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RESEARCH ARTICLE

Phytoremediation of mercury-contaminated Soil by *Vigna radiata* L. plant in companion with bacterial and fungal biofertilizers

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Abstract

Mercury is one of the most toxic pollutants that has drawn the attention of scientists. This study investigates the phytoremediation capabilities of *Vigna radiata* L. in conjunction with microbial biostimulators. The inoculated seeds were cultivated in soil under controlled greenhouse conditions. The concentration of Hg, biomass, and photosynthetic pigments was investigated under amendment factor including EDTA, bacterial, fungal (Mycorrhiza and *Trichoderma*), biochar, and combined levels, as well as the pollution factor with three levels of $HgCl_2$ as two factorial experiments. Results showed that Plant Growth-Promoting Microorganisms (PGPMs) influenced mercury absorption and distribution in different plant organs. Aside from biochar, all stimulators increased the plant's Hg concentration. Although EDTA greatly increased mercury accumulation in plants, it reduced biomass. Fungal and bacterial treatments increased total mercury in the plant but decreased its concentration in the leaves. The combination of bacteria and fungi resulted in the highest mercury absorption, while the biochar in combination with PGPMs produced the greatest biomass. Analysis of mercury concentration in seeds indicated that *V radiata* effectively prevented its contamination in seeds. The results disclosed that microbial combinations of bacteria and fungi could increase the plant's potential to cope with heavy metal pollution. This improvement is due to the different roles of these two organisms, like nitrogen fixation by bacteria and phosphorus absorption by mycorrhiza fungi. Moreover, biochar as a soil amendment and microorganism carrier was noticed. Finally, considering the plant's inherent capacity to stabilize mercury in the roots, phytostabilization with the benefit of combined levels of biochar and microorganisms can be introduced as the best approach.

Keywords Biochar · Pseudomonas · AMF · Mercury · Food safety

Introduction

A significant environmental concern is the introduction of heavy metals into the soil through various human activities (Chamba et al. 2017; Natasha et al. 2020; Mousavi et al. 2022). Key sources of this contamination include industrial and domestic wastewater, metal smelting, mining operations, fuel production, and the use of agricultural chemicals (Li et al. 2022). Industrial activities often release heavy metals as byproducts, which can subsequently enter the soil via atmospheric deposition or direct discharge. Furthermore, agricultural practices exacerbate the problem when synthetic fertilizers, pesticides, and herbicides containing heavy metals leach into the soil (Maftouh et al. 2024). Improper management and disposal of industrial and municipal waste also contribute to the accumulation of heavy metals (Ali et al. 2013). Additionally, emissions from vehicles further contaminate the soil, as metals from exhaust and tire wear

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settle on the ground (Wang et al. 2018). These activities lead to the accumulation of heavy metals in the soil, which poses risks to both plant and animal health and may enter the food chain, ultimately impacting human health.

Mercury (Hg) is a highly toxic substance that plays no role in biological processes (Tangahu et al. 2011; Mohammadi et al. 2021). Human exposure to Hg has been linked to several harmful effects on the immune, digestive, and neurological systems, in addition on the kidneys, lungs, and skin (WHO 2019). In some circumstances, they can be fatal and cause mental retardation, seizures, loss of eyesight and hearing, delayed development, and cognitive impairments (Chamba et al. 2017). As one of the top ten chemicals of major public health concern, it is crucial to manage Hg in soil–plant-human systems effectively (Natasha et al. 2020).

To address specific environmental issues and prevent further pollution, it is crucial to develop more efficient, eco-friendly, rapid, and sustainable methods for remediating heavy metals from contaminated soils (Harindintwali et al. 2020). Evolutionary plants are unique for cleaning up the contamination and improving the environmental health since the earliest stages of life on Earth. Additionally, microorganisms play a key role in supporting these vital remediation efforts.

Phytoremediation offers a promising alternative to chemical and physical remediation methods, being more efficient, cost-effective, and environmentally friendly (Mucha et al. 2011; Rocha et al. 2016; Mousavi et al. 2021). The primary phytoremediation methods include phytoextraction, where contaminants are absorbed by plant roots and accumulate in aerial parts; phytostabilization, which immobilizes contaminants in the soil; phytodegradation, involving the breakdown of pollutants by plants; rhizofiltration, the purification of water through plant roots; and phytovolatilization, where contaminants are released into the atmosphere via transpiration (Ashraf et al. 2019; Edenborn et al. 2015).

For the Hg removal, phytoextraction and phytostabilization are the most relevant methods (Natasha et al. 2020). Enhancing Hg bioavailability through chemicals like EDTA can aid in plant absorption and stabilization of Hg in plant tissues (Liu et al. 2020a). Increased plant biomass is a crucial requirement for phytoremediation, with a large amount of Hg accumulating within tissues without hindering plant growth (Natasha et al. 2020; Liu et al. 2020b). Hg severely affects the growth, seedlings and roots, as well as the efficiency of photosynthesis and metabolic equilibrium, which results in a loss of plant mass (Tiodar et al. 2021). In this situation, using plant growth-promoting microorganisms (PGPMs) to clean up a mercury-contaminated site is a desirable solution.

Studies have shown that fungi and plant growth-promoting rhizobacteria (PGPR) can improve plant development and resistance to heavy metal stress (Harindintwali et al. 2020; Tiodar et al. 2021; Maheshwari et al. 2019; Maddahi et al. 2022). These microorganisms contribute to plant adaptation and stress mitigation through various mechanisms (Tiodar et al. 2021; Alizadeh et al. 2021). In addition to increasing metal tolerance, bacteria also produce plant growth promoters (PGP) and have antibacterial activity against phytopathogens (Rajkumar et al. 2009; Ullah et al. 2015; Rocha et al. 2016; Ganeshan and Kumar 2005). Bacteria from the genus *Pseudomonas* are found in diverse environments and have shown beneficial effects when associated with plants (Maheshwari et al. 2019; Karimi et al. 2022). However, research on their role in bioremediation is limited (Rocha et al. 2016).

Mycorrhizal fungi, which form symbiotic relationships with Over 80% of plant species, enhance nutrient uptake, alter nutrient bioavailability, and shields roots from nematodes and parasitic fungi (Smith et al. 2011). They also show resistance to heavy metal stress (Debeljak et al. 2018).

Plants employ a range of strategies to cope with heavy metal stress, which can be categorized into avoidance and tolerance mechanisms. Avoidance strategies include the exclusion of metals by restricting their entry through root barriers and immobilizing them in the rhizosphere (Fashola et al. 2024; Ali et al. 2013). Tolerance mechanisms involve chelation and sequestration, where plants produce metal-binding compounds such as phytochelatins and metallothioneins to neutralize toxicity, and transport these complexes into vacuoles. Antioxidative defense mechanisms, both enzymatic (e.g., superoxide dismutase, catalase) and non-enzymatic (e.g., glutathione, ascorbate), combat oxidative stress induced by heavy metals (Mustafa and Komatsu 2016). Plants also modify their metabolic pathways, produce stress proteins, and use specific transport proteins to compartmentalize metals into less sensitive parts of the cell (Ullah et al. 2015; Mahar et al. 2016; Tangahu et al. 2011).

Research has demonstrated that biochar enhances microbial degradation of contaminants and supports climate change mitigation by sequestering carbon in the soil (Huggins et al. 2014; Ahmad et al. 2014; Abbasi et al. 2023). Adding biochar to agricultural soils increases soil organic matter availability, microbial activity, water retention, soil remediation, and crop production while mitigating anthropogenic climate change (Harindintwali et al. 2020). This multifaceted approach underscores biochar's effectiveness in addressing various environmental challenges and enhancing sustainable agricultural practices. Biochar, a stable carbon-rich material produced by pyrolysis of organic matter, is highly effective for soil remediation, particularly in immobilizing heavy metals and treating gasoline contamination (Sashidhar et al. 2020; Brassard et al. 2019; Harindintwali et al. 2020). Biochar's porous structure and negatively charged surfaces enable it to adsorb and immobilize metal ions like lead, cadmium, and arsenic, reducing their bioavailability and mobility in the environment. Additionally, the functional groups on biochar can complex with heavy metals, precipitating them as less soluble forms. This process significantly lowers the risk of these contaminants entering the food chain or leaching into groundwater (Premalatha et al. 2023). Furthermore, biochar's large surface area allows it to adsorb harmful hydrocarbons such as benzene, toluene, ethylbenzene, and xylene (BTEX), thereby reducing their concentration in the soil. Biochar can enhance microbial activity, particularly that of hydrocarbon-degrading bacteria, further breaking down these pollutants into less harmful substances. This dual action of adsorption and microbial enhancement makes biochar an eco-friendly and sustainable solution for remediating soils contaminated. (Premalatha et al. 2023; Wei et al. 2024).

Vigna radiata L was chosen for this study due to its exceptional attributes that make it an excellent candidate for phytoremediation. Its nitrogen-fixing capability, rapid growth, high biomass production, deep root system, and heavy metal tolerance allow it to flourish in contaminated soils while improving soil health (Baza et al. 2022). Additionally, *V. radiata*, a legume of significant economic importance in various regions, is relevant for research aimed at enhancing agricultural practices and environmental health (Zulfiqar et al. 2022; Parveen et al. 2024; Baza et al. 2022). Its dual function as a phytoremediator and a valuable food source underscores its potential to remediate contaminated soils and ensure the safety of its edible parts.

Although, *V. radiata* has demonstrated resistance to several heavy metals (Rahdarian et al. 2022), plants are not universally resistant to all heavy metals, they often exhibit resistance to specific metals based on their physiological and biochemical characteristics (Singh et al. 2016). Plants have evolved mechanisms to tolerate, accumulate, or detoxify specific metals, making them suitable for targeted phytoremediation. Many plants show selective tolerance and uptake capabilities, with resistance to metals such as Hg but not to others like lead (Pb) or arsenic (As) (Tangahu et al. 2011). The mechanisms of metal tolerance and detoxification are often metal-specific, involving the production of chelating agents or antioxidants tailored to particular metals (Ashraf et al. 2019). Therefore, selecting plant species for phytoremediation depends on their effectiveness in managing the specific contaminants present in the soil (Ali et al. 2013).

In this experiment the Hg phytoremediation potential of *V. radiata* plant association with bacterial (*Pseudomonas*) and fungal (*Trichoderma* and mycorrhiza) treatments, single and in combined, was investigated. Moreover, biochar and EDTA (ethylenediaminetetraacetic acid) was added, biochar as organic carbon capture material and soil amendment, and EDTA to better view in comparison of PGPM and chemical treatment. The study analyzed the biomass and Hg accumulation in various plant parts, including roots, stems, leaves, and seeds. Photosynthetic pigments were examined as key indicators of photosynthesis, a critical life process. Additionally, the concentration of Hg in seeds was assessed to evaluate the food safety of *V. radiata* grown in Hg-contaminated soils. This study hypothesizes that combining microbial biostimulators with *V. radiata* will enhance phytoremediation efficiency in mercury-contaminated soils, making it a competitive alternative to chemical treatments such as EDTA. Specifically, it is expected that biochar will improve the survival and activity of microorganisms by serving as a carrier. This combined approach is anticipated to produce greater biomass compared to using biochar or microorganisms alone.

Material and methods

Soil collection and preparation

The soil was collected from an agricultural area on the Urmia University campus with the properties listed in Table 1. The soil was mixed with river sand in a ratio of 2:1, soil crop and sand respectively. Each plastic pot, 19 cm in height and diameter, was filled with 6 kg of the soil-sand mixture. Biochar was added at a concentration of 5% to the respective pots.

Plant cultivation and microbial inoculation

After soaking the seeds for 8 h, microbial inoculation liquates were prepared for different levels and then applied to *V radiata* seeds carefully. Three seeds were planted per pot and grown under greenhouse conditions. During the third leaf stage, Hg contamination was introduced via $HgCl_2$ irrigation at three levels (0, 20, 40 mg/L) twice a week. After

Table 1 Soil properties

EC	0.48 ds/m
pH	7.72
Lime	11.4%
Organic mater	0.46%
Clay	35%
Silt	29%
Sand	36%
Tex	C.L
N	0.05%
P	11.6 mg/Kg
K	305 mg/Kg
Fe	10.2 mg/Kg
Zn	0.38 mg/Kg
Mn	15.38 mg/Kg
Cu	1.77 mg/Kg

14 weeks and 6 times of Hg application, the plants were harvested.

The experiment involved seven levels of treatments: EDTA (E), Bacteria (Ba), *Trichoderma* (T), *Trichoderma* + Mycorrhiza (TM), Bacteria + *Trichoderma* + Mycorrhiza (BTM), Biochar (Bi), Bacteria + *Trichoderma* + Mycorrhiza + Biochar (MiBi) and three Hg concentration (0, 20 and 40 mg/liter) as two factor factorial experiments in completely randomized design. The PGPMs including Bacteria (*Pseudomonas putida* strain P13*, Pseudomonas koreansis* strain S14*, Pseudomonas vancouverensis* strain S19*, Pseudomonas japonica* fz.21–1*, Pantoea agglomerans* Q4) and Mycorrhiza (*Glomus entunicatom, Glomus mosseae, Glomus intraradices*) and *Trichoderma haziantum* prepared from Green Biotech Incorporation.

The preparation of biochar and EDTA application

Dehydrated wood was pyrolyzed in anaerobic conditions for 14 h at 550°C. After pyrolysis, it was ground and sieved through a 2-mm mesh. A 1:20 (w/v) biochar-to-distilled water suspension was prepared and shaken for two hours to measure pH and electrical conductivity (EC). pH was measured with a pH meter (Corning, 7, UK), and EC was measured with a conductivity meter (Hanna, HI 8819, Portugal). Biochar was heated in a muffle furnace at 550°C and 450°C to determine ash and volatile matter concentrations, respectively. Organic matter concentration was assessed using the loss on ignition method at 550°C (Wu et al. 2012; Abbasi et al. 2023). Nutrient content was determined by digesting biochar with HNO_3 and $HClO_4$ at a 3:1 ratio. The characteristics of apple wood biochar were: EC = 0.79 dS/m, pH = 8.35, N = 0.9%, P = 0.23%, K = 0.41%, organic content = 53%, and C/N ratio = 58.8.

EDTA treatment was applied via irrigation at a concentration of 0.5 mM, four times throughout the growth period, in conjunction with HgCl2 levels.

Plant sampling

For biomass measurement, one representative plant per pot was selected, and its root and aerial parts were weighed separately. Leaf samples for photosynthetic pigment analysis were stored at -80°C.

Measurement of Hg and translocation factor

The plant organs (root, stem, leaf, seed) were washed three times with ultrapure water and dried for 72 h at 65°C and turned into powder. A combination of three replicates of each treatment was analyzed as one sample for Hg concentration using ICP-MS (Elan 9000 DRCe, Perkin Elmer) in Zarazma Laboratory, Tehran, Iran. The detection limit was 100 ppb.

The translocation factors were calculated as follows (Mohammadi et al. 2021):

Root to stem = C in stem/C in root
Stem to leaf = C in leaf/C in stem
Root to shoot = C in shoot/C in root

where C represents Hg concentration (mg/kg).

Total Hg content per plant was calculated as:

Total Hg per plant = Concentration of Hg in plant (mg/kg) × Biomass (kg)

The percentage of Hg fixed in the roots relative to the total Hg in the plant was determined as:

(Root Hg / Total Hg) × 100

The measurement of photosynthetic pigments

Photosynthetic pigments were measured using the method described by Lichtenthaler and Wellburn (1983). Leaf samples were extracted with pure acetone, centrifuged at 2500 rpm for 10 min, and the absorbance of the supernatant was measured at 662 nm, 645 nm, and 470 nm using a UV/Vis spectrophotometer (Halo DB 20 Double beam). Pigments concentrations were calculated as follows:

$\text{Chl } a = 11.75\ A_{662} — 2.350\ A_{645}$
$\text{Chl } b = 18.61\ A_{645} — 3.960\ A_{662}$
$C_{X+C} = (1000\ A_{470} — 2.270\ \text{Chl } a\ -81.4\ \text{Chl } b)\ /227$

Statistical analysis

The study will involve analyzing samples from the roots and aerial parts of 72 pots, which constitute the statistical population. A completely randomized design with 21 treatments will be utilized in a factorial experiment, with each treatment replicated three times. The primary parameters measured will include the translocation factor (TF), Hg concentration in various plant tissues, and photosynthetic pigments. Data will be subjected to analysis using SAS software, with ANOVA employed to determine significant differences among treatments. Tukey's test will be applied for post-hoc analysis, with a significance threshold set at $P \leq 0.05$. Graphical representations of the results were created using Microsoft Excel to facilitate the interpretation and presentation of the data.

Results

Biomass

The comparison between controls (Cs of three Hg levels) showed that Hg pollution decreased both shoot and root biomass, however, the decline in the root was more pronounced (Fig. 1). Under non-polluted level, the fungal treatment of TM produced less biomass than bacteria, but TM produced the most biomass under stress conditions, particularly in the root. The maximum dry weight recorded in MiBi, in combination biochar, in all three levels of Hg contamination.

Under the high pollution level of Hg40, both bacterial and fungal treatments individually and in combination, increased root and shoot biomass significantly. EDTA decreased the biomass under Hg40, so EDTA recorded the lowest amount in both root and shoot.

Hg concentration

Hg concentrations in different plant parts treated with various stimulators are shown in Fig. 2. PGPMs notably increased Hg concentration in the stems compared to controls, with the maximum increases observed at Hg20 and Hg40, where bacteria and fungi elevated stem Hg levels by 114.5% and 102%, respectively. Conversely, these microorganisms decreased Hg content in the leaves, particularly under Hg20. The lowest leaf Hg concentrations were recorded with bacterial and TM treatments, which reduced Hg levels by 34% and 25%, respectively, compared to the control of Hg20 level. Additionally, under Hg40, the combined treatments of BTM and MiBi further reduced leaf Hg by 21% and 46%, respectively.

EDTA treatment resulted in the highest Hg concentration in plants at Hg20 compared to other treatments, while at Hg40, the highest concentration was observed with BTM. Phytostabilization in the roots was most effective with BTM treatment, where 90% of the total Hg was concentrated in the roots under Hg40. Microbial combinations were more effective in accumulating Hg in the roots than single treatments. Biochar, whether used alone or in combination with microorganisms, reduced Hg levels in plants, particularly in the roots. Overall, all amendments, except for biochar, increased the total Hg concentration in plants exposed to mercury.

Total Hg concentrations in plants at Hg20 were ranked as follows: E > BTM > TM > Ba > T > MiBi > Bi with concentrations of 81, 69, 59, 55, 54, 43, and 27 mg/Kg DW respectively. At Hg40, the rankings were BTM > E > TM > Ba > T > MiBi > Bi, with concentrations of 240, 193, 167, 159, 154, 79, and 72 mg/kg DW respectively.

Mycorrhiza combined with bacteria improved the plant's remediation capability more than either alone.

Hg remediation per plant and transfer factor

In addition to concentration, the amount of total Hg in each plant was examined. This calculation highlights the critical role of biomass in a plant's potential for Hg remediation. The analysis revealed that while the concentration in the plants decreased following MiBi treatment, the overall total Hg content increased, indicating an enhanced remediation capacity (Fig. 3).

At moderate contamination level, Hg accumulation per plant was highest in BTM (3.02 mg), followed by TM (2.98 mg), E (2.9 mg), MiBi (2.51 mg), Ba (2.39 mg), T (2.04 mg), C (1.59 mg), and Bi (1.06 mg). Similarly, at high contamination level, BTM again exhibited the highest accumulation

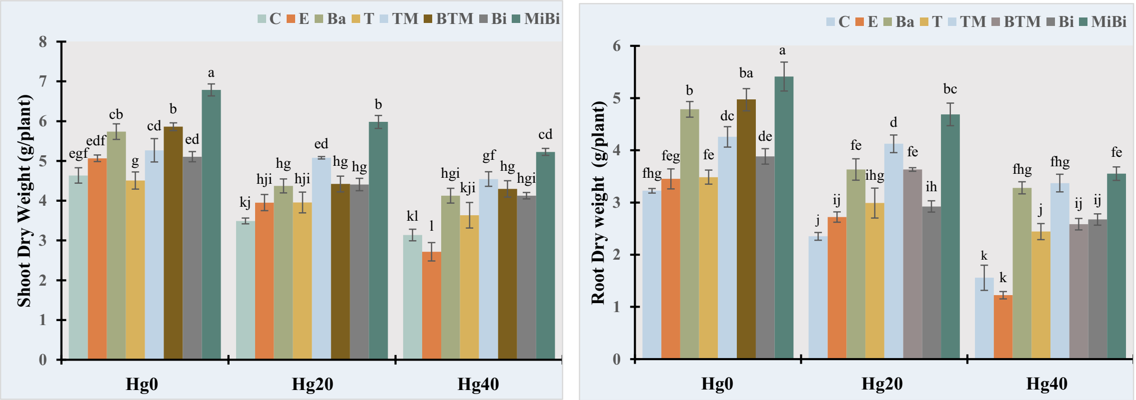

Fig. 1 Root and shoot dry weight of munge bean plant (*V. radiata*) under three levels of Hg pollution; Hg0 (without Hg), Hg20 ($Hg_{20mg/L}$), Hg40 ($Hg_{40mg/L}$), and seven levels of stimulator; E = EDTA; Ba = bacteria; T = *Trichoderma*; M = mycorrhiza; BTM = bacteria + *Trichoderma* + mycorrhiza; Bi = biochar; MiBi = bacteria + *Trichoderma* + mycorrhiza + biochar and C = control. Different letters show a significant difference at the $P < 0.05$ level based on Tukey's test

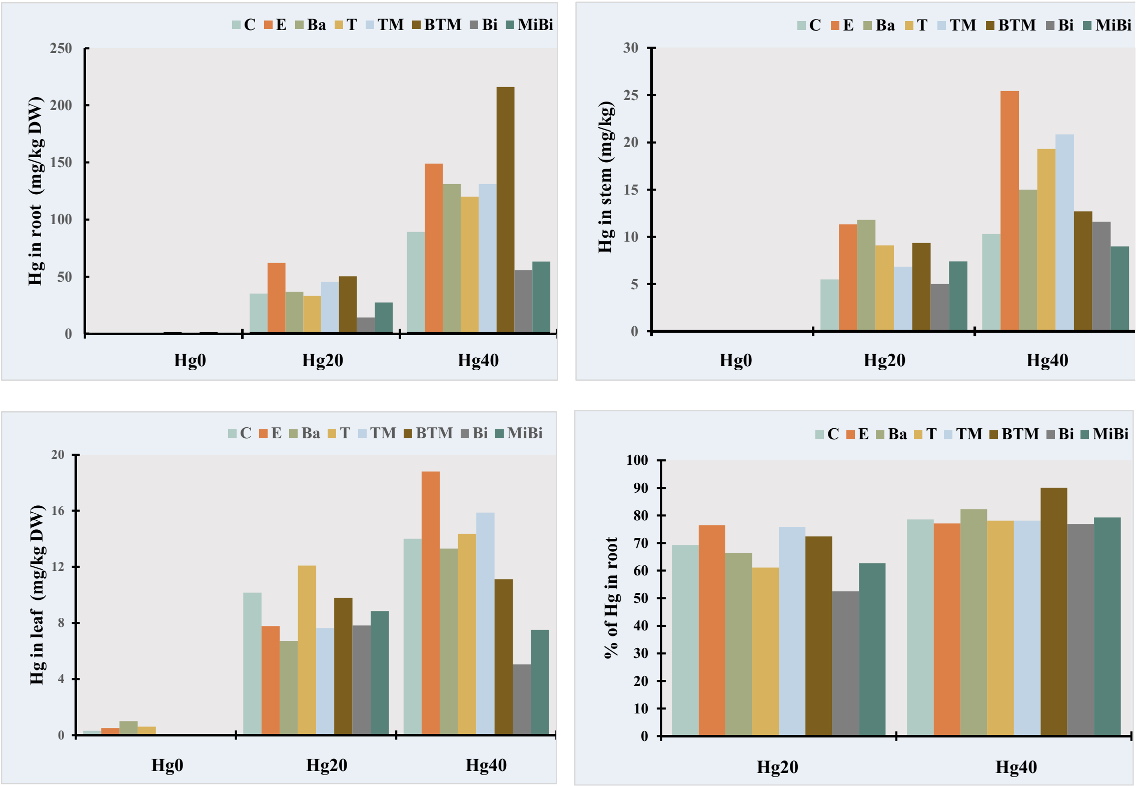

Fig. 2 Hg concentration in different organs of munge bean plant (*V. radiata*) under three levels of Hg pollution; Hg0 (without Hg), Hg20 ($Hg_{20mg/L}$), Hg40 ($Hg_{40mg/L}$), and seven levels of stimulator; E = EDTA; Ba = bacteria; T = *Trichoderma*; M = mycorrhiza; BTM = bacteria + *Trichoderma* + mycorrhiza; Bi = biochar; MiBi = bacteria + *Trichoderma* + mycorrhiza + biochar; and C = control

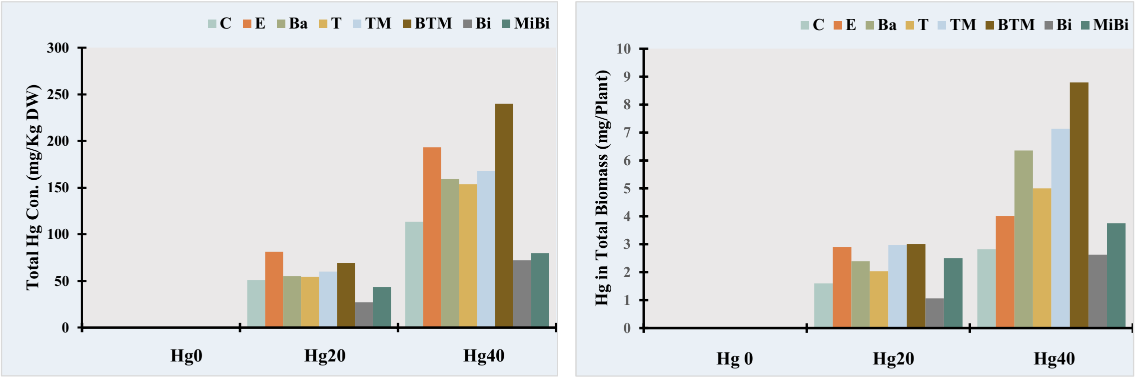

Fig. 3 Total Hg concentration in plant and total Hg accumulation per plant in munge bean (*V. radiata*) under three levels of Hg pollution; Hg0 (without Hg), Hg20 ($Hg_{20mg/L}$), Hg40 ($Hg_{40mg/L}$), and seven levels of stimulator; E = EDTA; Ba = bacteria; T = *Trichoderma*; M = mycorrhiza; BTM = bacteria + *Trichoderma* + mycorrhiza; Bi = biochar; MiBi = bacteria + *Trichoderma* + mycorrhiza + biochar; and C = control

(8.8 mg), followed by TM (7.14 mg), Ba (6.36 mg), E (5 mg), MiBi (4.02 mg), C (3.75 mg), and Bi (2.63 mg).

A significant shift in the translocation of pollutants within the plants occurs when the pollution level in the soil is increased. All treatments at Hg40 exhibited a lower root-to-shoot TF compared to Hg20, except for EDTA (Fig. 4). Among the treatments, BTM strongly reduced TF at high contamination level.

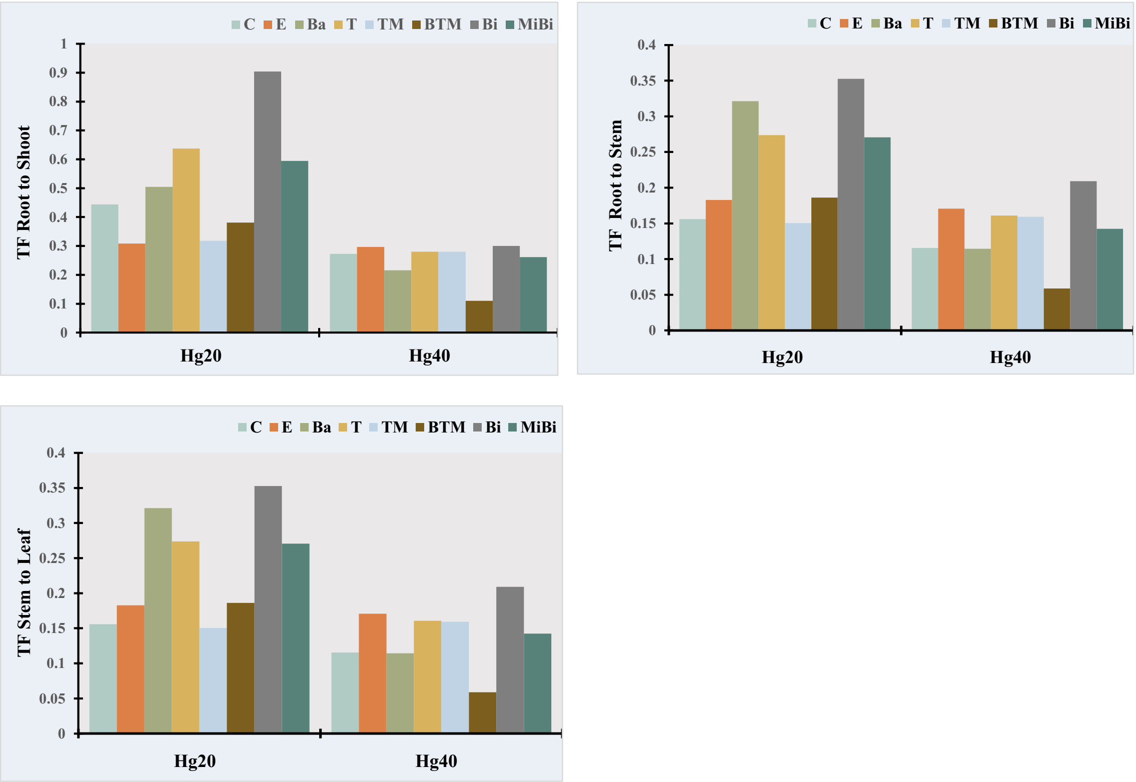

Fig. 4 Hg transfer factor (TF) between deferent organs of munge bean (*V. radiata*) under three levels of Hg pollution; Hg0 (without Hg), Hg20 ($Hg_{20mg/L}$), Hg40 ($Hg_{40mg/L}$), and seven levels of stimulator; E = EDTA; Ba = bacteria; T = *Trichoderma*; M = mycorrhiza; BTM = bacteria + *Trichoderma* + mycorrhiza; Bi = biochar; MiBi = bacteria + *Trichoderma* + mycorrhiza + biochar; and C = control

Photosynthetic pigments

At Hg20, chlorophyll *a* (Chl *a*) decreased more than chlorophyll *b* (Chl *b*) compared to controls (Fig. 5). All biostimulant treatments increased both Chl *a* and Chl *b* under Hg20 and Hg40, particularly in bacterial treatments and BTM combinations. TM fungi enhanced Chl *a*, Chl *b*, and carotenoid content with increasing pollution. At Hg40, all microbial treatments and EDTA increased Chl *b* content compared to controls. BTM showed decreased Chl *b* content at High pollution level compared to moderate. EDTA, bacteria, and BTM enhanced pigment content under Hg20 compared to Hg0, but decreased under Hg40 compared to Hg20.

Carotenoid content increased with Hg pollution, with Hg40 > Hg20 > Hg0. Bacteria reduced carotenoid content at Hg0 but increased it at Hg20. EDTA, Trichoderma, TM fungi, and BTM showed no significant differences at Hg0. However, at Hg20, bacteria, T, BTM, and Biochar increased carotenoid content compared to controls. TM fungi and BTM exhibited the highest carotenoid content at Hg40, with carotenoid content increasing in TM fungi and MiBi in parallel with Hg pollution.

Discussion

Phytoremediation relies on plant species capable of tolerating and removing pollutants over extended periods. This study evaluates the effectiveness of various treatments, including biochar, microbial inoculants, and chemical amendment EDTA, in enhancing the mercury-removal capacity of *V. radiata*. Given that no plant species is recognized as a hyperaccumulator of Hg (Tiodar et al. 2021), leveraging the synergistic effects of plants and microorganisms offers a viable approach.

Biomass

Our study observed that plant biomass decreased with increasing Hg concentration in the soil. Hg is readily absorbed by plants through mechanisms similar to those for essential micronutrients (Zhang et al. 2017). In non-inoculated (control, and EDTA) plants exposed to the pollution, biomass decreased, which impacted the overall phytoremediation efficiency.

Marrugo-Negrete et al. (2016) examined the impact of Hg on *Jatropha curcas* plants grown in hydroponic cultures

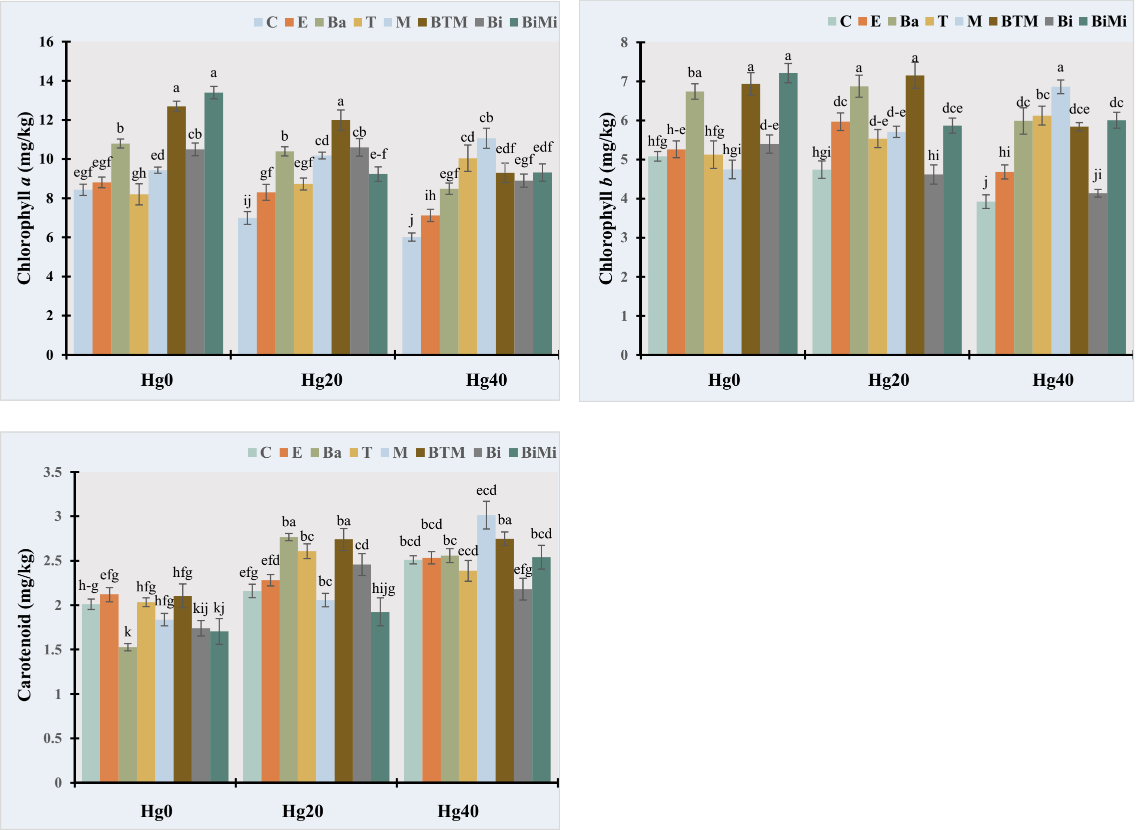

Fig. 5 Photosynthetic pigmen in munge bean plant (*V. radiata*) under three levels of Hg pollution; Hg0 (without Hg), Hg20 ($Hg_{20mg/L}$), Hg40 ($Hg_{40mg/L}$), and seven levels of stimulator; E = EDTA; Ba = bacteria; T = *Trichoderma*; M = mycorrhiza; BTM = bacteria + *Trichoderma* + mycorrhiza; Bi = biochar; MiBi = bacteria + *Trichoderma* + mycorrhiza + biochar; and C = control. Different letters show a significant difference at the $P < 0.05$ level based on Tukey's test

supplemented with various amounts of 5, 10, 20, 40, and 80 g/mL of Hg. The findings revealed a decrease in biomass, reduced development, and photosynthetic inhibition (Natasha et al. 2020). When *Brassica juncea* was exposed to 25 and 50 mM Hg, the dry biomass fell by 25% and 37%, respectively (Ansari et al. 2009).

However, in our study, bacterial treatments led to an increase in plant biomass even in exposure Hg concentrations. The plant survival and growth under toxic metal stress is facilitated by interactions with PGPB, beside increasing the uptake of heavy metals (Harindintwali et al. 2020; Tiodar et al. 2021). PGPB are a varied collection of prokaryotes that live in a variety ecological niche. These microbes may be free-living in the rhizosphere (rhizobacteria), occupying root nodules (rhizobia), or residing inside the tissues of plants (endophytes) (Tiodar et al. 2021). PGPB increased plant health in different ways including: provide valuable nutrients (e.g., fixed N, Fe, P, Zn), signals for induction of systemic resistance, e.g., volatile organic compounds, hormones (e.g., abscisic acid, ethylene, jasmonate, cytokinins, gibberellins, indole-3-acetic acid), enzymes (e.g., 1-aminocyclopropane-1-carboxylate deaminase, chitinases, cellulases, proteases, lipases), antibiotics or siderophores (Glick 2012; Ma et al. 2016; Naik et al. 2019; Tiodar et al. 2021; Moradzadeh et al. 2021).

The previous publishers have fully documented the growing effects of AM on plant biomass. In this study, AM was treated along with *Trichoderma* to look at the association effects of these two fungi. Our results show that plants treated with TM fungi increased root to shoot biomass ratios strongly. Increasing root biomass and pollution accumulation enhanced phytostabilization capacity in the plant. This aligns with previous research highlighting the role of mycorrhization in improving plant biomass and metal stabilization (Smith et al. 2011; Moradzadeh et al. 2021).

Hg content and translocation

The combination of bacteria and fungi plays a significant role in Hg dynamics within plants. While this microbial synergy increased the total Hg content in plants, it notably reduced Hg accumulation in leaves, which are crucial for photosynthesis. This reduction in leaf suggests an adaptive mechanism to minimize toxicity in the most sensitive parts of the plant.

PGPMs enhance metal ion bioavailability by altering soil pH and producing chelators (Kumar Yadav et al. 2018; Franchi et al. 2017; Liu et al. 2020a). Additionally, the mycelia of filamentous fungi facilitate bacterial spread in the soil, enhancing ecological processes like biodegradation and nutrient cycling (Banitz et al. 2013). Fungal exudates provide carbon sources that promote bacterial growth and can also act as signaling molecules, inducing bacterial phosphatase gene expression and further strengthening the interaction between these microorganisms (Zhang et al. 2018; Jiang et al. 2021). This microbial synergy significantly enhances the plant's ability to manage and stabilize the pollution. The PGPM, particularly the BTM amendment, proved most effective in Hg remediation and distribution. Under high Hg contamination, 90%, and under moderate contamination, 72% of the total Hg was sequestered in the roots, resulting in the root-to-shoot translocation factor (TF) decreasing from 0.38 under Hg20 to 0.11 under Hg40 with BTM treatment. Indicating a strategic reduction in Hg movement from roots to shoots as contamination levels increased. plant adaption is improved in association with PGPMs strategy to limit the Hg translocation and protecting aerial parts. This mechanism is crucial for mitigating toxicity and is supported by the role of heavy metal-resistant microbes in converting toxic heavy metals into less hazardous forms (Harindintwali et al. 2020; Azubuike et al. 2016). Interestingly, EDTA was found to be more effective in increasing Hg concentration in plants at moderate pollution than at higher contamination level. However, PGPM was effective in both stress levels due to increasing plant resistance with PGP production.

Plants can be classified based on their interaction with heavy metals into several categories. Hyperaccumulators are capable of absorbing and concentrating extremely high levels of metals, such as *Thlaspi caerulescens* for zinc and cadmium (Cosio et al. 2004), and *Pteris vittata* for arsenic (Zhao et al. 2023a, b). Excluders limit metal uptake and maintain lower metal concentrations in their tissues. Accumulating plants take up moderate amounts of heavy metals (Ali et al. 2013), such as our selected plant *V radiata*. These classifications aid in selecting appropriate plants for targeted phytoremediation strategies.

Comparative efficacy of EDTA and PGPM

In phytoremediation, both EDTA and PGPM are employed to enhance the removal of heavy metals from contaminated soils. Each offers distinct advantages, but also presents different trade-offs. EDTA, a chemical chelator, enhances Hg uptake by forming strong complexes with Hg ions, which increases their solubility and mobility in the soil. These Hg-EDTA complexes are more bioavailable for plant uptake and can be translocated to various plant tissues, thereby improving Hg absorption (Liu et al. 2020b; Abbasi et al. 2023; Mousavi et al. 2022). However, this study found that while EDTA was effective at lower contamination level, it also led to the lowest biomass among all treatments with increasing the toxicity. previous reports indicating that EDTA, despite its efficacy in enhancing heavy metal uptake, can be detrimental to plant health and may pose environmental risks, such as secondary contamination and disruption of soil microbial communities. (Rodríguez et al. 2016). On the other hand, PGPM offers a more sustainable and eco-friendly approach to phytoremediation. Though it may be slower and less predictable in its efficacy, PGPMs improve plant and soil health with minimal environmental impact (Beltyukova et al. 2023).

Combining EDTA with PGPM, or selecting the appropriate treatment based on specific site conditions and remediation goals, can optimize phytoremediation outcomes. However, the use of EDTA should be carefully managed due to its environmental risks, and its application should be tailored to the specific plants and mercury-contaminated soils involved (Tiodar et al. 2021; Lebrun et al. 2023). This study suggests that PGPM may offer a more effective and sustainable solution for phytoremediation, particularly in severe contaminated areas.

Impact of biochar on phytoremediation

Biochar, both alone and in combination with microorganisms, showed distinct effects compared to other amendments. Specifically, biochar reduced the Hg concentration in plants, acting as a stabilizer. This eco-friendly amendment was associated with the highest biomass production across all contamination levels, supporting the role of biochar in enhancing plant growth while minimizing metal uptake (Liu et al. 2020a; Harindintwali et al. 2020). Biochar does not reduce the total amount of heavy metals in the soil, it does decrease their bioavailability and phytotoxicity, thereby enhancing phytostabilization when combined with metal-immobilizing plants (Edenborn et al. 2015). Additionally, biochar has proven to be an effective carrier for microbial agents, providing a suitable habitat that protects introduced microorganisms from desiccation and predation (Edenborn et al. 2015; Harindintwali et al. 2020). Abbasi et al. 2023 used biochar to investigate Zea mays L.'s capacity to absorb metals, that resulted in an increase in plant length and dry weight relative to non-amended treatment, suggesting that biochar amendment could lessen Hg phytotoxicity.

Understanding the interactions between biochar, PGPM, and plants is crucial for managing heavy metal-contaminated environments in an ecologically and economically sustainable way. This knowledge also opens up the potential for developing novel green technologies for environmental remediation.

Photosynthetic pigments

Exposure to Hg in higher plants disrupts several biochemical and physiological processes, including photosynthesis and chlorophyll synthesis (Jain and Gadre 2004). In our study, the highest content of Chl *a* and Chl *b* were observed in plants treated with BTM and mycorrhiza. Nevertheless, Mondal et al. (2015) reported a reduction in all photosynthetic pigments, including carotenoids, in *V. radiata* under Hg toxicity. Debeljak et al. (2018). found that mycorrhization of plants in mercury-contaminated soil enhanced chlorophyll concentrations, suggesting a protective role of mycorrhiza against the toxicity. Remarkably, our study observed an increase in carotenoid content under pollution conditions.

Photosynthesis depends on Chl *a* and *b*, which are extremely vulnerable to environmental stresses such heavy metals (Ekmekçi et al. 2008). In our study, PGPM application enhanced photosynthetic pigment content that are sporting to energy and growth improvement. High Hg exposure altered chlorophyll content and reduced net photosynthesis rates (Teixeira et al. 2018). Lower levels of photosynthetic pigments were observed in the control plants exposed to Hg40, which correlated with reduced plant biomass. The decline in chlorophyll content under Hg stress could be attributed to decreased uptake of essential elements like Mn and K, oxidative stress, and the substitution of metal ions by Hg in photosynthetic pigments (Cho and Park 2000).

Root properties

Our study found that *V. radiata* accumulated significant amounts of pollution in the roots, with minimal translocation to the shoots. Studies by Moreno et al. (2005) on *Phaseolus vulgaris*, *Brassica juncea*, and *Vicia villosa* also reported that Hg is primarily retained in the roots to prevent its translocation to the shoots. Xu et al. (2021), demonstrated that root properties play a crucial role in Hg accumulation and stabilization. However Different plant species vary in their ability to store contamination in their tissues. The root architecture, including increased root biomass and surface area, enhances the plant's ability to immobilize Hg in the rhizosphere.

Fungal interactions with plants showed varied responses under different stress levels, offering protection to plant roots from the toxicity. Specifically, *V. radiata* roots retained 69% of pollution under Hg20 and 79% under Hg40. PGPM significantly improved this plant property, effectively limiting Hg transfer within the plant. Although research on Hg localization within plant organs is limited, it is likely bound to cell wall components or sequestered in root cell vacuoles. (Debeljak et al. 2018). The uptake, transport, and sequestration of Hg by roots are influenced by factors such as plant phenophase, soil characteristics, and contamination levels (Debeljak et al. 2018). Further research is needed to identify the transporters involved in its uptake from the soil.

The role of root exudates in phytostabilization cannot be overlooked. These exudates, composed of organic acids, sugars, and other compounds, can alter the soil pH, chelate metal ions, and foster beneficial microbial communities (Zhao et al. 2023a, b; Montiel-Rozas et al. 2016), which collectively contribute to reducing heavy metal bioavailability. The soil–plant transfer factor provides insight into how Hg behaves within the soil–plant system which is influenced by soil properties and Hg speciation. (Clayden et al. 2013; Natasha et al. 2020; Mohammadi et al. 2021).

Risk of Hg being transferred to the food chain

One of the primary concerns when using edible crops for phytoremediation is the potential for contaminants to enter the food chain (Ha et al. 2017). This study carefully evaluated the risk of Hg transfer to the food chain. The results indicated that while *V. radiata* plant could absorb and accumulate Hg in their tissues, the concentration in the seeds remained below 0.1 PPM as measured by ICP-MS. This suggests that *V. radiata* possess mechanisms to limit the contamination translocation to the seeds, thereby reducing the risk of entering the food chain.

To ensure food safety, it is crucial to monitor and manage toxicity levels in organs of plants used for phytoremediation. Implementing measures such as crop rotation, soil amendments, and post-harvest processing can further mitigate the risk of Hg contamination in the edible parts of the plant (Khanam et al. 2020; Xu et al. 2021). Detailed studies on the mechanisms of its uptake, translocation, and sequestration in *V. radiata* and similar crops are essential for developing effective strategies for safe phytoremediation practices.

Conclusion

This study demonstrates two effective approaches for managing soil Hg contamination using *V. radiata* (Fabaceae Family) in conjugation with the various amendments: phytoextraction, which removes Hg from the soil, and phytostabilization, which limits its mobility. The use of PGPMs and EDTA significantly enhanced Hg uptake, while biochar reduced internal levels and stabilized the contaminant within the soil. Microorganisms played a crucial role in boosting the plant's phytoremediation capacity, showcasing an environmentally sustainable strategy. However, the application of EDTA, while effective at the low concentrations, must be carefully managed due to its potential environmental risks.

V. radiata has shown the potential to reduce the accumulation in above-ground parts, prevent its accumulation in seeds, and thereby contribute to food security.

This study highlights the promise of *V. radiata* for safe phytoremediation, but it also emphasizes the need for ongoing monitoring and further research to better understand Hg uptake and sequestration mechanisms. In order to improve the quality of biochar as a soil amendment and microbial carrier, as well as various aspects of the plant–microbe-microbe interactions, can be given more attention in future research.

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Authors' contributions Shirwan Malaie: Data collection, Methodology, Formal analysis, Writing—Original Draft, Writing—review and editing. Latifeh Pourakbar: Conceptualization, Supervision, Design, Project administration, Methodology and Material preparation, Writing—review and editing. Sina Siavash Moghaddam: Supervision, Validation, Resources, Writing—review and editing. Jianbo Xiao: Visualization, Writing—review and editing. Nabi Khezrnejad: Investigation, Writing—review and editing. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Declarations

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