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Trichoderma Inoculation Alleviates Cd and Pb-Induced Toxicity and Improves Growth and Physiology of *Vigna radiata* (L.)

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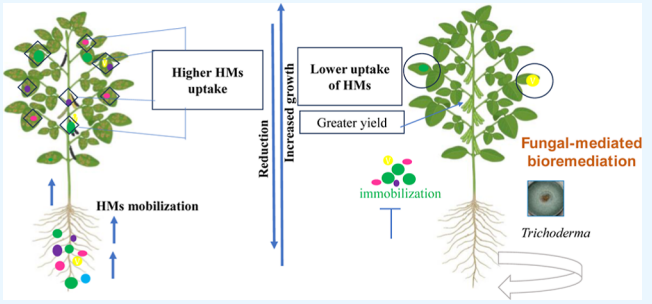
ABSTRACT: Heavy metals (HMs) pose a serious threat to agricultural productivity. Therefore, there is a need to find sustainable approaches to combat HM stressors in agriculture. In this study, we isolated *Trichoderma* sp. TF-13 from metal-polluted rhizospheric soil, which has the ability to resist 1600 and 1200 μg mL^{-1} cadmium (Cd) and lead (Pb), respectively. Owing to its remarkable metal tolerance, this fungal strain was applied for bioremediation of HMs in *Vigna radiata* (L.). Strain TF-13 produced siderophore, salicylic acid (SA; 43.4 μg mL^{-1}) and 2,3-DHBA (21.0 μg mL^{-1}), indole-3-acetic acid, ammonia, and ACC deaminase under HM stressed conditions. Increasing concentrations of tested HM ions caused severe reduction in overall growth of plants; however, *Trichoderma* sp. TF-13 inoculation significantly ($p \leq 0.05$) increased the growth and physiological traits of HM-treated *V. radiata*. Interestingly, *Trichoderma* sp. TF-13 improved germination rate (10%), root length (26%), root biomass (32%), and vigor index (12%) of *V. radiata* grown under 25 μg Cd kg^{-1} soil. Additionally, *Trichoderma* inoculation showed a significant ($p \leq 0.05$) increase in total chlorophyll, chl a, chl b, carotenoid content, root nitrogen (N), and root phosphorus (P) of 100 μg Cd kg^{-1} soil-treated plants over uninoculated treatment. Furthermore, enzymatic and nonenzymatic antioxidant activities of *Trichoderma* inoculated in metal-treated plants were improved. For instance, strain TF-13 increased proline (37%), lipid peroxidation (56%), catalase (35%), peroxidase (42%), superoxide dismutase (27%), and glutathione reductase (39%) activities in 100 μg Pb kg^{-1} soil-treated plants. The uptake of Pb and Cd in root/shoot tissues was decreased by 34/39 and 47/38% in fungal-inoculated and 25 μg kg^{-1} soil-treated plants. Thus, this study demonstrates that stabilizing metal mobility in the rhizosphere through *Trichoderma* inoculation significantly reduced the detrimental effects of Cd and Pb toxicity in *V. radiata* and also enhanced development under HM stress conditions.



INTRODUCTION

Heavy metal (HM) pollution in the soil ecosystem has become a serious concern due to its detrimental effects on humans, ecology, and agricultural productivity.[1] Globally, 20 million hectares of soil have been contaminated by HMs, which has become a serious concern especially in developed countries.[2] Like other HMs, Cd and Pb affect most of the agriculturally important crops. *Vigna radiata* L. (mung bean/green gram) is a well-known legume crop used as a common traditional food across the globe. In India, it is commonly consumed as sprouts, a vegetable food.[3] Among the several species affected, *V. radiata* (L.) has been used as a representative indicator of HM contamination due to its sensitivity to such environmental stressors.[4] HMs like cadmium (Cd), lead (Pb), zinc (Zn) and copper (Cu) are very often found in soil/water systems through various anthropogenic activities, including industrial discharge, mining, and agricultural practices.[5] These toxic elements can accumulate in plant tissues, compromising their growth, development, and nutritional value.[6] Among HMs, Cd is one of the most genotoxic metals known to cause chromosomal abnormalities, including the formation of micronuclei, and the inhibition of root growth in plants. Numerous investigations have shown that Cd is one of the most genetically harmful HMs as it can target the DNA structure and function. Similarly, despite not being a necessary component for plants, Pb is readily absorbed and accumulates in plant organs. Excess Pb causes various toxicity symptoms like stunted growth, blackening of roots, and chlorosis. Also, it (Pb) inhibits photosynthesis, distresses nutrient uptake and water balance, changes hormonal status, and affects membrane structure and permeability. *V. radiata* (L.), with its rapid growth and extensive root system, has long been a subject of research in the context of metal stress, serving as a model plant to investigate the consequences of HM contamination.[7] The adverse effects of HMs on *V. radiata* include reduced germination rates, stunted growth, altered nutrient uptake,

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and oxidative stress-induced damages.[8] These outcomes not only threaten the productivity of this essential legume crop but also raise concerns about the potential transfer of HMs through the food chain.[9]

Beneficial rhizosphere soil microorganisms also known as 'plant growth promoting rhizobacteria' (PGPR) improves plant growth by producing an array of bioactive compounds, such as siderophores, organic acids, phytohormones, and hydrolytic enzymes in adverse environmental conditions like HM stress.[10−16] Interestingly, PGPR strains have evolved special resistance mechanisms, such as cell membrane modification, through the release of exopolysaccharides, an efflux pump, producing heat shock proteins and osmo-protective molecules, which enables them to persist under unfavorable environmental conditions.[17]

Due to their increased metal tolerance, the ability to thrive in harsh environments under variable pH, temperature, and nutrition resources, filamentous fungi have an advantage over soil bacteria/rhizobacteria for use as HM remediators.[18] Among the beneficial microbial HM remediators, growth-promoting saprophytic fungi have not been fully explored for their HM remediation potential.[19] Several fungal genera, including *Trichoderma*, *Penicillium*, *Aspergillus*, and *Phoma*, have been reported to involve in bioremediation of metal-contaminated sites.[20] Fungi can resist and detoxify HMs by several mechanisms, including precipitation, biosorption, complexation, intracellular compartmentation, sequestration chemical modification, reduced intake, or efflux.[21] Biomineralization or bioprecipitation are other mechanisms by which filamentous fungi neutralize the harmful substances/metals.[22]

In recent agricultural practices, *Trichoderma* species have gained special recognition for their ability to enhance plant growth, suppress plant pathogens, and improve nutrient acquisition[23] via the release of siderophores,[24] phosphate-solubilizing enzymes,[25] and phytohormones.[26] Moreover, their ability to withstand and detoxify the higher level of HMs make them a suitable candidate for bioremediation of contaminated sites. Several species of *Trichoderma* sp. are known to play vital roles in decomposition,[27] mycoparasitism,[28] and even in cellulose degradation.[29] Under adverse environmental conditions, including metal stress, *Trichoderma* inoculation detoxifies the metal-induced toxicity and improves the growth, physiology, and oxidative stress status of numerous crops. However, despite promising individual studies, there exists a substantial knowledge gap in the collective understanding of the intricate relationship between *Trichoderma* and *V. radiata* under HM stress.

Considering these, current research delves into the multifaceted aspects of HM toxicity to *V. radiata*, shedding light on the physiological, biochemical, and molecular responses of this plant to HM exposure. Furthermore, it explores the potential of *Trichoderma* sp. TF-13 strain to counteract the Cd- and Pb-induced toxicity, aiming to elucidate the mechanisms underlying their beneficial effects. This work was carried out with the following specific objectives: (i) isolating *Trichoderma* from contaminated rhizosphere soils, evaluation of HM tolerance, and plant growth regulating activities of *Trichoderma* sp. TF-13 under Cd and Pb stress, (ii) inoculation impact of metal-resistant TF-13 strain on growth, dry biomass, leaf pigments, and nutrient uptake in HM-treated *V. radiata*, and (iii) assessing inoculation of *Trichoderma* on oxidative stress and antioxidative enzymatic profile of HM-treated *V. radiata*.

RESULTS AND DISCUSSION

Fungal Recovery and HM Tolerance. Because HM pollution frequently interferes with plant development, nitrogen (N) uptake, photosynthesis, and other physiological processes, it poses a serious threat to future food security. In this regard, we searched for HM-tolerant soil fungal isolates that may be used as microbial mediators to improve the crop performance and production under HM stress. At this juncture, 20 *Trichoderma* isolates were recovered from metal contaminated rhizosphere soil, and their cultural and biochemical characteristics were evaluated. Furthermore, the ability of *Trichoderma* isolates to synthesize/produce different bioactive compounds that enhances plant developmental and adaptive traits were further studied. Additionally, each recovered fungal isolate displayed a different level of metal tolerance. Among the fungal isolates, strain TF-13 showed a maximum HM tolerance at different concentrations for example, Cd (1600 μg mL^{-1}), Pb (1200 μg mL^{-1}) Cr (1000 μg mL^{-1}), and Ni (1600 μg mL^{-1}) (Table 1). Due to the maximum tolerance ability, strain TF-13 was used as a metal bioremediator in this study. The ability of the fungal strain to survive under high HM concentrations is an important characteristic because they can be used as important drivers to boost the crop output under HM-contaminated soil conditions. Similar to the current finding, it has been reported that soil fungal isolates from metal-polluted soil revealed a different HM tolerance profile. Many metal-tolerant fungi, such as *Trichoderma* sp.,[24] *Aspergillus welwitschiae*,[30] *Alternaria* sp., *Chaetomium globosum*, *Epicoccum nigrum*,[31] *Polygonum acuminatum*, and *Aeschynomene fluminensis*,[32] *Trichoderma brevicompactum* QYCD-6[33] etc., have been described for their vital functions in HM remediation and agricultural crop improvement. Interestingly, global study and research are being conducted to determine why microbial populations vary so greatly in their capacity to withstand dangerous contaminants like HMs. The reason why microbial populations differ so much in their ability to survive such harmful pollutants as HMs

Table 1. HM Tolerance in *Trichoderma* Isolates

Trichoderma isolates	heavy metal tolerance (μg/mL)			
	Cd	Pb	Cr	Ni
TF-1	800	100	400	800
TF-2	400	200	200	200
TF-3	1000	400	100	100
TF-4	200	200	200	50
TF-5	1200	100	600	50
TF-6	400	800	50	100
TF-7	800	400	50	200
TF-8	1200	200	200	200
TF-9	400	400	400	100
TF-10	400	600	50	50
TF-11	200	200	100	400
TF-12	400	800	400	800
TF-13	1600	1200	1000	1600
TF-14	1200	200	800	800
TF-15	800	50	100	400
TF-16	400	50	200	800
TF-17	800	200	50	1000
TF-18	1000	400	50	1200
TF-19	200	200	50	1200
TF-20	200	800	400	800

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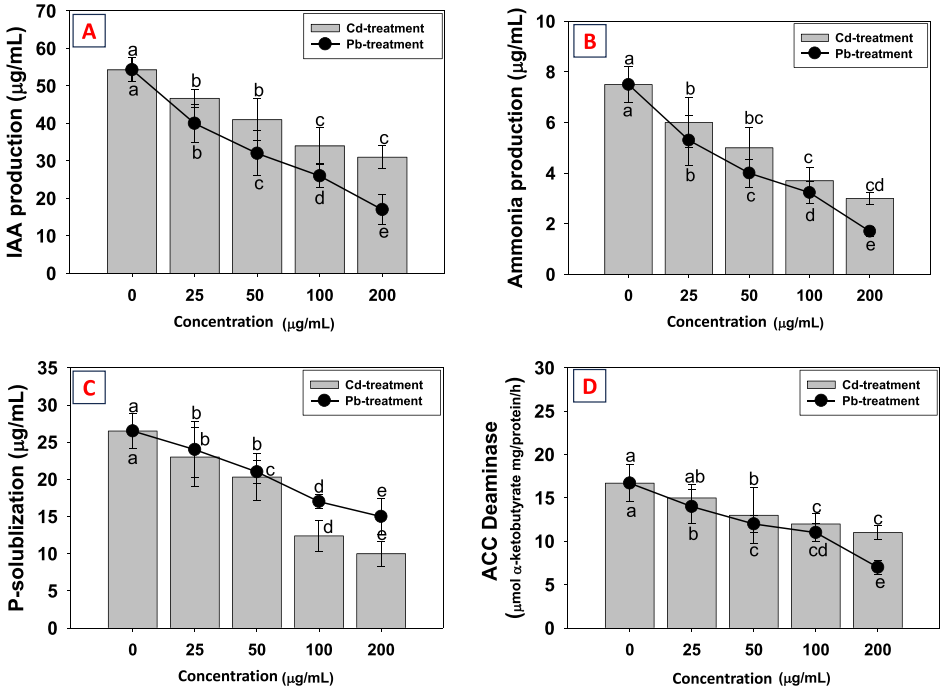

Figure 1. Effect of increasing doses (25, 50, 100, and 200 μg mL^{-1}) of Cd and Pb on plant growth-regulating substances synthesized by *Trichoderma* sp. TF-13 strain; IAA (A), ammonia production (B), P-solubilization (C), and ACC deaminase (D). In this and succeeding figures, bar and line diagrams represent the mean (mean ± S. D) values of three replicates (n = 3). The Duncan's multiple range test (DMRT) indicates that mean values differ significantly when represented by different letters.

is a topic of study and research on a global scale. Some studies suggest that various microorganisms respond differently to different HMs depending on their metal tolerance. The cause may be due to (a) differences in the macro- and micronutrients found in soil and other growing media that support fungal growth and development,[34] (b) genetic differences among microbial strains, and (c) growth conditions or environmental factors that have an impact on the growth of microorganisms.[35]

Plant Growth-Promoting Compounds Produced by *Trichoderma* sp. TF-13 during Metal-Stressed Conditions. *Indole-Acetic Acid and Ammonia Production, P-Solubilization, ACCD and Siderophore.* Several genera of soil microorganisms including fungi synthesize phytohormones such as auxin. Interestingly, synthesis of indole-acetic acid (IAA) by bacterial and fungal strains modulates root growth under stress conditions. In our investigation, *Trichoderma* sp. TF-13 produced a significant amount of IAA (54.3 ± 3.2 μg mL^{-1}), which, however, decreased in the presence of increasing concentrations of Cd and Pb. For instance, under doses of 25, 50, 100, and 200 μg/mL Cd, the production of IAA was markedly decreased by 5.64, 45.70, and 76.13%, respectively, over the control (Figure 1A). Similarly, Pb at 200 μg/mL, significantly reduced the levels of fungal secreted IAA by 68.5%, over untreated control (Figure 1A). These results demonstrate that fungal strain TF-13 utilizes the indole moiety from tryptophan, a necessary precursor compound, to produce IAA. These findings demonstrate that *Trichoderma* sp. TF-13 also follows the tryptophan-dependent pathway. This may be the cause of TF-13 strain's abrupt decrease in indole-3-acetic acid synthesis at higher concentrations of tested metal ions; Cd and Pb. Phytohormones help plants develop and function more efficiently. IAA produced by soil microorganisms may have a direct impact on plants by promoting cell extension and division, which will promote root growth.[36] Lower IAA concentrations enhance primary root development.[37] In contrast, greater IAA concentrations cause the development of lateral and adventitious roots. Likewise, IAA and other phytohormone-synthesizing soil fungal isolates, including *Trichoderma,* are reported to improve the lateral root formation and growth of numerous crops.[38,39]

The generation/release of ammonia by strain TF-13 was quantified in the presence and absence of different Cd and Pb concentrations. Under a controlled medium (absence of metals), *Trichoderma* sp. TF-13 strain produced 7.5 ± 0.7 μg mL^{-1} of ammonia, which, however, decreased to 6.0, 5.0, 3.7, and 3.0 μg/mL when treated with 25, 50, 100, and 200 μg mL^{-1} of Cd, respectively (Figure 1B). By expanding the root surface area, plant growth promoting microbes, including fungi, can help plants absorb nitrogen more efficiently. According to our research, denitrification-related metabolic pathways are inhibited when exposed to Cr and Pb stress, which results in a considerable drop in the fungal release of ammonia. The Pb toxicity, however, has little effect on the rate of ammonia generation at higher concentrations (200 μg mL^{-1}), and this may be because ammonia is assimilated during the amino acid biosynthesis process. Also, there is an increase in extra proton H^+-ion activity of fungal cell, which causes the surrounding media to become acidic. This makes it easier for microorganisms to survive and maintain ammonia production stability under a metal-stressed situation.[40] Additionally, proton excretion helps dissolve insoluble phosphates.

In the presence or absence of both tested metals, i.e., Cd and Pb, *Trichoderma* TF-13 produced phosphate that was easily soluble. Even at higher metal doses, strain TF-13 could solubilize the insoluble P in a liquid medium treated with Cd and Pb (Figure 1C,D). The majority of plants do not directly use phosphorus from the soil. By releasing several enzymes, such as phosphatases, phosphonates, C−P lyases, and several

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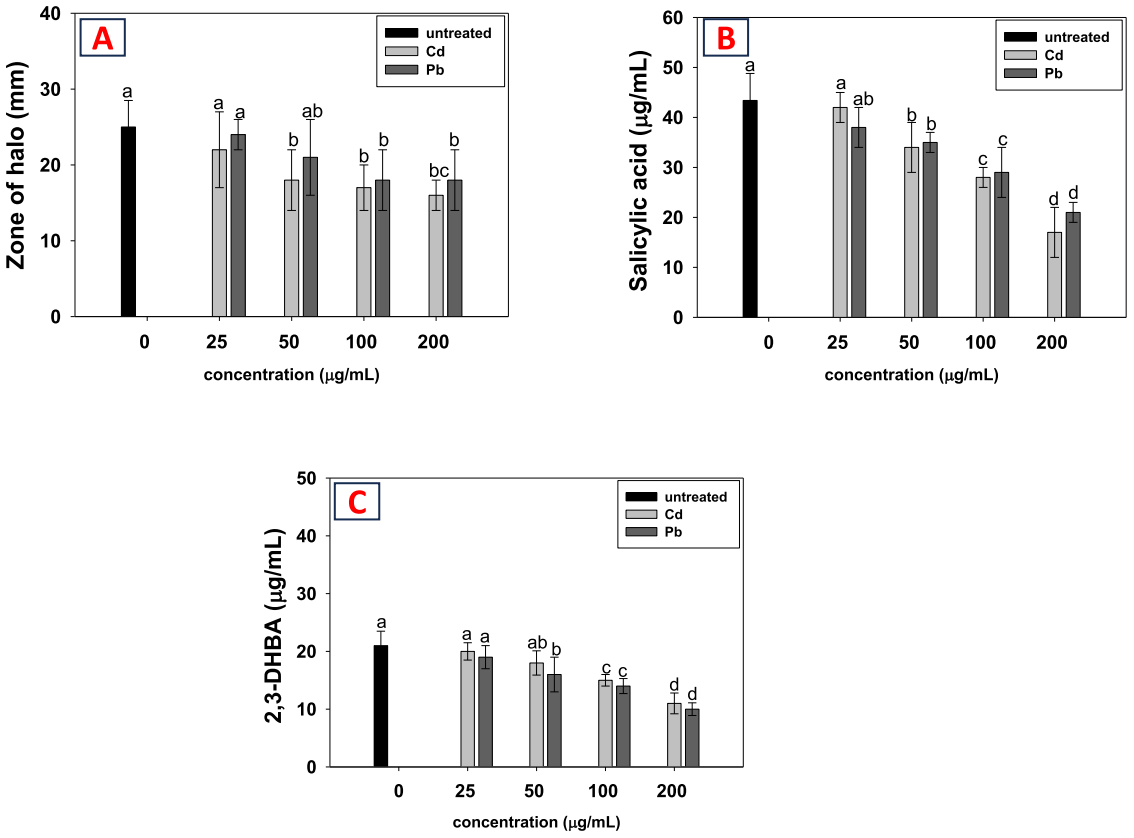

Figure 2. Siderophore production activity of *Trichoderma* sp. TF-13 strain under metal stressed conditions; zone of halo (mm) on CAS agar plates under increasing Cd and Pb doses (A), production of salicylic acid (B), and 2, 3-DHBA (C) under metal stressed conditions.

organic acids (α-ket-gluconic, lactic, oxalic, acetic, formic, citric acids etc.).[13,41] Under these circumstances, soil microbes, including fungi, makes it easier for P to be available at the rhizosphere, which could improve plant growth by delivering enough P at the rhizosphere.[42] With the aid of exchange reactions, acidification, chelation, and the release of substances that dissolve or complex minerals, these enzymatic activities transform the insoluble form of phosphates into a soluble/readily available form. Due to the solubilized P, HM absorption and translocation are facilitated by the formation of HM-phosphate complexes on fungal cell walls.[43]

The siderophore production ability of *Trichoderma* sp. TF-13 strain (Figure S1A) was assessed by growing the culture on a chrome azurol S (CAS) agar plate (Figure S1B). The strain showed a positive response to siderophore production as revealed by the change in the color of medium from bluish green to orange/reddish pink (Figure S1C,D). The siderophore production was variably affected when cultivated with increasing concentrations (25−200 μg mL^{-1}) of Cd and Pb. The increasing concentrations of both Cd and Pb decreased the zone of halo (Figure 2A). Furthermore, siderophores produced by TF-13 were quantitatively evaluated under metal stress. The strain TF-13 produced a considerable amount of salicylic acid (SA) (43.4 ± 5.6 μg mL^{-1}) and 2,3-DHBA (21.0 ± 2.5 μg mL^{-1}) under a metal free medium, which, however, significantly ($p \leq 0.05$) decreased with cumulative increases in Cd and Pb concentrations (Figure 2B). For instances, the production of 2,3-DHBA was reduced to 19 ± 2.0, 16 ± 3.0, 14 ± 1.3, and 10 ± 1.1 μg mL^{-1} in the presence of 25, 50, 100, and 200 μg Pb mL^{-1} (Figure 2C). Similarly, Cd at 200 μg mL^{-1}, maximally and significantly ($p \leq 0.05$) decreased the fungal production of SA and 2,3-DHBA by 60.4 and 47.6%, respectively, over the untreated control.

Iron (Fe) insufficiency and nutrient imbalance cause a loss of nutrient uptake because the solubility of Fe(III) is considerably lower than Fe requirements for plants and microorganisms under stressful conditions.[44] Plants have evolved mechanisms to overcome Fe metal deficit in order to deal with this stress. To chelate the insoluble iron (Fe) and release siderophores, microorganisms and plants have developed specific mechanisms. Plants will then consume the iron−siderophore complexes.[45] Because metal stress may decrease the ability of plants to absorb iron from saline soils by preventing phyto-siderophore production, soil microorganisms may be crucial in promoting plant iron (Fe) uptake under Fe-limiting circumstances.[46] However, some soil microbes, including soil fungi, may also emit siderophores in order to survive, which can aid in the plant's uptake of iron.[47] The vital roles that siderophore-secreting fungal genera plays in the growth and development of plants includes promoting the solubility, availability, and uptake of Fe and other vital micronutrients while impeding the mobility of toxic HMs.[48] For instance, siderophore generated by *Trichoderma harzianum* boosted the availability of Pb, Cd, and Hg while also alleviating the oxidative stress brought on by HMs in *Solanum melongena*.[49] In iron-limited environments, siderophore-producing *Trichoderma* sp. and *Penicillium aculeatum* have been shown to improve the growth of *Sorghum bicolor* (L.), while improving the phytoremediation efficiency of mine tailing soil.[50] Therefore, one hypothesis is that fungal siderophores observed in the current investigation could chelate insoluble

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Table 2. Effect of Metal Tolerant Siderophore Producing *Trichoderma* sp. TF-13 Strain on Seedling Germination, Growth, Biomass, and Vigor Indices of *V. radiata* (L.) Cultivated in Pot Soils Treated with Increasing Cd and Pb Concentrations[a]

treatments	concentrations (μg/kg soil)	seed germination (%)	plant length (cm)		fresh weight (gm)		dry biomass (g/plants)		seedling vigor index (SVI)
			roots	shoots	roots	shoots	roots	shoots	
control (UC)	0	90	21 ± 3.6^{b}	25 ± 4.1^{b}	5.7 ± 1.4^{b}	7.8 ± 1.5^{b}	1.8 ± 0.2^{c}	3.4 ± 0.8^{c}	4534 ± 145^{b}
control (IC)	Trichoderma sp. TF-13	100	29 ± 5.3^{a}	36 ± 5.6^{a}	7.3 ± 1.8^{a}	10.2 ± 2.3^{a}	2.9 ± 0.0^{a}	5.3 ± 1.0^{a}	5571 ± 67.3^{a}
Cd	25 μg kg^{-1} soil	85	17 ± 2.1^{c}	20 ± 2.7^{c}	4.1 ± 0.7^{c}	6.5 ± 0.6^{bc}	1.5 ± 0.4^{d}	3.0 ± 1.2^{cd}	4213 ± 142^{bc}
	50 μg kg^{-1} soil	75	14 ± 1.6^{d}	17 ± 1.2^{d}	3.2 ± 0.6^{d}	5.3 ± 0.4^{d}	1.2 ± 0.1^{e}	2.2 ± 0.7^{e}	3896 ± 97^{bc}
	100 μg kg^{-1} soil	60	12 ± 0.7^{e}	13 ± 0.8^{e}	2.4 ± 0.4^{e}	4.0 ± 0.7^{e}	0.8 ± 0.0^{f}	1.5 ± 0.6^{f}	3421 ± 234^{d}
	200 μg kg^{-1} soil	40	5.1 ± 0.8^{f}	10 ± 0.0^{f}	1.0 ± 0.0^{g}	2.4 ± 0.3^{f}	0.4 ± 0.1^{ga}	1.0 ± 0.0^{g}	2523 ± 435^{f}
	Trichoderma TF-13 + 25 μg kg^{-1} soil	95	23 ± 4.2^{b}	27 ± 4.7^{b}	5.8 ± 1.6^{b}	8.0 ± 1.4^{b}	2.2 ± 0.6^{b}	4.4 ± 0.3^{b}	4732 ± 542^{ab}
	Trichoderma TF-13 + 50 μg kg^{-1} soil	85	20 ± 1.7^{b}	21 ± 3.1^{c}	4.4 ± 1.0^{c}	7.0 ± 0.9^{c}	1.8 ± 0.4^{c}	3.0 ± 0.9^{cd}	4212 ± 721^{b}
	Trichoderma TF-13 + 100 μg kg−^{1} soil	70	18 ± 3.0^{c}	19 ± 2.6^{d}	3.6 ± 0.7^{c}	5.4 ± 0.6^{d}	1.3 ± 0.5^{d}	2.2 ± 0.1^{e}	3876 ± 317^{d}
	Trichoderma TF-13 + 200 μg kg^{-1} soil	50	10 ± 0.6^{e}	15 ± 1.5^{e}	1.7 ± 0.3^{f}	2.8 ± 0.5^{f}	0.9 ± 0.2^{f}	1.6 ± 0.5^{f}	2761 ± 118^{f}
Pb	25 μg kg^{-1} soil	90	19 ± 1.5^{c}	22 ± 2.8^{c}	4.6 ± 0.9^{c}	6.0 ± 0.7^{c}	1.7 ± 0.0^{c}	2.7 ± 0.4^{d}	4023 ± 231^{c}
	50 μg kg^{-1} soil	70	14 ± 2.0^{d}	18 ± 1.4^{d}	3.7 ± 0.7^{c}	5.1 ± 0.0^{d}	1.6 ± 0.3^{d}	2.2 ± 0.2^{de}	3436 ± 187^{e}
	100 μg kg^{-1} soil	60	12 ± 1.0^{e}	14 ± 2.0^{e}	2.8 ± 0.5^{e}	3.5 ± 0.4^{e}	0.7 ± 0.4^{f}	1.0 ± 0.0^{g}	3121 ± 543^{e}
	200 μg kg^{-1} soil	35	7.2 ± 0.8^{f}	10 ± 0.8^{f}	0.8 ± 0.3^{g}	2.0 ± 0.2^{f}	0.5 ± 0.2^{g}	0.6 ± 0.0^{h}	2236 ± 321^{f}
	Trichoderma TF-13 + 25 μg kg^{-1} soil	95	24 ± 3.4^{b}	27 ± 4.7^{b}	5.5 ± 0.1^{b}	6.9 ± 0.7^{b}	2.4 ± 0.4^{b}	3.3 ± 0.7^{c}	4678 ± 187^{ab}
	Trichoderma TF-13 + 50 μg kg^{-1} soil	85	19 ± 2.7^{c}	23 ± 3.9^{c}	4.2 ± 0.8^{c}	5.8 ± 0.4^{d}	2.0 ± 0.1^{c}	2.6 ± 0.2^{d}	3844 ± 256^{c}
	Trichoderma TF-13 + 100 μg kg^{-1} soil	70	15 ± 1.8^{d}	18 ± 2.6^{d}	3.6 ± 0.4^{c}	4.2 ± 0.6^{e}	1.2 ± 0.0^{d}	1.5 ± 0.0^{f}	3500 ± 421^{d}
	Trichoderma TF-13 + 200 μg kg^{-1} soil	50	11 ± 2.1^{e}	14 ± 2.1^{e}	1.2 ± 0.0^{f}	2.6 ± 0.5^{f}	0.8 ± 0.0^{g}	1.0 ± 0.0^{g}	2612 ± 187^{e}

[a]Values are the mean of different replicates performed in triplicates. According to the DMRT test, mean values that are followed by distinct letters are significantly different ($p \leq 0.05$).

iron from the environment to aid metal-stressed plants in absorbing and utilizing the Fe^{3+}.

The ACC deaminase enzyme is released by a variety of soil microorganisms, including bacteria[51] and fungi.[52] This enzyme is essential for lowering ethylene (ET) in plants and fostering plant growth even under challenging circumstances. As a result, it was determined if fungal strains could produce ACC deaminase when exposed to metal stress. In metal-free environments, *Trichoderma* sp. TF-13 could generate 16.7 μmol α-ketobutyrate mg g^{-1} protein h^{-1} ACCD. This strain showed ACC deaminase activity regardless of the Cd and Pb levels present; however, with increasing metal concentrations, the ACCD activity was gradually diminished. When compared to the untreated control, the ACC deaminase activity of strain TF-13 significantly ($p \leq 0.05$) decreased by 31.2 and 58% at 200 μg mL^{-1} of Cd and Pb, respectively. It is noteworthy that the capacity of fungal strain to produce α-ketobutyrate during Cd and Pb HM stress further supports the notion that they trigger the transcription of ACC deaminase coding genes under metal-stressed conditions. Prior research has also noted that the ability of some fungal strains to release the enzyme ACC deaminase even in the presence of metal stress may be useful for promoting not only the growth and adaptive responses but also boost crop production under HM stress.[53−55]

Bioinoculation Impact of *Trichoderma* sp. TF-13 Strains on *V. radiata* under Metal-Contaminated Soils. *Germination and Vigor Indices.* At 6 days of sowing (DAS), the effectiveness of *Trichoderma* sp. inoculated *V. radiata* seedling germination was measured in soil pots containing different concentrations of Cd and Pb. In the case of uninoculated control treatments, seeds were maximally germinated; however, 200 μg Cd kg^{-1} soil significantly declined the germination (55.5%) and vigor index (44.3%) of *V. radiata*. In contrast, inoculation of metal tolerant fungal strains improved/increased the seed germination and seedling vigor index (SVI) of plants by detoxifying the metal-induced phytotoxicity. For instance, the germination rate and SVI of 25 μg Pb kg^{-1} plants increased by 11 and 12%, respectively, following *Trichoderma* sp. TF-13 inoculation (Table 2). Similarly, *Sesbania sesban* (L.) cultivated in soil treated with increasing dosage of Cd and Cr showed improved germination efficiency and growth characteristics when exposed to the metal-tolerating microbial strain.[56]

Growth and Dry Biomass of Metal-Treated and Trichoderma-Inoculated V. radiata. The Cd and Pb exposed *V. radiata* plants experienced variable adverse/toxic effects. The tested metals Cd and Pb at 200 μg kg^{-1} soil highly decreased the root length by 76.1 and 65.7%, respectively, above the untreated control (Table 2). But, soil inoculation of HM-tolerant *Trichoderma* sp. TF-13 strain relieved the phytotoxicity of tested metals and improved the plant growth. For instance, the highest increases in root (21%) and shoot (54.5%) lengths were observed in plants inoculated with *Trichoderma* sp. TF-13 in the presence of 25 μg Pb kg^{-1} soil (Table 2). In addition, among metals, Pb at 200 μg kg^{-1} soil maximally decreased the fresh weight of root (86%) and shoot (83%), which, however, improved by inoculating *Trichoderma*

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Table 3. Role of HM Tolerant *Trichoderma* sp. on Leaf Pigments and Nutrient Acquisition in *V. radiata* (L.) Cultivated in Varied Levels of Cd- and Pb-Stressed Soil[a]

heavy metal	concentration (μg/kg soil)	chlorophyll pigments (μg g^{-1} fw)				N content (μg g^{-1})		P content (μg g^{-1})	
		Chl a	Chl b	total Chl	carotenoid	roots	shoots	roots	shoots
control	uninoculated	25.5 ± 3.6^{b}	15.2 ± 2.7^{b}	34.2 ± 7.8^{b}	105 ± 11.2^{b}	9.2 ± 1.4^{c}	18.2 ± 4.2^{c}	3.2 ± 0.2^{c}	7.1 ± 1.3^{b}
	Trichoderma sp. TF-13	37.8 ± 4.1^{a}	21.4 ± 3.5^{a}	48.9 ± 9.0^{a}	123 ± 13.0^{a}	17.3 ± 2.5^{a}	27.2 ± 5.1^{a}	7.0 ± 1.1^{a}	9.6 ± 2.3^{a}
Cd	25 μg Cd kg^{-1} soil	23.0 ± 2.6^{c}	14.0 ± 3.7^{c}	32.4 ± 5.6^{b}	98.9 ± 9.8^{c}	8.6 ± 1.6^{c}	17.3 ± 2.2^{c}	3.0 ± 1.0^{c}	6.2 ± 0.8^{c}
	50μg Cd kg^{-1} soil	19.5 ± 1.7^{d}	11.3 ± 0.6^{d}	26.7 ± 4.4^{c}	93.2 ± 7.8^{d}	7.1 ± 0.8^{d}	15.6 ± 1.4^{d}	2.4 ± 0.0^{d}	4.2 ± 0.0^{e}
	100 μg Cd kg^{-1} soil	16.0 ± 2.4^{e}	7.6 ± 0.8^{e}	23.6 ± 3.8^{d}	91.0 ± 6.3^{d}	5.7 ± 0.4^{e}	12.1 ± 1.1^{e}	2.0 ± 0.4^{e}	3.5 ± 0.3^{f}
	200 μg Cd kg^{-1} soil	10.0 ± 1.6^{f}	4.3 ± 0.0^{g}	17.2 ± 2.1^{f}	81.0 ± 5.3^{f}	3.2 ± 0.0^{f}	7.3 ± 0.6^{g}	1.2 ± 0.2^{g}	1.7 ± 0.1^{h}
	Trichoderma TF-13 + 25 μg Cd kg^{-1} soil	29.0 ± 2.8^{b}	19.0 ± 3.0^{a}	42.6 ± 5.9^{a}	107.9 ± 4.8^{b}	14.7 ± 3.1^{b}	24.4 ± 2.8^{b}	5.4 ± 1.2^{b}	8.4 ± 0.9^{a}
	Trichoderma TF-13 + 50 μg Cd kg^{-1} soil	23.5 ± 2.3^{c}	15.0 ± 0.9^{b}	33.4 ± 5.5^{b}	99.7 ± 5.8^{c}	11.4 ± 1.0^{c}	19.6 ± 1.7^{c}	4.0 ± 0.5^{c}	6.2 ± 1.0^{c}
	Trichoderma TF-13 + 100 μg Cd kg^{-1} soil	20.0 ± 2.8^{c}	9.7 ± 0.5^{e}	27.2 ± 2.0^{c}	96.4 ± 5.2^{c}	9.7 ± 0.9^{c}	15.2 ± 1.3^{d}	3.0 ± 0.7^{c}	5.5 ± 0.4^{d}
	Trichoderma TF-13 + 200 μg Cd kg^{-1} soil	12.0 ± 1.0^{f}	6.4 ± 0.5^{f}	20.0 ± 0.0	85.0 ± 6.1^{e}	5.1 ± 0.4^{e}	9.4 ± 0.8^{f}	1.9 ± 0.1^{e}	2.3 ± 0.4^{g}
Pb	25 μg Pb kg^{-1} soil	21.0 ± 4.1^{c}	12.5 ± 0.8^{d}	30.1 ± 4.9^{b}	87.3 ± 12.0^{e}	6.8 ± 1.3^{d}	14.1 ± 2.9^{d}	2.5 ± 0.7^{d}	6.0 ± 0.9^{c}
	50 μg Pb kg^{-1} soil	16.7 ± 2.6^{e}	8.8 ± 1.0^{e}	25.3 ± 6.4^{d}	79.2 ± 6.9^{f}	4.4 ± 0.7^{e}	11.3 ± 2.1^{e}	2.0 ± 0.3e	4.4 ± 0.3^{e}
	100 μg Pb kg^{-1} soil	9.3 ± 1.8^{g}	4.0 ± 0.3^{g}	19.2 ± 3.2^{e}	65.2 ± 7.6^{g}	2.6 ± 0.1^{g}	6.2 ± 0.0^{g}	1.0 ± 0.1g	2.0 ± 0.1^{g}
	200 μg Pb kg^{-1} soil	5.2 ± 1.8^{h}	1.6 ± 0.1^{h}	13.1 ± 3.0^{f}	43.2 ± 5.0^{h}	1.12 ± 0.3^{h}	3.6 ± 0.5^{h}	0.3 ± 0.0^{h}	0.67 ± 0.2^{h}
	Trichoderma TF-13 + 25 μg Pb kg^{-1} soil	25.7 ± 1.8^{b}	15.5 ± 1.4^{b}	34.7 ± 2.4^{b}	95.2 ± 5.4^{c}	9.4 ± 1.1^{c}	18.4 ± 2.7^{c}	3.6 ± 0.6^{c}	7.8 ± 1.7^{b}
	Trichoderma TF-13 + 50 μg Pb kg^{-1} soil	20.1 ± 1.7^{d}	11.5 ± 0.7^{d}	28.4 ± 2.2^{c}	84.2 ± 8.0^{e}	6.7 ± 0.5^{d}	13.5 ± 2.2^{d}	2.7 ± 0.4^{d}	5.6 ± 0.8^{d}
	Trichoderma TF-13 + 100 μg Pb kg^{-1} soil	11.2 ± 0.7^{f}	6.6 ± 0.4^{f}	22.0 ± 0.0^{e}	68.9 ± 5.8^{g}	3.3 ± 0.3^{f}	7.4 ± 1.0^{f}	1.5 ± 0.3^{f}	2.9 ± 0.6^{f}
	Trichoderma TF-13 + 200 μg Pb kg^{-1} soil	7.1 ± 1.4^{g}	1.9 ± 0.2^{g}	16.5 ± 4.0^{g}	47.8 ± 9.0^{g}	2.1 ± 0.4^{g}	4.3 ± 0.7^{g}	0.9 ± 0.0^{g}	1.32 ± 0.3^{g}

[a]Values are the mean of different replicates performed in triplicates. According to the DMRT test, mean values that are followed by distinct letters are significantly different ($p \leq 0.05$).

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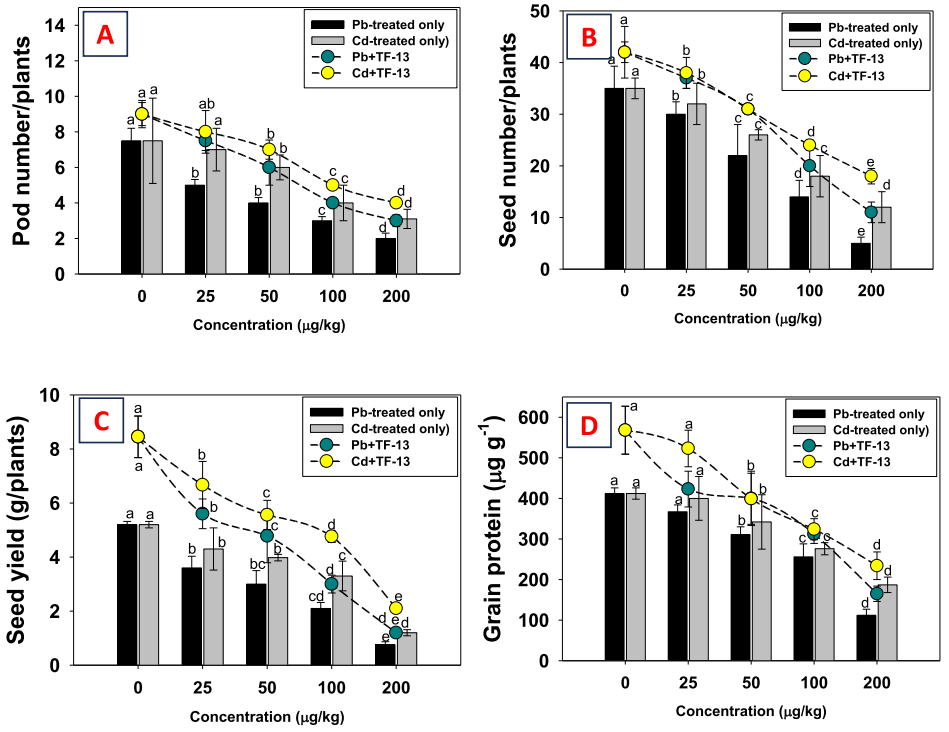

Figure 3. Yield traits in *Trichoderma* sp. TF-13 inoculated with Cd and Pb-treated *V. radiata* plants; number of pods (A), seed/grain number per plants (B), SY (C), and GP (D). Three replicates ($n = 3$) are used to represent the mean in each bar and scatter plot. The standard deviation of the replicates is shown by the corresponding error bars. According to the DMRT test, different letters indicate that the mean values differ significantly.

sp. to *V. radiata*. After inoculation of *Trichoderma* sp. TF-13 to 200 μg Pb kg^{-1} treated plant, a greater increase in the root length (29.3%) and shoot length (19%) was recorded over uninoculated but metal-treated plants (Table 2). Similar to this, Cd at 200 μg kg^{-1} soil decreased the root and shoot dry matters of *V. radiata* by 77.7 and 70.5%, respectively, over the control. Contrarily, even at 25 μg Cd kg^{-1} soil, the dry biomass of *Trichoderma* sp. TF-13-inoculated roots and shoots showed increases of 31.8 and 32%, respectively, compared to noninoculated but cadmium-treated *V. radiata* (Table 2). Like our observation, an earlier study also found similar results like significant reduction of biological parameters in *Brassica campestris* (L.) plants grown in soil-treated with Cd, Cr, Pb, and Hg.[57] Additionally, a dose-dependent reduction in the germination rate, organ length, dry and fresh biomass, nutrient uptake, and yield attributes of *V. radiata* (L.) growing in the presence of increasing concentrations of Cu has been found.[58] However, microbe-mediated metal reduction, detoxification, and growth improvement in several crops raised in metal-polluted soils has been reported by several workers. In this regard, Cao et al.[59] in a study, documented a considerable improvement in biological traits, like the germination rate, length, and biomass in *Brassica juncea* (L.) seedlings exposed to Ni and Cd stress after inoculation of *Trichoderma atroviride* (a biocontrol and metal-tolerant filamentous soil fungus). Also, the fungal strain increased the metal phytoextraction efficiency of plants. These findings suggest that the inoculation of *Trichoderma* sp. TF-13 to *V. radiata* (L.) reduced/eliminated HM-induced toxicity because of its metal detoxification ability and increased the root fresh weight and dry biomass of plants grown in metal-treated soils.

Trichoderma sp. TF-13 Improved Leaf Pigments and Nutritional Contents in Metal-Stressed V. radiata. Under metal stress, total chlorophyll, and carotenoid levels of *V. radiata* leaves were dramatically reduced. For instance, among the tested metal species and their concentrations, 200 μg Pb kg^{-1} soil reduced the chlorophyll a, b, total chlorophyll, and carotenoid content by 79.6, 87, 61.7, and 59%, respectively, over untreated control (Table 3). The toxicity of metals causes generation of reactive oxygen species (ROS), which results in photo-oxidative damage and photoinhibition in the chloroplasts of *V. radiata* and due to which the leaf pigments were declined. Also, the Calvin cycle is impacted by HM ions in the chloroplast, which slows down the photosynthetic pigments and changes the rate of photosynthesis. Additionally, it limits the amount of light that can be harvested and may inhibit the photosynthesis in order to minimize the damages caused by metal stress. However, it was interesting to note that fungal inoculation enhanced the level of leaf pigments in plants. Contrarily, when strain TF-13 was administered to *V. radiata* plants grown in soil treated with 25 μg Pb kg^{-1} soil, an increase of 18.3, 20, 13.5, and 9% was recorded in chl a, chl b, total chlorophyll, and carotenoid content, respectively, compared to the uninoculated control (Table 3). Furthermore, *Trichoderma* sp. inoculation efficiently bioremediated the experimental soil that had been exposed to metal by increasing the chlorophyll a, chl b, total chlorophyll, and carotenoid content by 20.6, 26.3, 24, and 8.3%, respectively, even at 25 μg Cd kg^{-1} soil. Several species of plant growth promoting soil fungi, including *Trichoderma* are reported to enhance the growth, leaf pigment characteristics, and fruit quality of edible crops growing in different agroclimatic regions.[60] Likewise, single and dual applications of metal tolerant and siderophore synthesizing *Trichoderma* sp. with *Pseudomonas fluorescence* positively influenced the content of leaf pigments in Cd-treated chickpea plants.[24] A pot study conducted by Sun et al.[61] showed that the addition of Pb-tolerant *Trichoderma asperellum* SD-5 enhanced the growth and biomass in *Lolium perenne* (L.) under Pb stress,

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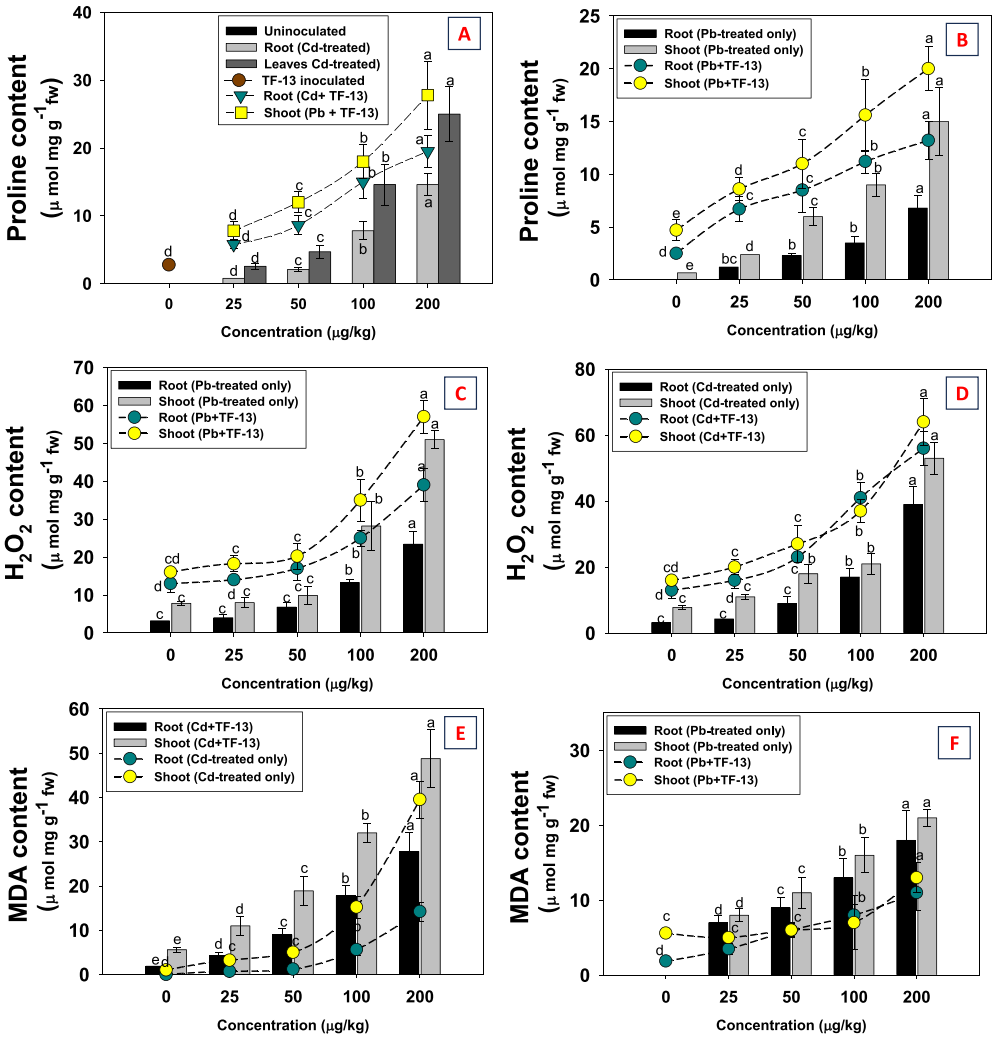

Figure 4. Stress biomarkers in *V. radiata* bioinoculated with *Trichoderma* sp. TF-13 and treated with metals; proline content accumulated in Cd (A), Pb-treated (B) plant organs, accumulation of H_2O_2 content (C,D), and MDA content in plant exposed to Cd (E) and Pb (F). The DMRT indicates that mean values differ significantly when represented by different letters.

in addition to dramatically boosting the photosynthesis by enhancing the leaf chlorophyll content. Further, we assessed the effectiveness of metal-tolerant fungal strain in elimination of HM-induced toxicity and increased nutrient uptake in order to comprehend the harmful effect of HMs on the nutritional values of legumes. The increasing Cd and Pb concentrations significantly declined the nutrient (N and P) uptake in *V. radiata* raised in metal-polluted soil. The higher doses (200 μg kg^{-1} soil) of Cd and Pb maximally declined the roots N (65.2%) and shoot N (80.2%) compared to untreated *V. radiata*. However, *Trichoderma* inoculation caused the detoxification of metal-induced toxicity and maximally and significantly ($p \leq 0.05$) improved the root N (27.6%) and shoot N (24%) of plants treated with 25 μg Pb kg^{-1} soil, over uninoculated control (Table 3). Similarly, strain TF-13 inoculated, and 25 μg Cd kg^{-1} soil exposed *V. radiata* showed an increase in the root P (44.4%) and shoot P (26%) contents over uninoculated but treated with identical metal doses (Table 3).

Inoculation Impact of Trichoderma sp. TF-13 on Yield Attributes of Metal-Treated V. radiata. The effect of increasing Cd and Pb doses on grain quantity and quality of *V. radiata* was inconsistent. With the steady increase in metal concentrations, seed yield (SY) and grain protein (GP) of *V. radiata* was significantly ($p \leq 0.05$) declined and the maximum pronounced effect was recorded at higher doses. For instance, among metals and their concentrations, 200 μg Cd kg^{-1} soil greatly reduced the pod number (PN) (Figure 3A), seed number (SN) (Figure 3B), SY (Figure 3C), and GP (Figure 3D) by 73.3, 85.7, 83.2, and 72.8%, respectively, over untreated control. HM uptake and translocation into edible plant parts may be the cause of the harmful effects of such metal ions on the seed characteristics. Therefore, the uptake of metals within the grains of *V. radiata* may pose a serious threat to human health. Contrarily, metal-tolerant *Trichoderma* sp. TF-13 mitigated the metal-induced toxicity and improved/increased the seed features of *V. radiata,* even raised in soil contaminated with low and high levels of Cd and Pb. For instance, TF-13 increased the PN, SN, SY, and GP of 25 μg Cd kg^{-1} soil-exposed *V. radiata* by 33.3, 19, 40, and 14%, when compared to non-inoculated plants growing under similar metal doses.

Proline, MDA, and H_2O_2 Content in Trichoderma-Inoculated and Metal-Treated V. radiata. Under high metal load, proline is necessary to preserve cellular osmolarity. It (proline) assists successfully in reducing the oxidative damage brought on by ROS during metal detoxification in plants[62] and

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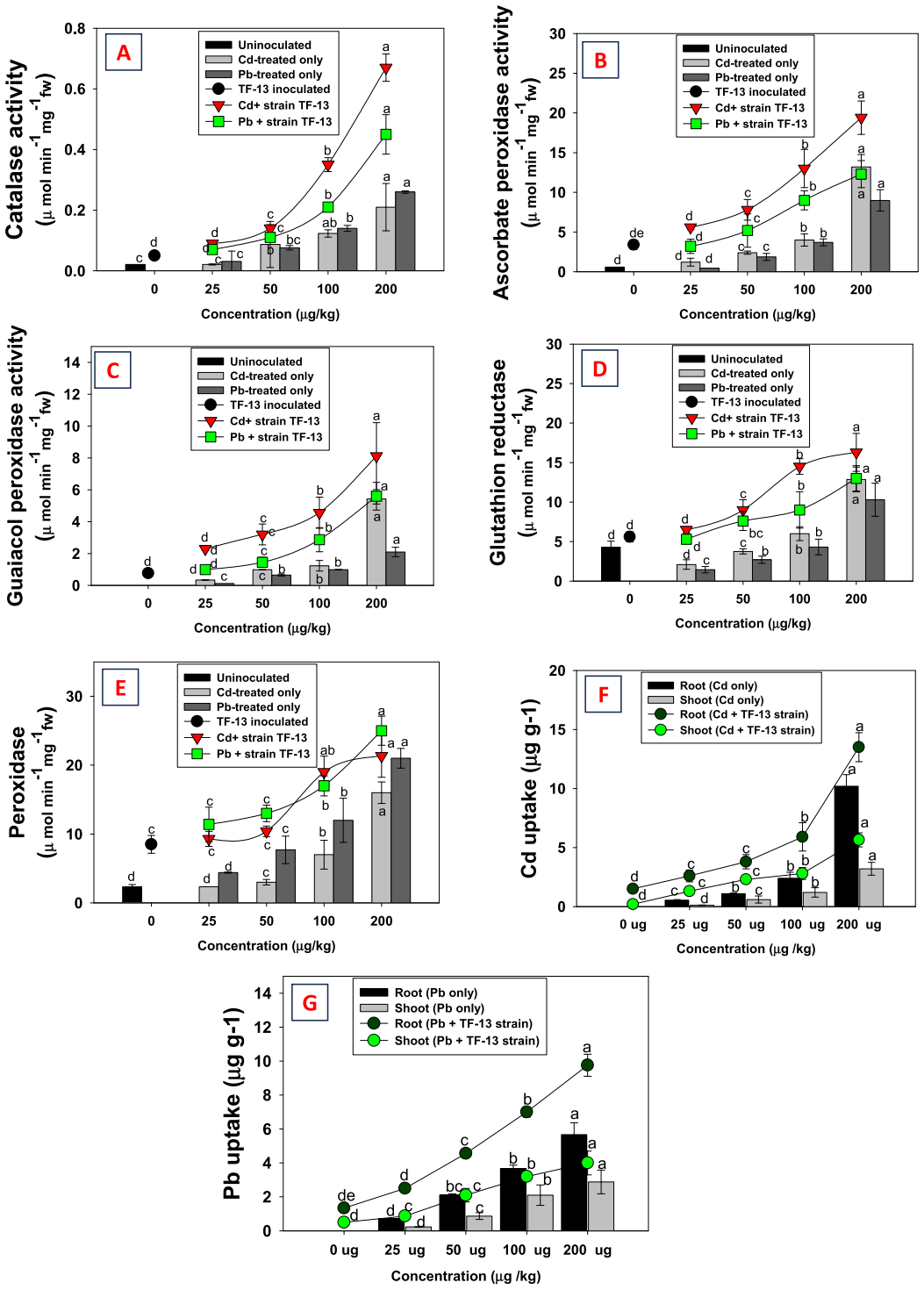

Figure 5. Antioxidant enzymatic responses of *Trichoderma* sp. TF-13 inoculated with Cd and Pb-treated *V. radiata* plants; CAT (A), ascorbate POD activity (B), guaiacol POD activity (C), GR (D), and POD activity (E). Panels (F,G) represent the uptake of CD and Pb in *V. radiata* exposed with increasing doses (0, 25, 50, 100, and 200 μg kg^{-1} soil) and inoculated with metal-resistant *Trichoderma* sp. TF-13. The DMRT indicates that mean values differ significantly when represented by different letters.

serves as a marker for environmental stressors.[63] In our investigation, it was discovered that *V. radiata* plants treated with varying levels of Cd and Pb had higher proline and H_2O_2 levels than untreated control plants. Generally, proline levels in plants raised by HMs aid in defense by adjusting osmotic pressure, scavenging ROS, acting as a redox buffer for reductants (NADH and NADPH), supplying energy for reductants, and establishing connections with critical pathways associated with abiotic stress management procedures. Under HM stress, H_2O_2 raises extremely hazardous OH radicals by Fenton and Haber−Wiess reactions with the assistance of free redox active Cd and Pb ions.

Under HM stress, H_2O_2 raises extremely hazardous OH radicals by Fenton and Haber−Wiess reactions with the assistance of free redox active Cd and Pb ions. By oxidizing a target molecule, H_2O_2 serves as the major form of ROS as a

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signaling molecule in plants.[64] The plant organs of several agronomically important plant species, such as *Zea mays*,[65,66] *Cajanus cajan*,[67] *Persicaria hydropiper*,[68] *Solanum tuberosum*,[69] and (*S. bicolor*)[70] have shown an increased accumulation of proline and H_2O_2 when exposed to different metals. However, inoculation of *Trichoderma* sp. TF-13 strain mitigated the toxicity and lowered the level of proline and hydrogen peroxide in metal-stressed *V. radiata*. For instance, it is interesting to note that the application of *Trichoderma* sp. TF-13 under the influence of 200 μg kg^{-1} soil Cd treatment greatly reduced the proline content in the roots and leaves of *V. radiata* by 66.4 and 76.1%, respectively, over the untreated control (Figure 4A). Also, proline content in 200 μg Pb kg^{-1} soil cultivated *V. radiata* lowered the level of root proline (from 6.7 μ mol mg g^{-1} fw to 1.2 μ mol mg g^{-1} fw) and shoot proline (from 8.6 μ mol mg g^{-1} fw to 2.4 μ mol mg g^{-1} fw) when inoculated with *Trichoderma* sp. TF-13 strain (Figure 4B).

In a similar manner, the H_2O_2 content of fungal-inoculated plants were considerably declined in metal-treated *V. radiata*. The maximum reduction was recorded at lower Cd and Pb doses. For instance, maximum reduction in H_2O_2 content roots (63.6%) and shoots (58%) of *Trichoderma* sp. inoculated 25 μg Cd kg^{-1} soil-treated *V. radiata* was recorded over uninoculated treatment (Figure 4C). Similarly, strain TF-13 has been shown to maximally decrease H_2O_2 content of 25 μg Pb kg^{-1} soil-exposed roots (from 8.0 μ mol mg g^{-1} fw to 4.0 μ mol mg g^{-1} fw) and shoots (from 18.2 μ mol mg g^{-1} fw to 14.0 μ mol mg g^{-1} fw) of *V. radiata* (Figure 4D). The fungal soil isolate, which disrupts the H_2O_2 signaling cascade by regulating the levels of antioxidant enzymes under metal stress, may be the cause of this positive regulation of proline and H_2O_2. Similar to our investigation, soil inoculation of mycorrhizal fungi modulated the tolerance of Cr to *Capsicum annuum* (L.) plants by regulating the metabolism of proline. The fungal isolates caused a significant reduction in metal stress by lowering the level of stress biomarkers.[71] In addition, a report claimed that Cd induced oxidative stress and increased proline accumulation in the soil cultivated, and arbuscular mycorrhizal fungi-inoculated *Solanum lycopersicum* (L.) were modified *S. lycopersicum* (L.) via induction of acquired systemic tolerance.[72] Also, HM tolerance in *Triticum aestivum* (L.) were increased when plants were subjected to beneficial soil fungal isolates.[73]

HM stress, which generates ROS and impacts lipid peroxidation (LPO) in cellular membranes, has an adverse effect on a variety of plants, including legumes. In plant tissues, malondialdehyde (MDA) is a byproduct of LPO and therefore acts as a sign of stress in plants. In light of this, the amount of MDA produced by *V. radiata* leaves exposed to various Cd and Pb dosages was examined. Based on our findings, MDA content was usually higher in leaves with higher metal dosages. Plant organs (leaves and roots) typically contained more MDA when exposed to higher metal dosages. For instance, Cd at 200 μg kg^{-1} soil significantly ($p \leq 0.05$) augmented the formation of MDA in roots (73.4%) and shoots (66.8%) over the uninoculated control compared to the control (Figure 4E,F). But even with 200 μg Cd kg^{-1} soil, *Trichoderma* sp. TF-13 successfully lowered the metal stress, which in turn decreased the amount of MDA by 43 and 49% in roots and shoots, respectively, compared to uninoculated but treated control (Figure 4E,F). Like our study, single and dual inoculation of saprophytic fungal consortium and *Rhizophagus irregularis* potentially ameliorated the metal-induced toxicity in *S. lycopersicum* (L.) grown under higher doses of Cd, Cu, Zn, Pb, and As and significantly decreased the MDA level in plants.[74] In another study carried out by Ikram et al.,[75] HM-stress-induced *T. aestivum* (L.) displays increased levels of MDA and proline than untreated plants. However, these contents decreased after the plants were inoculated with IAA producing and Cu, Cd, Pb, Ni, and Zn-tolerant endophytic fungus *Penicillium roqueforti* to metal-exposed wheat plants.

Inoculation Impact of Trichoderma sp. TF-13 on Antioxidant Enzymatic Responses of Metal-Treated V. radiata. The antioxidant enzyme activity in the metal-exposed *V. radiata* plants were significantly impacted by inoculation of *Trichoderma* sp. The increasing metal concentration enhanced the antioxidant enzymes in plants. At higher metal doses, the extent of accumulation of antioxidant enzymes was higher. For instance, activity of catalase activity (CAT), APX, guaiacol peroxidase activity (GPX), glutathione reductase (GR), and peroxidase (POD) were found to be 0.021, 0.60, 0.011, 4.30, and 2.34 μ mol min^{-1} mg^{-1} fw, respectively, in the *V. radiata* plant under controlled conditions, which, however, maximally increased to 0.67 (96.8%) (Figure 5A), 19.4 (97%) (Figure 5B), 8.12 (99.8%) (Figure 5C), 16.3 (75%) (Figure 5D), and 21.34 (90%) (Figure 5E) μ mol min^{-1} mg^{-1} fw, when plants were detached from soil cultivated with 200 μg Cd kg^{-1} soil.

The higher accumulation of metals in *V. radiata* growing in Cd and Pb-treated soil could account for these elevated antioxidant enzymes. By blocking the passage of hydrogen peroxide (H_2O_2) from the cytosol, CAT and POD eliminate generated H_2O_2 through purine catabolism, photorespiration, and oxidation of fatty acids. Additionally, earlier research found that under varying metal stress, the antioxidant enzymatic activities like CAT, POD, APX etc. in *Oryza sativa*,[76] *Lactuca sativa*,[77] *S. lycopersicum*,[78] *Phaseolus vulgaris*,[79] and *Hordeum vulgare*.[80] During metal poisoning, excessive ROS generation alters plant mitochondrial function and disrupts electron transport to NADH- and NADPH-dependent systems. As a result of this inhibition, H_2O_2 levels increases, which in turn elevates the metabolism of free radicals and has a deleterious effect on cellular metabolic processes. Nonenzymatic LPO, for example, may indirectly aid in facilitating the accommodation of oxidative burst in numerous cell locations. When metal-tolerant strain TF-13 were administered to Cd and Pb-treated *V. radiata*, the accumulation of antioxidant enzymes was considerably ($p \leq 0.05$) reduced in the HM-treated plants. For instance, plants treated with 25 μg Cd kg^{-1} and inoculated with TF-13 were evaluated against uninoculated plants. The maximum drop (65%) in CAT activity was also observed when *Trichoderma* sp. TF-13 was subjected with lower Cd doses. This advantageous result could be ascribed to the capacity of TF-13 strain to provide an array of benefits to host plants such as nutrition and improve stress resilience even in the presence of metal toxicity. The evident reduction in antioxidants caused by metal-tolerant fungal inoculation resulted in the overall growth of *V. radiata* being greatly boosted even in soils with metal contamination. Like our study, after inoculating sunflower plants with *Trichoderma* sp., Abeed et al.[81] claimed that oxidative stress in metal-treated plants declined and antioxidant enzyme activity were significantly reduced. Similarly, combined application of *T. asperellum* with biochar improved the physiological and oxidative stress properties of Ni- and Cu-stressed *Z. mays* plants.[82] In addition, phytohormone synthesizing endophytic fungus *P. roqueforti* considerably

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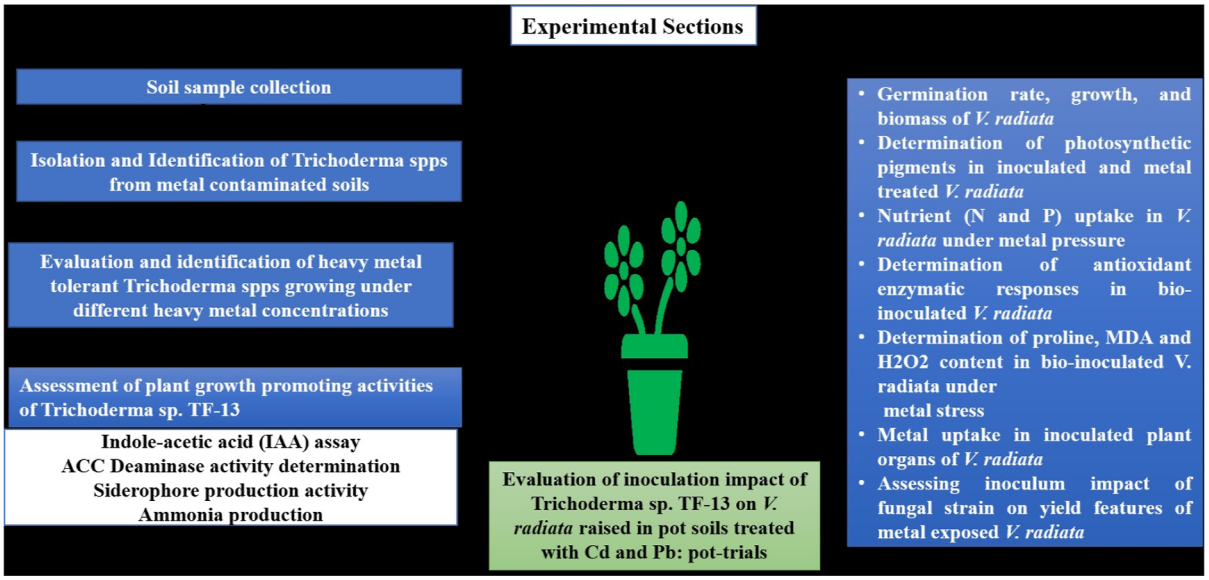

Figure 6. Layout depicting the overall experimental research plan and design of study conducting throughout the finding.

declined antioxidant enzymatic activities of *T. aestivum* (L.) growing in metal stressed soil.[83]

Metal Uptake in Trichoderma-Inoculated V. radiata. Despite large variations in the degree of metal uptake in roots and shoot tissues of *V. radiata*, increasing Cd and Pb dosages consistently resulted in higher metal concentrations. Among the species and doses of metals, 200 μg Cd kg^{-1} soil caused the maximum metal uptake in roots (13.5 μg g^{-1}) and shoot (5.6 μg g^{-1}) tissues (Figure 5F). Fascinatingly, following inoculation of metal-tolerant *Trichoderma* sp. TF-13, the accumulation of Cd and Pb were significantly reduced in plant organs of *V. radiata*. For instance, following inoculation with *Trichoderma* sp. TF-13, the Cd and Pb absorption by roots taken from *V. radiata* growing at 25 μg Cd kg^{-1} soil was reduced by 79.2 and 90.7%, respectively, over uninoculated plants. Similarly, strain TF-13 maximally and significantly ($p \leq 0.05$) the Pb content in roots (72%) and shoots (74%) when applied to *V. radiata* cultivating in soil contaminated with 25 μg Pb kg^{-1} (Figure 5G). Despite being a very dangerous element, some metal ions are transported easily from the soil to plant parts. This may be brought on by the synthesis of protons, root exudates, and other metabolites that make it easier for metal ions to dissolve, most likely by the development of metal-chelating complexes. As a result, *V. radiata* inoculated with metal-tolerant fungal isolate may have displayed less metal deposition in each organ, likely as a result of the metal-tolerant strain accumulating the majority of the metal component present in the soil. Therefore, plants could only absorb a smaller amount of metal. Thus, *Trichoderma* sp. TF-13 strain-inoculated *V. radiata* had a better chance of surviving even in soil contaminated with Cd and Pb as a result of the metal pressure being relieved. Also, this finding suggests that TF-13 strain supplementation decreased the uptake of Cd and Pb in *V. radiata* through decreasing its bioavailability at the rhizosphere. Rhizosphere microorganisms typically have the capacity to limit the mobility of metals at the rhizosphere by entrapping them with cell−surface polymeric compounds, rendering them unavailable to the plants. Similar to this, the metal-resistant *T. harzianum* decreased Cd concentrations in *H. vulgare* (L.) by reducing the metal uptake.[84] In a study, *T. atroviride* significantly improved the phytoextraction efficiency of Ni- and Cd-exposed *B. juncea* plants by lowering the metal accumulation in plant organs.[59]

CONCLUSIONS

To increase the crop output and management, agricultural activities in HM-contaminated areas require special consideration. The recovered putative multimetal-tolerant Cd- and Pb-reducing *Trichoderma* sp. TF-13 was notably capable of synthesizing growth-regulating substances even when subjected to metal pressure. *Trichoderma* inoculation led to a significant increase in growth, biomass, chlorophyll formation, nutrients, and yields of Cd- and Pb-treated *V. radiata*. Further, metal uptake, stress markers, and antioxidant enzymatic activities in HM-treated plants were improved following *Trichoderma* inoculation. Thus, it was found that strain TF-13 is an efficient/possible candidate that might be employed to clean up HM-contaminated soils. Due to its commendable metal tolerance ability and other unique growth-promoting characteristics, metal-resistant *Trichoderma* sp. may therefore be used as a potent fungal inoculant that can be used in an easy, inexpensive, and environmentally friendly way to remediate metal-contaminated soils. Additionally, the study suggests minimizing the irrigation of food crops with industrially released effluents. In summary, use of HM-tolerant microorganisms in agriculture could be a viable strategy for removing HM contamination in agriculture soils. In the future, transferring genes from these HM-resistant microorganisms to other beneficial microbes or plants through genetic engineering can be an effective way for mitigating HM stressors in sustainable agriculture.

EXPERIMENTAL SECTIONS

Fungal Isolation and Metal Tolerance Evaluation. For fungal isolation, we collected rhizosphere soil samples from vegetables growing in contaminated agricultural fields near the industrial area, Chinhat, Lucknow, (26.8744° N, 81.0350° E) UP (India). Serial dilution (10^{-3} and 10^{-4}) of soil samples were prepared and further spread on Potato Dextrose agar (PDA; Hi Media, Mumbai, India) medium plates then kept at

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28 ± 2 °C for 3−4 days. In this study, we isolated 20 *Trichoderma* strains and assessed for their cultural and biochemical properties. By using microscopic examination, the fungus was provisionally identified at the genus level. Recovered fungal isolates were cultured on metal-treated PDA plates to test their tolerance ability of HMs, like Cd, Pb, Cr, and Ni. The freshly cultured fungal isolates were spotted on metal-supplemented PDA and incubated at 28 ± 2 °C for 3−4 days and observed for their survival ability. Metal-resistant fungal isolates that have survived the highest concentrations of metals are referred to as metal-tolerant fungal strains (see Figure 6).

Assessment of Plant Growth-Promoting Activities of *Trichoderma* sp. TF-13. *Assay for IAA.* In this experiment, we followed the procedure of Bric et al.[85] to determine IAA levels secreted by *Trichoderma* sp. TF-13 strain under HM stress (refer to Supporting Information, Section S2.2.1).

ACC Deaminase Activity Determination. In order to measure the ACC deaminase activity under metal stressed conditions, strain TF-13 was grown in a 50 mL TSB medium and incubated at 28 °C and activity was determined (for detailed descriptions, see Supporting Information Section S2.2.2).[86,87]

Siderophore Production Activity. The $FeCl_3$ test was used to estimate the siderophore qualitatively by cultivating the strain TF-13 in a liquid broth amended with increasing Cd and Pb doses. Additionally, the previously described methodology was used to assess the siderophore production,[88] which entailed spot-inoculating newly formed cultures of strain TF-13 onto Cd and Pb-treated CAS agar plates. After incubation, the plates were checked for the appearance of a yellow to orange zone (halo), which denotes the release of siderophores, around fungal growth. A quantitative evaluation of siderophores was performed using Modi media. To do this, we inoculated a fungal cell suspension (100 μL) in a metal-supplemented Modi medium kept for a 5 day incubation period at 28 ± 2 °C. The phenolate siderophores SA and 2, 3-dihydroxybenzoic acid (DHB) was quantified.[89]

Ammonia Production. Under metal-stressed conditions, the ammonia production activity of strain TF-13 was evaluated according to previously published protocols.[90] For the test, strain TF-13 was inoculated in metal-added peptone water and were then incubated for 4 days at 28 ± 2 °C. Upon receiving a good response, the color change from yellow to reddish brown was recorded as positive for ammonia production.

Evaluation of Inoculation Impact of Trichoderma sp. TF-13 on V. radiata Raised in Pot Soils Treated with Cd and Pb: Pot-Trials. Seeds (healthy and uniform sized) of *V. radiata* (L.) were obtained from the local seed market. Seeds were with 4% sodium hypochlorite (NaOCl; Sisco Research Laboratory Pvt. Ltd., Mumbai, India) for 3 min. After that, seeds were washed six or seven times in sterile water. Using a 1% guar gum powder as an adhesive, the seeds were primed with metal-tolerant *Trichoderma* asp. TF-13 Furthermore, uncontaminated seedlings that had just been immersed in sterile water for the control. The bioprimed and uninoculated seeds were planted in earthen pots in a 3.0 kg of nonsterilized alluvial sandy clay loam soil. Two days before the sowing of seeds, increasing doses (0, 25, 50, 100, and 200 mg/kg soils) of Cd and Pb were added to soil. These Cd dosages were employed in the experimental trials in order to reproduce the amounts observed in the soil samples tested. There were 18 treatments with 3 replications (refer to Supporting Information, Table S1).

The pots were arranged using a totally random block design. Each container could only support two plants at a time until harvest. The pots were kept outside (open-air settings) and given sporadic tap water irrigations. The tests were run several times over a period of 2 years in order to confirm the precision and repeatability of the results.

Germination Rate, Growth, and Biomass of V. radiata. After 7−8 DAS, the germination efficiency of Cd and Pb-treated and *Trichoderma* sp. TF-13 primed *V. radiata* was recorded. The plant organs (roots and shoots) were carefully removed from the soil. Deionized water was used to clean the plant roots in order to eliminate any debris from their surface. A digital balance was used to measure the length of the plant shoots, roots, and fresh material. In order to calculate the dry biomass, every plant part was oven-dried (York Scientific Industries, Pvt. Ltd. India) at 70 °C until a consistent weight was attained.

Determination of Photosynthetic Pigments in Inoculated and Metal-Treated V. radiata. The content of chlorophyll (a, b and total) and carotenoid in Cd/Pb-treated and *Trichoderma* sp. TF-13 inoculated *V. radiata* plants were examined[91,92] (for detailed descriptions, refer to Supporting Information, Section S2.4).

Nutrient (N and P) Uptake in V. radiata under Metal Pressure. The root and shoot samples were detached from bioinoculated and metal-treated *V. radiata* plants. The uptake of N and P in plant samples were determined following the methods of Lindner[93] and Jackson[94] (for detailed descriptions, refer to Supporting Information, Section S 2.5).

Determination of Antioxidant Enzymatic Responses in Bioinoculated V. radiata. The antioxidant enzymes in *V. radiata* plants exposed to increasing concentrations of Cd and Pb and inoculated with TF-13 strains was determined. For the assessment, leaf samples (0.5 g) were homogenized in a 50 mM phosphate buffer (pH = 7.0) containing 1% polyvinylpyrrolidone (PVP; Hi-Media Pvt. Ltd., Mumbai, India). The mixtures were homogenized (at 15,000*g* for 10 min and at 4 °C), and the resulting filtrate was used to measure the antioxidant enzymes.

POD Activity. In order to measure the POD activity, 0.1 mL of the enzyme extract was mixed with pyrogallol, phosphate buffer (pH = 6.8), and 1% H_2O_2. In order to determine absorbance at 420 nm, a UV−visible spectrophotometer was employed.[95]

CAT Activity. Using the method proposed by Abei,[96] the CAT was calculated to measure the removal of H_2O_2 at the start of the reaction. The reaction mixture for the sample was made up of 100 μL of enzyme extract, 50 mM phosphate buffer (pH 7.0), and 15 mM hydrogen peroxide. The decrease in optical density at 240 nm for 2 min with a break of 30 s at 25 °C paralleled the decrease in H_2O_2 levels. As a control, a test tube was filled with all of the aforementioned solutions except the enzyme extract.

GPX Activity. With a few minor modifications, the method of Sánchez et al.[97] was used to assess the GPX in metal-treated and fungal inoculated *V. radiata*. The reaction mixture for the sample was made up of 100 μL of enzyme extract, 50 mM of phosphate buffer (pH 7.0), 20 mM of guaiacol, and 1.5 mM of H_2O_2. By observing the absorbance at 436 nm for 1 min at 25 °C, the GPX activity was determined.

GR Activity. The GR in metal contaminated and *Trichoderma* sp. inoculated *V. radiata* was assessed following the method of Cribb et al.[98] The reaction mixture was started

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by adding glutathione disulfide (GSSG) to the cuvette, then using a UV−visible spectrophotometer, the drop-in absorbance at 405 nm was measured for 1 min at 30 °C. A unit of GR activity is defined as the quantity of enzyme catalyzing the reduction of 1 μM of NADPH per minute.

Determination of Proline, MDA, and H_2O_2 Content in Bioinoculated *V. radiata* under Metal Stress. *Proline Content Estimation in Metal-Contaminated V. radiata.* Using the procedure outlined by Bates et al.,[99] proline content accumulated in plant organs of Cd and Pb-treated, and *Trichoderma* TF-13-inoculated *V. radiata* was determined (refer to Supporting Information, Section S2.6).

MDA Content Determination. The previously utilized methods[100−104] were used to calculate LPO as evaluated by MDA content in metal exposed and *Trichoderma* sp. inoculated *V. radiata*. In order to conduct the test, roots were homogenized in 0.1% trichloroacetic acid (TCA; Sisco Research Laboratory Pvt. Ltd., Mumbai, India) and centrifuged for 15 min at 10,000*g*. The supernatant was mixed with thiobarbituric acid (TBA; Sisco Research Laboratory Pvt. Ltd., Mumbai, India; 20% TCA solution, in 0.5% TBA). The solution was then heated for 30 min at 95 °C. Following cooling, the supernatant was centrifuged at 1000*g* for 15 min at 4 °C. The obtained resultant was measured at 532 nm.

Determination of Hydrogen Peroxide (H_2O_2) Concentrations. The procedure outlined by Patterson et al.[105] was employed in order to determine the hydrogen peroxide (H_2O_2) concentrations inside the leaf and root tissues of inoculated and Cd and Pb exposed *V. radiata*. For this, 500 mg of inoculated and treated plant material was homogenized in 3.0 mL of 50 mM phosphate buffer (pH 6.8) and the homogenate was centrifuged for 25 min at 6000*g*. From this obtained homogenate, 3.0 mL of the extract was combined with 0.1% titanium chloride produced in 20% (v/v) H_2SO_4, and the combination was again centrifuged at 6000*g* for half an hour. At 410 nm, the absorbance of the colored solution was measured. The H_2O_2 content was calculated using a standard curve with known H_2O_2 concentrations on a fresh mass basis.

Metal Uptake in Inoculated Plant Organs of V. radiata. The uptake and accumulation of Cd and Pb in dried plant organs (root and shoots) of *Trichoderma* sp. TF-13 inoculated *V. radiata* was evaluated.[106,107] In this study, 100 mg of dried plant samples were separately digested in a 4:1 solution of nitric acid (HNO_3) and perchloric acid ($HClO_4$). Following digestion, the residual suspensions were filtered using Whatman no. 2 filter paper, and the volume was increased to 100 mL using ddH_2O. The concentrations of Cd and Pb in each sample was evaluated using a double beam flame atomic absorption spectrophotometer (FAAS; GBC 932B Plus, GBC Scientific Equipment Ltd., Braeside, Australia).

Assessing Inoculum Impact of Fungal Strain on Yield Features of Metal Exposed V. radiata. At 80 DAS, *V. radiata* growing in soils inoculated with strain TF-13 and treated/untreated with increasing doses of Cd and Pb were eventually harvested, and SY was noted. The protein content in grains was measured.[108] Briefly, 500 mg of seeds were steeped in pH 7.4 phosphate buffer and then extracted in 3.0 mL of pH 7.8 phosphate buffer that contained 2% w/v polyvinylpyrrolidone (PVP) and 1.0 mM EDTA. This was done to estimate the amount of protein in the seeds. The supernatant from the extract's spin at 5742 g for 10 min at 4 °C was further used for protein analysis.

Statistical Analysis. The trials were set up in a completely randomized design and performed in triplicate (n = 3). An analysis of variance was carried out using Statistics 8.1 to review the collected data. In order to statistically examine the results, One-way ANOVA and Minitab-17.1 were used. To compare the treatment means, DMRT was also run on the data at a 5% confidence level.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c10470.

Details on assay for indole-3-acetic acid, ACC deaminase and determination of photosynthetic pigments, nutrient (N and P) uptake, proline accumulation in *Trichoderma* inoculated and metal-treated *V. radiata* plants under pot soils condition, experimental plan for metal treatment and *Trichoderma* inoculation to pot soils, and qualitative and quantitative siderophore production ability of *Trichoderma* sp. TF-13 (PDF)

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Notes

The authors declare no competing financial interest.

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