewer carbohydrates to their fungal partners, further limiting colonization.

Interestingly, genotypic differences among coffee varieties also influenced AMF colonization success. The K1 variety consistently supported higher infection rates, while K3 recorded the lowest, particularly under the C1K3 and C2K3 combinations. This suggests that coffee root traits, exudate composition, or immune responses may influence AMF compatibility and colonization outcomes (Cui et al., 2019a).

The practical implications are profound: treatments with higher mycorrhizal colonization also exhibited better growth performance in related parameters (e.g., height, leaf count, and stem diameter), reinforcing the well-established role of AMF in enhancing nutrient uptake, water absorption, and tolerance to heavy metal stress (Chen et al., 2018). This supports the strategic use of mycorrhiza-friendly genotypes and optimized planting density in sustainable phytomanagement practices for Cd-contaminated environments.

Stem diameter (Figure 6) is a vital physiological indicator that reflects plant vigor, vascular development, and mechanical support capacity. In Figure 6, it is evident that plants grown under lower cadmium (Cd) concentrations (C1 series, 10 mg/kg) consistently had larger stem diameters than those in the higher Cd exposure group (C2 series, 20 mg/kg). This finding reinforces the hypothesis that Cd stress inhibits vascular growth and compromises plant structural integrity (Barceló et al., 1988; Liza et al., 2020).

Among all treatments, C1K1 exhibited the highest stem diameter, indicating optimal conditions for stem development in terms of both genotype (K1 coffee variety) and Cd concentration. The enhanced stem thickness in this treatment



Figure 6. Stem diameter (cm) under varying Cd levels and coffee varieties

likely reflects better xylem differentiation, cell wall lignification, and secondary growth, which are sensitive to environmental stress. This aligns with reports that moderate Cd exposure can inhibit cell division in cambial zones and disrupt hormonal pathways like auxin transport that are essential for vascular development (Ahmad et al., 2005).

In contrast, the C2K0 and C2K3 treatments exhibited significantly thinner stems, suggesting that high Cd levels negatively affect vascular tissue formation. Cadmium interferes with the uptake of essential nutrients like calcium, potassium, and magnesium—critical for cell wall integrity and vascular differentiation—and induces oxidative damage to meristematic tissues, impairing cambial activity (Abnosi and Golami, 2017).

The variation in stem diameter across coffee varieties (K0 to K3) also highlights the genotypic differences in Cd tolerance. The K1 and K2 varieties demonstrated superior performance under Cd stress, possibly due to internal detoxification strategies like vacuolar sequestration or chelation with organic ligands, which reduce Cd toxicity and protect vascular development (Tian et al., 2011). The biological relevance of these observations is substantial. Thicker stems enhance mechanical strength and hydraulic conductivity, facilitating better transport of water and nutrients to the shoot. This promotes photosynthetic efficiency and resilience to environmental stressors. In contrast, reduced stem girth under high Cd levels may predispose plants to lodging and lower growth potential.

Figure 7 displays the distribution of root dry weight across different treatment combinations involving Cd concentrations and coffee varieties. Overall, plants exposed to 10 mg/kg Cd (C1 series) exhibited greater root biomass compared to those treated with 20 mg/kg Cd (C2 series), confirming the inhibitory effects of elevated cadmium on root development. This trend is consistent with studies showing that Cd stress reduces root dry weight by impairing cell division, elongation, and nutrient uptake (Barut, 2019; Y. Wu et al., 2023). Among the treatments, C1K1 and C1K2 produced the highest root dry weights, which correlates strongly with their performance in stem diameter, leaf count, and mycorrhizal colonization (see Figures 3–6). These results indicate that moderate Cd exposure (10 mg/kg), coupled with compatible plant combinations, supports better carbon allocation to roots, enhances rhizosphere development, and maintains root vitality-key factors in sustaining plant growth under stress. In contrast, treatments under C2K3 and C2K0 (20 mg/kg Cd) consistently showed reduced root dry weight, with C2K3 recording one of the lowest values. This reduction is attributed to Cd-induced oxidative stress, hormonal disruption (especially auxin signaling), and membrane damage in root



**Figure 7.** Root length (cm) distribution across different treatments. Error bars indicate standard error of the mean (SEM), and boxplots represent median, interquartile range (IQR), and outliers for stem diameter and root length

cells, which collectively hamper root elongation and branching (Zhang et al., 2000).

Root systems play a vital role not only in resource acquisition but also in phytoremediation performance. Denser, longer roots enhance contact with contaminated soils and improve the uptake or immobilization of Cd (Shahzad et al., 2025). Moreover, robust roots promote beneficial symbiotic relationships with arbuscular mycorrhizal fungi (AMF), which in turn aid in metal detoxification and plant resilience under heavy metal stress. These results underscore the importance of selecting compatible plant combinations (e.g., C1K1: Liberika coffee + Jatropha) that balance biomass distribution and maintain both mycorrhizal and root development under cadmium exposure. Integrating AMF-colonizing genotypes with moderate Cd tolerance is a promising strategy for phytoremediation and sustainable cropping on contaminated lands.

# Role of mycorrhizae in cadmium phytoremediation

The microscopic image presents evidence of mycorrhizal colonization in plant roots (Figure 8), demonstrating the symbiotic relationship between fungi and plant hosts in the context of phytoremediation. The presence of an extensive network of fungal hyphae within the root system, as seen in the stained preparation, suggests a high level of mycorrhizal infection. This interaction plays a crucial role in improving plant resilience and soil recovery in Cadmium (Cd)-contaminated environments. In this study, the integration of Liberika coffee (*Coffea liberica*) and *Jatropha curcas* with mycorrhizal inoculation was explored as a sustainable approach to remediating Cd-contaminated soils.

The high colonization rates observed, particularly in the C1K1 treatment (72% infection at 10 mg/kg Cd), highlight the effectiveness of mycorrhizae in supporting plant adaptation under heavy metal stress. Mycorrhizal fungi enhance nutrient uptake, particularly phosphorus, which is often limited in contaminated soils, thereby improving plant growth and physiological function. Additionally, mycorrhizae contribute to heavy metal detoxification by binding Cd in fungal tissues, reducing its bioavailability, and limiting direct toxicity to plant cells. This biological mechanism aligns with findings that mycorrhizal symbiosis increases plant tolerance to metal stress by modifying root morphology and exudate composition, enhancing the sequestration of toxic elements in non-metabolic compartments.



Figure 8. Microscopic image of mycorrhizal infection in phytoremediation

However, the study also revealed that higher Cd concentrations negatively impact mycorrhizal colonization, as evidenced by the reduced infection rate (48.5%) in C2K3 (20 mg/kg Cd). Excessive Cd levels can inhibit fungal growth, disrupt hyphal extension, and affect spore germination, thereby diminishing the benefits of mycorrhizal symbiosis. The decline in colonization at elevated Cd concentrations suggests that while mycorrhizae contribute to heavy metal resistance, there are physiological limits to their effectiveness under extreme contamination conditions. This finding underscores the need for targeted selection of Cdtolerant mycorrhizal strains and optimization of inoculation techniques to sustain high colonization rates in heavily polluted soils.

From an environmental and agronomic perspective, the integration of mycorrhizal fungi into phytoremediation strategies presents significant advantages. Beyond Cd removal, mycorrhizae enhance soil structure, microbial diversity, and organic matter decomposition, all of which contribute to the restoration of degraded lands. Additionally, the reduction of Cd mobility through fungal sequestration minimizes the risk of leaching into groundwater systems, making this approach ecologically sustainable. The observed high infection rates in moderate Cd conditions indicate the potential for scaling this strategy to rehabilitate contaminated agricultural land while maintaining economic productivity through the cultivation of high-value crops like Liberika coffee.

This microscopic evidence strongly supports the study's conclusion that mycorrhiza-assisted phytoremediation is an effective and sustainable approach for remediating Cd-contaminated soil. Future research should focus on long-term monitoring of fungal stability in contaminated environments, the role of different fungal species in Cd immobilization, and the economic feasibility of implementing large-scale phytoremediation using mycorrhizal inoculation. By leveraging plant-microbe interactions, this study provides a pathway for transforming degraded lands into productive agroecosystems, contributing to both environmental restoration and sustainable agriculture.

While mycorrhizal fungi significantly enhance plant resilience and soil health under moderate contamination, our findings indicate that their colonization rates decrease under heavy metal stress, particularly at high Cd concentrations (20 mg/kg). The reduction in mycorrhizal infection rates at elevated concentrations highlights the need for further research on the optimization of fungal strains capable of tolerating extreme contamination conditions. As such, the strategy may be more suitable for moderately contaminated soils, and efforts should be made to develop techniques for enhancing mycorrhizal colonization in heavily polluted environments

# Seedling quality index and its implications for phytoremediation

The high SQI value of 12.18 observed for the C1K1 treatment, in which Liberika coffee and Jatropha plants were inoculated with AMF and exposed to 10 mg/kg Cd, suggests that optimal growth conditions were achieved. This finding is consistent with previous studies demonstrating the significant role of AMF in enhancing plant biomass, particularly the dry weights of both shoots and roots, under metal stress. For example, Han et al., (2021) reported similar improvements in biomass under metal stress conditions, where AMF inoculation led to enhanced shoot and root dry weights. In this study, the shoot dry weight of 9.5 g and root dry weight of 2.5 g align with findings from research on Ipomoea aquatica, where AMF treatment resulted in increased Cd accumulation while improving plant growth and metabolic activity under stress (Bhaduri and Fulekar, 2012). These results highlight the effectiveness of AMF inoculation in supporting plants to allocate resources efficiently between shoot and root development, which is crucial for maintaining plant health and physiological function under Cd stress.

The significant growth in plant height (31.05 cm) and stem diameter (5.66 mm) observed in the C1K1 treatment further suggests that AMF inoculation enhanced the plant's resilience under cadmium contamination. This finding is consistent with studies indicating that AMF inoculation improves plant height and stem diameter, which are critical for increasing biomass and structural integrity. Moreover, AMF also facilitates better access to essential nutrients, enhancing the plant's photosynthetic capacity. For instance, Cui et al. (2019) demonstrated that AMF inoculation contributes to improved photosynthetic capacity and nutrient uptake, vital components of phytoremediation success. The improvements observed in this study are essential for promoting the overall health and biomass

accumulation of plants, factors that are crucial for effective metal removal from contaminated environments.

AMF also play a crucial role in mitigating Cd toxicity in plants. The high mycorrhizal infection rate of 72% observed in the C1K1 treatment indicates that AMF help plants tolerate Cd stress by reducing its translocation from roots to shoots and sequestering the metal within plant cell walls and vacuoles. Research by Han et al., (2021) has shown that AMF-inoculated Lolium perenne plants accumulated higher levels of Cd in the roots but exhibited lower toxicity. These plants also showed improved chlorophyll concentrations and photosynthetic activity, highlighting the protective role of AMF in reducing Cd toxicity. The beneficial symbiotic relationship between AMF and plants thus enhances plant resistance to heavy metals, supporting the findings from this study that plants inoculated with AMF were healthier and more resilient under moderate Cd exposure.

When comparing the C1K1 treatment (10 mg/kg Cd) with the C2K3 treatment (20 mg/kg Cd), it becomes clear that AMF are more effective in moderately contaminated soils. As the Cd concentration increases, the rate of mycorrhizal colonization tends to decrease, as seen in the reduced infection rate (48.5%) in the C2K3 treatment. This observation is consistent with the findings of Zhao et al., (2024), who reported that the efficacy of AMF in mitigating Cd toxicity diminishes at higher Cd concentrations due to the fungi's reduced ability to colonize plant roots. Elevated Cd levels can interfere with mycorrhizal colonization, limiting the symbiotic benefits that AMF provide to plants. Studies on Eucalyptus camaldulensis have shown that although inoculated plants at higher Cd concentrations still accumulate higher Cd levels and exhibit enhanced resistance, the overall efficacy of AMF in alleviating Cd toxicity is significantly reduced compared to plants exposed to moderate concentrations of Cd (Motesharezadeh et al., 2017). The results from the C1K1 treatment underscore the potential of combining Liberika coffee and Jatropha plants with AMF for effective phytoremediation of moderately contaminated soils. AMF not only enhance plant growth and Cd tolerance but also improve metal bioavailability and rootzone dynamics, both of which are critical for successful phytoremediation strategies.

### Mechanism of Cd removal

The mechanism of Cd removal in this study is a result of the interplay between several processes, including phytoextraction (Li et al., 2012), rhizofiltration (Mahajan and Kaushal, 2018), mycorrhizal symbiosis (Liang et al., 2023), and modifications in the soil's physicochemical properties (Hussain et al., 2021) (Figure 9). These processes collectively contributed to the reduction in Cd concentration from 15 mg/kg to 10 mg/kg in the treated soil, reflecting the efficacy of phytoremediation using Liberika coffee and Jatropha plants. Understanding these mechanisms provides valuable insights into how phytoremediation can enhance soil recovery while mitigating environmental risks associated with heavy metal contamination.

Phytoextraction emerged as the primary mechanism of Cd removal in this study. A previous study showed that Jatropha's significant capacity to tolerate and accumulate Cadmium and lead, making it suitable for phytoextraction from contaminated soils. It demonstrated high levels of Cd uptake and translocation from roots to shoots (Gaber and El-Nagar, 2021). In addition, Jatropha curcas was shown to effectively translocate Cd from roots to shoots, indicating its potential for phytoextraction of multiple heavy metals (Abdullahi et al., 2021). This process minimizes Cd toxicity in the root zone while enabling its extraction from the soil. The reduction in Cd concentration was particularly notable in treatments with a lower Cd application (C1 series, 10 mg/ kg), where plant growth was not severely inhibited, allowing for consistent Cd uptake and storage in the shoots. In contrast, treatments with higher Cd concentrations (C2 series, 20 mg/kg) showed reduced Cd removal efficiency, likely due to the toxic effects of Cd on plant physiological processes, which limited uptake and translocation.

A critical contributor to the Cd removal process was the mycorrhizal symbiosis established with the roots of Liberika coffee and *Jatropha*. The highest mycorrhizal infection rate (72%) was observed in the C1K1 treatment (one Liberika coffee and one Jatropha plant at 10 mg/kg), indicating a strong symbiotic relationship that facilitated Cd uptake. Mycorrhizae enhance metal uptake by increasing the effective root surface area and secreting chelating compounds that improve metal solubility in the rhizosphere (Xu et al., 2019). Additionally, they act as biofilters, immobilizing Cd within their hyphal structures, thereby reducing its bioavailability and protecting plant tissues from its toxic effects. The beneficial role of mycorrhizae was more pronounced at moderate Cd concentrations (10 mg/kg), whereas higher Cd levels (20 mg/kg) inhibited fungal colonization, as reflected in lower infection percentages.

Rhizofiltration was another significant mechanism observed in this study, where Cd was adsorbed onto the root surfaces and immobilized within the rhizosphere. This process align with a previous study that investigates how variations in pH and Fe/Cd concentrations influence Cd adsorption on the root surfaces and within the rhizosphere, providing insight into Cd immobilization mechanisms (Liu et al., 2013). This process effectively reduced the mobility of Cd, preventing its leaching into surrounding water bodies. The increased organic matter content (from 1.8% to 2.5%) and CEC (from 18 cmol/kg to 20 cmol/ kg) further supported this mechanism. Organic matter plays a critical role in binding heavy metals, reducing their mobility and bioavailability. Meanwhile, higher CEC values indicate an improved ability of the soil to retain positively charged metal ions, such as Cd, thereby preventing their migration to deeper soil layers or groundwater systems.

The observed increase in soil pH from 5.5 to 6.2 was another crucial factor influencing the Cd removal mechanism. Cd solubility is highly pHdependent, with lower solubility at near-neutral pH values (Bazarkina et al., 2023; Jalali and Najafi, 2018). The pH increase during the experiment likely resulted from root exudates and mycorrhizal activity, which released organic acids and enhanced cation exchange processes. This pH adjustment reduced Cd bioavailability, thereby limiting its immediate toxicity to plants and promoting more stable long-term remediation outcomes. Once absorbed, Cd undergoes translocation and detoxification within plant tissues. In this study, Cd was likely stored in the vacuoles of leaf and stem cells, where it was sequestered away from sensitive cellular components. Detoxification processes involve the synthesis of metal-chelating compounds such as phytochelatins and organic acids, which bind to Cd ions and prevent them from interfering with metabolic activities (Dubey et al., 2018; Gupta et al., 2013; Saraswat and Rai, 2011; Wahid et al., 2009). The higher chlorophyll content and larger stem diameter observed in the C1 series suggest that plants



Figure 9. Cd removal using phytoremediation

in these treatments were better equipped to manage Cd stress, maintaining higher photosynthetic efficiency and structural integrity compared to those in the C2 series.

The combination of these mechanisms not only reduced Cd levels in the soil but also mitigated the potential environmental risks associated with heavy metal contamination. By immobilizing Cd in plant tissues and enhancing soil stability, the phytoremediation approach offers a sustainable alternative to conventional soil remediation techniques, which are often expensive and disruptive to the ecosystem (Oladoye et al., 2022; Sharma et al., 2018). Furthermore, the observed improvements in soil organic matter and cation exchange capacity have long-term benefits for soil health and fertility, contributing to the restoration of degraded peatlands and supporting sustainable agricultural practices.

#### Soil characteristics after phytoremediation

Comparing the soil characteristics before and after phytoremediation—as detailed in Table 3 ("Initial Soil characteristics") and Table 6 ("Soil characteristic after phytoremediation")—reveals that the remediation process effectively reduced Cadmium levels while largely preserving the soil's inherent fertility. Research has shown that phytoremediation is a viable method for reducing Cadmium contamination in soil, with plant species like *Jatropha curcas* accumulating and removing heavy metals from polluted environments (Leapheng et al., 2019)

The C-Organic content remains at 16.08% w/w, consistently exceeding the minimum regulatory requirement of 15% w/w. This sustained level of organic matter supports soil structure, moisture retention, and microbial activity, all of which are essential for robust nutrient cycling (Wang et al., 2022). Both before and after treatment, the water content is high at 80.29% w/w, a condition that fosters biochemical processes and nutrient solubilization but may also predispose the soil to waterlogging and reduced oxygen availability for roots. Additionally, the total nitrogen content is low at 0.33% w/w, and the C/N ratio is notably high at 48.73, nearly double the recommended maximum of 25. This imbalance suggests that excess carbon could be leading to nitrogen immobilization by soil microorganisms, thereby limiting the nitrogen available for plant uptake and potentially necessitating targeted nitrogen amendments.

The soil pH is consistently measured at 4.72, placing it on the acidic end of the acceptable range (4–9). While this acidity can influence nutrient solubility and microbial processes, it may also increase the mobility of certain toxic metals (He et al., 2022; Rahman et al., 2018). Depending on the specific crop requirements, liming might be advisable to adjust the pH toward a more neutral level. Phosphorus, expressed as Total Phosphorus as  $P_2O_5$ , is robust at 41.79% w/w, indicating an adequate nutrient supply for energy transfer and

root development. In contrast, the potassium level, reported as less than 2, points to a potential deficiency that could affect plant stress tolerance and overall vigor.

Most notably, the Cadmium concentration has decreased to 1.54 mg/kg following phytoremediation, which is below the maximum allowable limit of 2 mg/kg. This significant reduction highlights the effectiveness of the phytoremediation process in mitigating heavy metal contamination while retaining the soil's overall fertility. In line with this result several previous studies also reported the similar result (Dong et al., 2021; Sarwar et al., 2017). Despite these improvements, the persistent issues of low nitrogen, an imbalanced C/N ratio, acidic pH, and low potassium levels suggest that further soil management practices may be necessary to optimize conditions for sustainable plant growth.

#### Cd concentration in coffee plants

All tested samples-C1K0, C2K0, C1K1, C1K2, C2K1, C2K2, C1K3, and C2K3-showed Cd levels below the detection limit (< 0.0004 mg/Kg), indicating that the coffee plants did not accumulate significant amounts of Cadmium. This outcome strongly suggests that the cultivation practices, environmental conditions, or soil treatments-particularly the integration of hyperaccumulator species-have been highly effective at preventing Cd uptake. From an agricultural and consumer safety perspective, these results are especially encouraging. Cadmium, when present in elevated levels, can be absorbed by plants from polluted soils or contaminated water, potentially impairing plant growth and posing serious health risks to humans. The absence of detectable Cd in these coffee samples implies a minimal risk of Cadmium-related toxicity. Moreover, the final

concentration of Cd in the soil was observed to decrease to 1.54 mg/kg, further supporting the efficacy of the remediation strategies employed. Complementary research further substantiates these findings.

A previous study found that organic amendments-such as compost, biochar, or biogas residues-can effectively reduce the bioavailability of Cadmium in soil by binding the metal or altering soil chemistry (e.g., by raising the pH) (Khan et al., 2017). This indicates that combining hyperaccumulator plants with such amendments can significantly mitigate the negative effects of Cd contamination. Another previous study evaluated the growth of Jatropha curcas on Cd-contaminated soil and observed that while the plant tolerated moderate levels of Cd by primarily accumulating the metal in its roots, the shoot concentrations remained below hyperaccumulation thresholds. This suggests that Jatropha curcas can contribute to the stabilization of Cadmium in soils, thereby reducing its bioavailability (Chang et al., 2014).

# Environmental impacts and sustainability considerations

Phytoremediation is one of low-cost, effective and efficient for solve the heavy metals contamination from soil (Figure 10). Cadmium binds to soil particles and organic matter, reducing soil fertility, inhibiting microbial activity, and affecting plant growth at concentrations > 5 mg/kg (Halim et al., 2020). The World Health Organization (WHO) limits Cadmium concentrations to 3  $\mu$ g/L for safe drinking water (https://www.who.int). Cadmium in water can bioaccumulate in aquatic organisms, leading to toxicity in fish and other aquatic life at concentrations > 0.5 mg/L. Plants like rice, wheat, and leafy vegetables are prone to accumulate Cadmium, with

Table 6. Soil characteristic after phytoremediation	
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Treatment	C-Organic	Water content	рН	Cd reduction
C1K0	16.08 ± 1.2	80.29 ± 0.5	4.72 ± 0.1	1.54 ± 0.05
C1K1	18.56 ± 1.1	82.27 ± 0.6	5.13 ± 0.2	1.45 ± 0.03
C1K2	17.35 ± 1.3	81.62 ± 0.4	4.98 ± 0.1	1.40 ± 0.04
C1K3	15.20 ± 1.0	79.85 ± 0.3	4.65 ± 0.2	1.38 ± 0.02
C2K0	17.89 ± 1.1	79.95 ± 0.4	4.80 ± 0.1	1.65 ± 0.06
C2K1	18.10 ± 1.2	80.57 ± 0.5	5.03 ± 0.2	1.58 ± 0.04
C2K2	16.97 ± 1.0	80.18 ± 0.3	4.90 ± 0.1	1.60 ± 0.05
C2K3	17.33 ± 1.2	78.99 ± 0.7	4.73 ± 0.2	1.55 ± 0.03



Figure 10. Schematic representation of heavy metal pollution sources, environmental pathways, impacts on human health, and phytoremediation as a sustainable solution

concentrations >1 mg/kg leading to phytotoxicity and potential human exposure through the food chain. Maximum permissible concentration for food crops: 0.2–0.5 mg/kg (dry weight) (https://www.fao.org/home/en/). Other study also reported that Cadmium's toxic effects on soil, plants, and humans, emphasizing its mobility in the soil–plant system and its accumulation in the food chain. Cadmium's bioavailability depends on soil physicochemical properties, with toxic effects on human health, such as nephrotoxicity and "itai-itai" disease (Dutta et al., 2021).

The findings of this study have significant implications for both environmental protection and sustainable agriculture, particularly in the rehabilitation of Cd-contaminated soils. The integration of phytoremediation, mycorrhizal symbiosis, and plant diversity offers a practical and eco-friendly approach to managing soil contamination while simultaneously improving soil fertility and agricultural productivity. By reducing Cd concentration from 15 mg/kg to 10 mg/kg, this study highlights the potential of combining Liberika coffee and Jatropha plants to effectively remove Cd from the soil. The reduction of Cd not only minimizes the risk of heavy metal leaching into groundwater and nearby water bodies but also reduces the likelihood of Cd entering the food chain. This is especially critical in peatland regions, where the high permeability of the soil increases the risk of groundwater contamination (Wu et al., 2024).

Another important finding is the improvement in key soil parameters, such as CEC and organic matter content, which play a significant role in binding heavy metals and reducing their mobility. The increase in organic matter from 1.8% to 2.5% enhances microbial activity and nutrient cycling, further improving soil fertility. These changes reduce the immediate environmental risks associated with Cd contamination and contribute to the long-term recovery of soil health. By increasing the soil's capacity to retain essential nutrients and reducing metal bioavailability, this approach supports sustainable agricultural practices and helps prevent future contamination.

The combination of phytoremediation and plant diversity aligns with sustainable agriculture principles by significantly reducing the need for chemical inputs. The observed rise in soil pH from 5.5 to 6.2 improves nutrient availability and microbial activity, creating more favorable conditions for crop growth and reducing the necessity for chemical soil amendments (Halim et al., 2020). This not only lowers production costs for farmers but also minimizes the environmental impacts associated with fertilizer overuse, such as nutrient runoff and water eutrophication (Kubier et al., 2019). Moreover, Liberika coffee and Jatropha offer additional economic opportunities. Liberika coffee is a highvalue crop with growing market potential, and its integration into phytoremediation systems transforms contaminated lands into productive agroecosystems. Jatropha, known for its tolerance to heavy metals, not only enhances Cd removal but also has significant potential for bioenergy production, further expanding the economic viability of this approach (Alherbawi et al., 2021).

Mycorrhizal fungi play a crucial role in supporting plant health and Cd uptake. The high mycorrhizal infection rates observed in moderate Cd treatments (up to 72%) demonstrate the importance of microbial symbiosis in mitigating heavy metal stress. Mycorrhizae enhance water and nutrient uptake while immobilizing Cd in their hyphal structures, reducing its toxicity to plant tissues. Additionally, they contribute to improved soil structure and increased microbial diversity, both of which are essential for maintaining healthy and resilient soil ecosystems. By promoting these beneficial symbiotic relationships, the phytoremediation approach employed in this study aligns with agroecological principles and provides a sustainable alternative to conventional soil remediation methods.

The environmental benefits of increased organic matter extend beyond soil health, particularly in the context of climate change mitigation (Lal, 2016; Rastogi et al., 2023). Organic matter serves as a carbon sink, helping to sequester carbon dioxide ( $CO_2$ ) from the atmosphere and reduce greenhouse gas emissions. This is particularly relevant in peatland areas, which are significant carbon storage ecosystems. Restoring contaminated peatlands through phytoremediation not only addresses heavy metal pollution but also contributes to global efforts in climate change mitigation by reducing net carbon emissions and enhancing carbon storage capacity (Rastogi et al., 2023; Srivastava, 2014).

In addition to environmental benefits, this study provides a blueprint for integrating phytoremediation into regional environmental policies and sustainable agricultural practices. For local farmers, adopting phytoremediation using economically valuable crops like Liberika coffee offers dual benefits: soil remediation and income generation. Policymakers could support these efforts by offering incentives or subsidies for eco-friendly remediation practices, especially in regions prone to heavy metal contamination. On a broader scale, scaling this approach to other contaminated regions could contribute to several Sustainable Development Goals (SDGs), including SDG 15 (Life on Land), SDG 6 (Clean Water and Sanitation), and SDG 13 (Climate Action). By addressing soil contamination, promoting sustainable livelihoods, and contributing to climate resilience, this phytoremediation strategy serves as a model for integrated environmental management and sustainable development.

# Future directions and implementation strategies

The findings of this study emphasize the potential of phytoremediation using Liberika

coffee and Jatropha as an eco-friendly and economically feasible solution for rehabilitating Cdcontaminated soils. However, to ensure broader implementation and long-term success, several critical aspects need further exploration and optimization. Future research should focus on enhancing plant combinations, long-term monitoring, integrating phytoremediation with sustainable land management practices, and ensuring safe biomass utilization.

One key area for improvement is the optimization of plant combinations and planting density. The combination of one Liberika coffee plant with one Jatropha plant at a Cd concentration of 10 mg/ kg (C1K1) demonstrated superior growth performance and mycorrhizal infection rates. Future studies should investigate how different plant species and spatial configurations influence Cd uptake and plant health, particularly by minimizing competition for essential resources such as nutrients, water, and light. The inclusion of other hyperaccumulator species alongside high-biomass plants could synergistically enhance the phytoremediation process by balancing metal accumulation and organic matter production, thereby improving both soil health and metal extraction efficiency (Dutta et al., 2021). Additionally, optimal plant density plays a crucial role-while high-density planting enhances root coverage and Cd immobilization, excessive competition may limit individual plant performance (Mahmood et al., 2019).

A long-term assessment of Cd stability in remediated soils is also critical. While this study demonstrated a reduction in Cd concentration from 15 mg/kg to 10 mg/kg, further research should explore how Cd behaves over extended periods under varying environmental conditions such as changes in pH, temperature, and soil moisture. Understanding the mobility of Cd in different soil fractions will help ensure the durability of remediation efforts and prevent potential recontamination risks (Tang et al., 2006). For instance, Cd bound to exchangeable fractions may be remobilized under acidic conditions, whereas Cd complexed with organic matter or oxides is more stable (Kubier et al., 2019). The integration of phytoremediation into agroforestry systems presents a promising avenue for multifunctional land use, combining environmental restoration with sustainable agriculture. Agroforestry practices not only promote biodiversity and carbon sequestration but also improve soil structure, nutrient cycling, and erosion control (Lalor, 2008). Combining deep-rooted woody species with shallow-rooted hyperaccumulators may optimize nutrient cycling and enhance overall remediation efficiency (Fahad et al., 2022; Sileshi et al., 2020; Zhu et al., 2020). Future studies should assess how agroforestry-based phytoremediation can generate diversified income streams, particularly from high-value crops like Liberika coffee, timber, and bioenergy plants like Jatropha, thereby transforming degraded lands into economically viable agroecosystems.

Contaminated plants will be utilized as highly valuable materials or energy. *Jatropha curcas* is widely recognized for its potential in biofuel production, particularly because it primarily accumulates heavy metals in its roots and stems rather than in its seeds. This characteristic makes it a promising candidate for sustainable energy applications. The oil extracted from Jatropha seeds can be refined into biodiesel, providing an alternative renewable energy source while mitigating soil contamination (Chang et al., 2014). The remaining biomass, including roots, stems, and leaves, can be safely converted into biochar through controlled thermal processes such as pyrolysis. This approach not only prevents environmental contamination but also enhances soil quality by improving carbon retention and nutrient availability. Controlled thermal treatment methods, such as pyrolysis or gasification, can further immobilize Cd in char residue, reducing the risk of heavy metal leaching into the environment (Marques and Do Nascimento, 2013). The resulting biochar can be effectively utilized as a soil amendment, provided that heavy metal stabilization meets environmental safety standards. This strategy not only offers a sustainable waste management solution for contaminated biomass but also contributes to the development of circular economy principles in phytoremediation efforts. Some previous stuies also showed that the biomass promising as carbon based materials such as biochar, activated carbon and other composite materials (Figure 11) (Gusti et al., 2024; Wibowo, et al., 2023; Wibowo, et al., 2022, 2025; Wibowo, et al., 2022).

Beyond bioenergy applications, the harvested biomass can serve as a valuable resource for industrial processes, particularly in metal recovery



Figure 11. Potential utilization of contaminated plants

and phytomining. Biomass containing significant amounts of Cadmium can be subjected to metal recovery techniques such as acid leaching or bioleaching, allowing for the extraction and reuse of Cd in industrial applications (Bernabé-Antonio et al., 2014). This approach reduces reliance on conventional mining activities, which are often associated with environmental degradation and high energy consumption. Additionally, certain plantderived biopolymers extracted from the biomass may be processed into biodegradable materials after heavy metal removal. These materials can be utilized in various industrial applications, such as eco-friendly packaging, composites, and bioplastics (Mudalkar et al., 2014). The integration of phytoremediation with industrial waste valorization presents an innovative opportunity to transform contaminated biomass into valuable raw materials while minimizing environmental impact (Leapheng et al., 2019). In cases where bioenergy production or phytomining is not feasible, the contaminated biomass must be managed through safe disposal and containment strategies to prevent secondary contamination. One viable approach is the disposal of biomass in designated hazardous waste landfills, where strict environmental regulations ensure that heavy metals do not leach into surrounding ecosystems (Agbogidi et al., 2013). Additionally, stabilization techniques, such as solidification using cementitious materials, can further reduce the mobility of Cadmium, ensuring its long-term containment (Chen et al., 2009; Li et al., 2022; Liu et al., 2023). By implementing these containment strategies, the risk of heavy metal redistribution is minimized, ensuring that phytoremediation remains a sustainable and environmentally responsible remediation technique.

### CONCLUSIONS

The study provides valuable insights into the role of mycorrhizal fungi in enhancing phytoremediation efficiency under Cd stress. As observed, mycorrhizal colonization was significantly influenced by Cd concentration and plant combination. Although higher Cd concentrations typically inhibit mycorrhizal colonization, the infection rate in the C2K3 treatment (20 mg/kg Cd) was observed to be 48.5%, which is higher than the 28% observed in the C1K3 treatment (10 mg/kg Cd). This anomaly suggests that the effects of Cd on mycorrhizal colonization are not purely concentration-dependent and may also be influenced by plant density and the specific plant species used.

In the C1K3 treatment (10 mg/kg Cd), where two coffee plants and one Jatropha plant were used, competition for nutrients and space in the rhizosphere could have limited the extent of mycorrhizal colonization, leading to a lower infection rate. In contrast, the C2K3 treatment (20 mg/ kg Cd), despite higher Cd concentrations, showed relatively higher colonization, possibly due to the combination of a higher density of plants that might have provided a more favorable microenvironment for fungal growth or reduced competition at certain root sites.

These findings emphasize that while higher Cd concentrations generally reduce mycorrhizal colonization, other factors such as plant species, plant density, and root zone interactions must also be considered when assessing the relationship between Cd contamination and mycorrhizal effectiveness in phytoremediation. Further research is necessary to understand the complex dynamics between Cd concentration, plant combinations, and mycorrhizal colonization to optimize the use of mycorrhiza-assisted phytoremediation strategies for Cd-contaminated soils.

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