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Assessing the Phytoextraction of Cd, Pb, and Zn from a Slag-Contaminated Soil by Legume Species Inoculated with Rhizobial Strains

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Abstract

Metal-polluted soils can pose a severe threat to the environment and need remediation. The work aimed at assessing the potential of jack bean (*Canavalia ensiformis* L.) and mucuna (Mucuna pruriens (L.) DC.) inoculated with rhizobial strains on the phytoremediation of soils contaminated by lead (Pb) smelting activities in Santo Amaro, Bahia state, the most severe case of Pb contamination in Brazil. Plants were grown in pots containing soils with three contamination levels for Pb, cadmium (Cd), and zinc (Zn) based on the distance from the chimney of the abandoned Pb smelter plant. The results showed that legumes and inoculated strains were tolerant to soil contamination. The BR 2811 strain is potentially indicated to increase jack bean biomass. The Cd, Pb, and Zn concentrations in plants were highest when grown on the soil with the highest contamination level. We found significant interactions between strains and soil contamination levels for phytoextraction efficiency. However, the very high metal contents of the soil can make phytoextraction unfeasible due to the time required to bring the metals to regulatory concentrations. The Cd, Pb, and Zn net removal from soil was proportional to increased contamination levels. The legumes did not show potential for Pb phytoextraction, but they have the potential for Zn phytoextraction and Cd phytostabilization. The BR 2811, BR 3501, and BR 7606 strains were the most promising, increasing the phytoremediation potential of jack bean and mucuna.

Highlights

- Rhizobia strains differ in their ability to protect plants against metal stress.
- Nodulation was directly related to plant metal tolerance.
- A specific legume-bacteria combination is required to enhance phytoremediation.
- Phytoextraction efficiency depends on the level of soil contamination.

Keywords Phytoremediation · Heavy metals · Trace elements · Soil pollution · *Bradyrhizobium*

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1 Introduction

Smelting activities can pose severe environmental threats, as waste containing heavy metals is generated during the operations. For instance, slag disposal from Pb smelting caused Brazil's most severe human metal contamination. The event occurred in Santo Amaro, Bahia state, where an abandoned Pb smelter plant processed galena (PbS - lead sulfide) to produce lead ingots for 33 years (Andrade and Moraes 2013). The activity left an estimated environmental liability of 500,000 tons of slags highly contaminated with Pb, Zn and Cd (Miranda et al. 2018; Silva et al. 2017). Epidemiological studies found mean Pb concentrations of 17.9 μ g dL⁻¹ in the blood of children 1 to 9 years old living in the town; this figure is threefold higher than the reference level by the Center for Disease Control and Prevention (CDC 2013). More than 18,000 inhabitants in Santo Amaro may have been victims of Pb poisoning resulting from this environmental contamination. The smelting activity also extensively added Zn and Cd to the surrounding soils, although studies on the toxicity of these metals to humans in the region are scarce. Silva et al. (2017) estimated that children's daily exposure to Zn and Cd in Santo Amaro exceeded the acceptable daily intake. Zinc is relatively nontoxic, but manifestations of overt toxicity symptoms (nausea, vomiting, epigastric pain, lethargy, and fatigue) will occur with high zinc intakes (Schoofs et al. 2024). Cadmium, on the other hand, is highly toxic, and long-term exposure can lead to various diseases, such as cancer, leukemia, and genetic toxicity (Charkiewicz et al. 2023).

The soils surrounding the Pb plant show contamination by multiple metals due to the slag disposal and the chimney metal emission, and require remediation to diminish human health risks. Thus, several remediation techniques can be applied depending on the concentration of metal in the soil. For example, phytoremediation, a technology that uses plants to remove, stabilize, degrade, or reduce the mobility of metals in the soil, is an approach suitable for areas with medium to moderate metal contamination (Shah and Daverey 2020; Ullah et al. 2015). Phytoextraction, one of the phytoremediation techniques, involves cultivating plants to concentrate metals in their shoot biomass that are removed from the site, leading to soil cleanup (Nascimento et al. 2021). The relatively low cost and public acceptance compared to other remediation strategies, such as soil removal, are the main advantages of phytoextraction. Such characteristics are essential for countries such as Brazil where resources for environmental remediation are insufficient (Nascimento and Xing 2006). Moreover, phyto-extraction can incorporate novel techniques such as chelating agents, genetic engineering, and microbial enhancement, further enhancing efficiency and sustainability (Nayeri et al. 2023; Rostami et al. 2023; Zhang et al. 2024).

Plants of the Fabaceae family, commonly known as legumes, are often used as pioneers for colonizing and restoring the quality and health of degraded and nitrogen-limited environments. These legume species have shown the potential to increase the concentrations of available phosphorus, nitrogen, and organic matter when grown on soils contaminated with metal tailings. Additionally, these plants establish symbiosis with diazotrophic bacteria, forming specialized structures called nodules where the biological nitrogen fixation process occurs. However, studies utilizing these legume species to stabilize or remove metals from contaminated soils are relatively scarce, underscoring the novelty and importance of our research.

The association between plants and microorganisms is critical to plant growth and survival in field conditions under toxic concentrations of heavy metals, and it can enhance phytoremediation (Navazas et al. 2022). In this scenario, bacterial-assisted phytoremediation has been gaining attention because it is eco-friendly, low-cost, and has made promising advancements (Montreemuk et al. 2024). Metal-contaminated soils affect soil chemistry and plant development, changing the community structure of naturally established microorganisms (Lin et al. 2019). Therefore, inoculation with previously selected bacteria can increase plant tolerance and biomass production, enhancing phytoremediation (Pajuelo et al. 2011). Such effects were reported for several legumes, such as *Lupinus albus*, soybean (Sánchez-Pardo and Zornoza 2014), *Mucuna deeringiana* (Boechat et al. 2016), *Medicago lupulina* (Jian et al. 2019; Kong et al. 2015), *Medicago sativa* (Chen et al. 2018), *Robinia pseudoacacia* (Fan et al. 2018), *Vicia faba, Sulla coronaria* (Saadani et al. 2019) and *Lathyrus sativus* (Abdelkrim et al. 2020).

Rhizobial strains can interact with legumes under heavy metal availability conditions in several ways besides nitrogen fixation since several other plant-growth and metal plant protection mechanisms are also displayed by a plethora of rhizobia strains. Briefly, massive rhizobial application (inoculation) could increase root protection from metals throughout exopolysaccharides production by rhizobia, forming a net of biofilm that binds the metals and protects the roots (Olenska et al. 2021). In addition, auxin production (a quite common characteristic of rhizobia) can be improved under metal toxicity, mainly by reducing the exogenous auxin threshold to plant growth promotion, increasing the ability of the auxin-producing strain to promote the growth of the host plant (Barazani and Friedman 1999). Once absorbed, the metals can interfere negatively with several cellular processes, mainly by increasing the reactive oxygen species (ROS) and other oxidative compounds. Rhizobia can increase the plant's antioxidative enzyme activity (Catalase, Superoxide dismutase, Ascorbate peroxidase, among others), reducing oxidative cell damage (Fatnassi et al. 2015).

Studies on legumes in heavy-metal-contaminated soils can lead to the discovery of resistant rhizobia, which could be used as inoculants in these harsh environments. However, a significant knowledge gap exists in our understanding of how legume-based phytoremediation works, especially with bacterial strains adapted to tropical conditions and metaltolerant. Also, research must focus on high-biomass legumes as metal removal from soil relies on both the metal concentration in shoots and biomass production (Schück and Greger 2020; Nascimento et al. 2021).

Given that the reclamation of large metal-contaminated areas is costly and should involve several approaches due to the high variability of metal concentrations across the sites, phytoextraction can provide an environmentally friendly and economically feasible approach to soils presenting low to moderate levels of metal contamination. Here, we assessed the potential of two high-biomass legume species (jack bean and mucuna) inoculated with previously selected rhizobia strains for phytoextraction of Pb, Cd, and Zn in soils from the vicinity of the Pb smelter plant in Santo Amaro showing different contamination levels. Few studies have examined rhizobia-inoculated legumes for phytoremediation of soils contaminated by multiple metals, especially in tropical conditions. Thus, this work provides information on the combination of high-biomass legumes and bacterial strains for removing or stabilizing metals from contaminated sites directly impacting our understanding of the phytoextraction processes enhanced by microorganisms.

2 Materials and Methods

2.1 Soil Sampling and Chemical Analyses

Soil samples were collected from the topsoil (0–20 cm) at three different sites around the Pb smelter plant in Santo Amaro, Bahia state, Brazil (12° 32' 48" S 38° 42' 43" W). Sampling sites were selected based on the distance from the source of contamination (chiminea) to obtain three levels of metal contamination (L1, L2, and L3) (Table 1). Soil samples were analyzed using standard soil methods (Teixeira et al 2017).

The concentration of metals in the samples was obtained by digesting aliquots of 0.500 g of soil (<0.15 mm) with an acid solution (HNO₃+HCl) in a microwave oven at 175 °C, according to method 3051 A (US EPA 2007). The Cd, Pb, and Zn concentrations were measured by optical emission spectrometry (ICP-OES/Optima 7000, Perkin Elmer), while those of chromium (Cr), iron (Fe), manganese (Mn), copper (Cu) and nickel (Ni) were determined by an atomic absorption spectrophotometer (Analyst 800 Perkin Elmer).

2.2 Pot Experiment

The experiment was conducted in pots containing one dm³ of soil, in which the herbaceous leguminous jack bean and mucuna were grown. The seeds were superficially disinfested in 98% alcohol for 3 min and 1% sodium hypochlorite for 3 min, with successive washings in distilled and autoclaved water to remove the sodium hypochlorite. Seedlings were thinned ten days after germination to leave one plant per pot.

Characteristic	Distance from the source of contamination						
	Area farthest from the source of contamination (L1)	Area of medium distance from the source of contami- nation (L2)	Area of shortest distance from the source of con- tamination (L3)				
pH (water, 1:2.5)	7.55	7.46	5.50				
Na ⁺ (cmol _c kg ⁻¹)	98.00	90.50	58.00				
K^+ (cmol _c kg ⁻¹)	36.00	30.50	41.50				
$P(mg kg^{-1})$	48.04	16.86	21.63				
$Fe (mg kg^{-1})$	32386.85	37301.85	46541.85				
Cu (mg kg ⁻¹)	33.08	46.83	171.33				
Ni (mg kg ⁻¹)	18.38	25.48	33.83				
Mn (mg kg ⁻¹)	352.68	540.23	711.23				
$Cr (mg kg^{-1})$	35.90	44.60	54.10				
$Cd (mg kg^{-1})$	1.45	8.00	89.30				
$Pb (mg kg^{-1})$	195.43	1004.58	5529.08				
$Zn (mg kg^{-1})$	218.53	490.68	1043.63				
Sand (%)	41.20	15.17	11.30				
Silt (%)	23.10	35.14	40.12				
Clay (%)	35.70	49.68	48.84				
Textural class	Clay loam	Clay	Clay				

 Table 1
 Chemical and physical characteristics of soil samples collected at varying distances from the chimney of a lead smelter plant in Santo Amaro, BA, Brazil

The bacteria were cultivated in a liquid yeast-mannitol (YM) medium under monoxenic conditions during adequate growth time for each bacterium to prepare the inoculants. The bacterial strains used were obtained from the bacterial collection of the Johanna Döbereiner Biological Resources Center (*CRB-JD*), Embrapa Agrobiologia: BR 2811 (*Bradyrhizo-bium elkanii*), BR 3501 (*Ensifer* sp.), BR 7606 (*Rhizobium leguminosarum* bv. Trifolii), BR 10,026 (*Rhizobium etli*) and BR 10,247 (*Bradyrhizobium neotropicale*). The choice of legumes and rhizobia strains was based on the species' adaptation to tropical soils (MAPA 2011). One day after sowing, the liquid inoculant was pipetted (2 mL seed⁻¹) into the soil next to the seeds. Inoculation was repeated ten days after sowing, right after thinning, placing 2 mL of inoculant in each pot close to the plant's stem. Plants were harvested 65 days after germination. Shoots and roots were collected, and nodules were counted and weighed. The root and shoots biomass were then obtained after drying in an oven at 65 °C.

The experiment was set up in a randomized block design with three replicates, totaling 108 experimental units. The data were subjected to ANOVA to compare biomass (shoots and roots), nodulation, and metal net removals from soils, considering two independent experiments for each legume species, both with a factorial arrangement (3×6), with three levels of soil contamination and six inoculation treatments (5 different rhizobia strains and one treatment without inoculation). Nodule number data was transformed into.

 $\frac{(x+1)^{0.51}}{2}$. Means were compared using Tukey's test ($p \le 0.05$).

2.3 Plant Analysis and Soil Metal Removal Efficiency

Cadmium, Pb and Zn in plant tissues were extracted by the 3051a method of US EPA and determined by ICP-OES as previously described. To evaluate the effectiveness of the phytoextraction process, the net removal of metals by plants was evaluated according to the following equation:

Net Removal (mg pot⁻¹) =
$$\frac{\text{Metalshoots} \times \text{biomass}}{1000}$$

where Metal_{shoots} is the metal concentration in the plant shoots (mg kg⁻¹), and biomass is each plant's mass (mg) for the metal concentration considered.

3 Results and Discussion

3.1 Concentrations of Metals in the Soil Samples

As expected, the Pb, Zn, and Cd concentrations of the soil samples increased with increasing distances from the abandoned Pb smelter plant (Table 2). The contamination levels (L1, L2, and L3, respectively) for the metals were 195.43, 1000.58, and 5529.08 mg kg⁻¹ (Pb); 1.45, 8.00, and 89.30 mg kg⁻¹ (Cd); and 218.53; 490.68 and 1043.63 mg kg⁻¹ (Zn). The high metal concentrations found seriously threaten soil quality in the region.

The Cd, Pb, and Zn contents in the L3 were 446, 140, and 83 times higher than the background concentration of these metals for soils in the region (Dos Santos et al. 2017). These figures are much higher than the prevention (PV) and investigation (IV) values established

collected in different sites around the pb smelter plant in Santo Amaro, Ba state, Brazil						
Metal	PV	IV (<i>R</i> ; I)	L1	L2	L3	
Cd (mg kg ⁻¹)	1.3	8; 20	1.4 (0.8)	8.0 (3.5)	89.3 (11.2)	
$Pb (mg kg^{-1})$	72	300; 900	195.4 (47)	1004.6 (321)	5529.1 (853.8)	
Zn (mg kg ⁻¹)	300	1000; 2000	218.5 (23.2)	490.7 (58.6)	1043.6 (354.5)	

Table 2 Prevention (PV) and investigation values (IV) for metals in two land-use scenarios (R=residential; I=industrial), and total and available (between parentheses) concentrations of cd, pb and zn in soil samples

Table 3 Shoots biomass of jack bean and mucuna inoculated with diazotrophic bacteria or non-inoculated cultivated in soil samples with different contamination levels collected in Santo Amaro, Bahia, Brazil (L1=lowest contamination level; L2=intermediate contamination L2=intermediate contamination level; L3=highest contamination level;	Shoots biomass	L1	L2	L3	Mean	
	Jack beans					
	Non-inoculated	3.69 ab	5.50 a	3.48 a	4.22 a	
	BR 2811	5.27 a	4.33 a	3.24 a	4.28 a	
	BR 3251	2.81 ab	3.10 a	4.46 a	3.46 a	
	BR 7606	1.15 b	2.87 a	1.99 a	2.01 a	
	BR 10,026	3.58 ab	4.85 a	3.76 a	4.01 a	
	BR 10,247	3.16 ab	2.75 a	2.89 a	2.93 a	
level. Means followed by the	Mean	3.28 A	3.89 A	3.30 A		
same capital letters in lines and lowercase letters in columns are not significantly different at 5% probability according to Tukey's test)	Mucuna					
	Non- inoculated	5.05 a	5.05 a	3.55 a	4.53 a	
	BR 2811	3.38 a	3.89 a	3.92 a	3.73 a	
	BR 3251	3.93 a	3.80 a	3.73 a	3.82 a	
	BR 7606	4.25 a	4.64 a	2.01 a	3.63 a	
	BR 10,026	4.78 a	6.05 a	2.93 a	4.59 a	
	BR 10,247	4.30 a	4.41 a	4.36 a	4.36 a	
	Mean	4.28 A	4.64 A	3.42 B		

by the Brazilian resolution for permissible levels of metals in agricultural soils (Conama 2009). The concentrations of these metals in the soil collected at the shortest distance from the smelter plant had values 70, 76, and 3.5 times above the PV (Table 2). Even the L1 soil, farthest from the waste disposal focus and with the lowest contamination level, had a PV for Pb above the allowable concentration. The concentrations for Pb in L2 and L3, and for Cd in L3, were also above the IV, which indicates the maximum tolerable concentrations for soils under residential or industrial land use (Conama 2009). According to the Brazilian guidelines for heavy metals in soils, sites with soil concentrations above the IV pose an unacceptable risk to humans and must be remediated.

3.2 Plant Biomass and Nodulation

The increasing contamination levels did not affect the jack bean shoots biomass (Table 3). Inoculation of plants with the BR 2811 strain promoted an over 40% increase in shoots dry matter of the plants growing in L1 compared to non-inoculated plants; such an increase was significantly higher than plants inoculated with the BR 7606 strain. Other studies have shown that inoculating metal-stressed plants leads to increased biomass. For instance, inoculated soybean plants had a 38% greater biomass compared to noninoculated plants (Reichman 2007). Our data showed that such an effect depends on the bacterial strain and the plant species, as the mucuna mean biomass was diminished in L3, and no significant inoculation effect was observed with the different strains or contamination levels.

The inoculations did not influence the N concentration in the shoot tissues. Therefore, N nutrition was not the reason for improved tolerance of the plants to heavy metals, although disturbances in the plant uptake and distribution of micro- and macronutrients are considered key factors related to metal phytotoxicity (Vazquez et al. 2020). Rhizobia is a plant growth-promoting bacteria that provides other roles besides N fixation, including P solubilization, phytohormone synthesis, and siderophore release that may promote legume growth while diminishing metal toxicity. Both species of plants produced nodules in all treatments. However, Jack beans had the highest number of nodules in the L2 (intermediate contamination level). In contrast, mucuna nodulation was reduced in the soil with the highest contamination level (Fig. 1). It is likely that higher nodulation promoted jack beans' higher metal tolerance compared to mucuna plants, especially when inoculated with the BR 2811 strain.

The ideal plant for phytoextraction use must have the ability to hyperaccumulate metals, preferably in the shoots, tolerance to high concentrations of metals in the soil, rapid growth and high biomass and easy harvesting (Marchiol et al. 2004; Nascimento and Xing 2006). Jack bean and mucuna are nodulating legumes capable of producing large amounts of aboveground biomass and providing symbiotically fixed nitrogen to systems in short growing cycles (Hauser and Nolte 2002; Dantas et al. 2019), but are still poorly studied about tolerance to heavy metal contamination in Brazilian soils (Melo et al. 2006; Da Silva et al. 2018). Despite the symptoms of chlorosis, the two species survived and produced aboveground biomass at the three soil contamination levels (Table 3), demonstrating that



Fig. 1 Nodule number of jack and mucuna (averages of inoculated with selected rhizobia strains and non-inoculated plants) cultivated in soil samples with different contamination levels collected in Santo Amaro, Bahia, Brazil. L1=lowest contamination level; L2=intermediate contamination level; L3=highest contamination level. Bars accompanied by the same capital letters for mucuna and lowercase letters for jack bean did not differ significantly at 5% probability by Tukey's test

they are relatively tolerant to high concentrations of heavy metals in the soils, which qualifies them for phytoremediation programs in the studied soils (Nascimento et al. 2021).

Both jack bean and mucuna nodulated in all treatments (Fig. 1), even at the highest soil contamination level, demonstrating the presence of naturally established rhizobia populations in the contaminated soils. All collected nodules were active (evaluation by reddish color indicative of leg-hemoglobin presence), indicating BNF occurrence (Uheda and Syono 1982). As they are tolerant to excess Cd, Pb, and Zn, these native bacteria demonstrated competitiveness and efficiency in forming a symbiosis with the two legume species, indicating a potential for future isolation and selection of specific strains to recommend inoculation in contaminated areas.

3.3 Metal Concentrations in Plants

The Cd, Pb, and Zn concentrations in the shoots and roots of both plant species showed a significant increase with the soil contamination levels (Table 3). Notably, Pb was more concentrated in roots than in shoots. Inoculation played a pivotal role in further elevating the concentration of metals in the plants, particularly in the highest level of contamination. The BR 7606 strain demonstrated the most consistent effect for the two legumes at L3, leading to higher Cd, Pb, and Zn concentrations in shoots compared to non-inoculated plants and those inoculated with the other strains.

Inoculation at L1 and L2 only influenced the Zn concentration in the jack bean roots, while plants inoculated with the BR 10,247 strain had lower concentrations than non-inoculated plants. For mucuna at L1 and L2, the inoculation effect on Zn concentrations was only observed in the shoots, with plants inoculated with BR 3051 and BR 7606 strains having lower Zn concentrations (Table 3).

Our results showed for the first time that although the BR3051 strain does not increase the production of jack bean biomass (Table 3), it promotes increased Cd concentration (Table 4), which may occur due to increased solubility. The jack bean generally concentrated more Cd in the shoots than in the roots, while the mucuna preferentially concentrated Cd in the roots. On the other hand, the immobilization of Cd in plant roots can also be associated with protection mechanisms activated by plants, including protection of the photosynthetic apparatus; in legumes, this mechanism can be used to protect the nitrogenase enzyme (Gómez-Sagasti and Marino 2015; Barba-Brioso et al. 2023). Cadmium usually shows high mobility in soil and plants; in the soils from Santo Amaro, Cd was associated with organic matter (labile fraction), providing greater potential for Cd availability (Da Silva et al. 2017), which may also have influenced the bioavailability of Cd in the soil. The highest Cd concentrations were observed in the roots of jack beans, which received inoculation with the BR 7606 strain in soil samples in L3 (Table 4).

Lead was more concentrated in roots when compared to metal extraction in shoot biomass (Table 4). Cadmium and Pb accumulations preferentially occur in the roots (Yang et al. 2014; Guarino and Sciarrillo 2017). The relatively low Pb concentrations in the shoots are due to the low Pb mobility and its high toxicity to plants, which can reduce the transport of Pb to shoots as a tolerance strategy (Aslam et al. 2021; Steliga and Kluk 2020). Lead compounds also have low solubility in the soil of Santo Amaro, which is retained in poorly available soil fractions, mainly associated with iron oxides and residual fractions (Da Silva et al. 2017). Mucuna plants, which received inoculation with the BR 3051 strain, and jack

In lines, for each legur	Jack bean			Mucuna					
	L1	1.2	1.3	L1	L2	1.3			
	Cd in Shoo	$\frac{22}{1000}$ (mg kg ⁻¹)		21					
Non-inoculated	5.43 aB	40.92 aB	244.36 abA	1.63 aB	6.09 aB	90.16 bcA			
BR 2811	8.83 aB	21.68 aB	186.86 bA	2.10 aB	9.86 aB	102.59 bcA			
BR 3051	3.25 aB	13.71 aB	285.52 aA	1.24 aB	5.49 aB	98.44 bcA			
BR 7606	3.96 aB	20.51 a B	189.23 bA	1.49 aB	8.34 aB	183.58 aA			
BR 10,026	3.38 aA	27.75 aA	2.14 cA	1.67 aB	10.82 aB	84.37 cA			
BR 10,247	3.39 aB	23.45 aB	223.98 abA	1.34 aB	4.33 aB	124.97 bA			
	Cd in Root	Cd in Roots (mg kg ⁻¹) 22000 and 100 kg ⁻¹							
Non-inoculated	1.33 aB	11.36 aAB	37.28 bA	2.36 aB	10.42 aB	76.84 bcA			
BR 2811	1.26 aB	3.85 aB	65.43 abA	0.95 aB	4.18 aB	121.17 abA			
BR 3051	0.8 aB	4.45 aB	67.13 abA	1.05 aB	8.41 aB	92.28 abcA			
BR 7606	1.21 aB	7.22 aB	100.11 aA	1.33 aB	13.53 aB	140.85 aA			
BR 10,026	0.55 aB	14.80 aAB	38.89 bA	1.30 aB	13.77 aB	60.00 cA			
BR 10,247	1.34 aB	4.33 aB	34. 93 bA	0.50 aB	6.13 aB	77.84 bcA			
	Pb in Shoots (mg kg^{-1})								
Non-inoculated	2.89 aB	23.72 aB	127.99 bcA	0.33 aB	1.35 aB	37.30 bA			
BR 2811	13.85 aB	11.48 aB	165.10 bcA	0.63 aB	2.15 aB	26.02 dA			
BR 3051	2.29 aB	6.74 aB	202.30 bA	0.25 aB	1.12 aB	26.42 dA			
BR 7606	2.58 aB	11.15 aB	404.57 aA	0.37 aB	1.58 aB	62.80 aA			
BR 10,026	11.35 aB	16.01 aB	107.86 cA	0.35 aB	1.45 aB	28.72 bcA			
BR 10,247	20.27 aB	7.91 aB	161.91 bcA	0.32 aB	0.82 aB	36.37 cdA			
	Pb in roots	$(mg kg^{-1})$							
Non-inoculated	24.21 aB	319.69 aAB	695.14 aA	2.72 aB	17.68 aB	292.45 aA			
BR 2811	101.51 aB	78.9B	1085.98 abA	5.11 aB	9.93 aB	226.40 abA			
BR 3051	27.70 aB	47.27 aB	1062. 41 abA	2.73 aB	3.60 aB	201.32 abA			
BR 7606	14.20 aB	78.61 aB	1430.96 aA	3.68 aB	8.48 aB	139.68 bA			
BR 10,026	9.60 aB	254.24 aAB	448.65 bA	2.90 aB	10.77 aB	202.90 abA			
BR 10,247	17.36 aB	141.3 aB	714.10 bA	0.30 aA	17. 68 aA	4.5 cA			
	Zn in shoo	ts (mg kg ⁻¹)							
Non-inoculated	148.91 aB	138.59 aB	490.11 aA	27.39 bB	99.69 aA	94.18 bA			
BR 2811	143.27 aB	119.96 aB	460.74 aA	142.01 aA	29.21 bB	131.13 abA			
BR 3051	85.83 a B	67.59 aB	584.41 aA	43.53 bB	16.47 bB	131.31 abA			
BR 7606	111.46 aB	105.96 aB	503.99 aA	41.25 bB	31.31 bB	149.74 aA			
BR 10,026	56.46 aB	149.36 aAB	241.63 bA	19.09 bB	99.81 aA	154.94 aA			
BR 10,247	79.30 aB	83.48 aB	550.41 aA	20.55 bB	38.89 bB	179.80 aA			
	Zn in roots	$(mg kg^{-1})$							
Non-inoculated	27. 39 bB	99.70 aA	92.18 bA	71.21 aB	160.99 aB	440.97 abA			
BR 2811	142.01 aA	29.21bB	131.13 abA	152.92 aB	130.91 aB	507.28 abA			
BR 3051	43.53 bB	16.47 bB	131.31 abA	48.07 aB	35.54 aB	532.30 abA			
BR 7606	41.25 aB	31.31 bB	149.74 aA	82.02 aB	161.39 aB	374.01 bA			
BR 10,026	19.09 bC	99.69 aB	154.94 aA	133.11 aB	138.16 aB	571.40 aA			
BR 10,247	20.55 bB	38.89 bB	179.80 aA	20.78 aB	201.41 aA	76.95 cAB			

 Table 4
 Concentrations of cd, pb and zn in shoots and roots of jack bean and mucuna, inoculated with diazotrophic bacteria or non-inoculated, cultivated in soil samples with different contamination levels collected in Santo Amaro, Bahia, Brazil (means followed by the same lowercase letter in columns and uppercase letter in lines, for each legume species, are not significantly different at 5% probability according to Tukey's test)

bean plants that received the BR 7606 strain, immobilized the greatest amounts of Pb, suggesting these strains can solubilize Pb from the soil.

The average Zn levels were higher in the shoots than in the roots (Table 4). As Zn is an essential element, its translocation to shoots is facilitated compared to Pb and Cd. We observed that inoculated jack bean plants were more efficient in accumulating Zn in the root system than plants that did not receive inoculation. The presence of bacteria in the soil has likely altered the rhizosphere of plants, modifying the solubility of heavy metals (Boechat et al. 2017; Zheng et al. 2023); however, more targeted studies of the rhizosphere of plants are needed to elucidate the immobilization of metals in roots. Plant-associated bacteria may also have influenced the Zn translocation in the plant, as they can aid phytoextraction by increasing the transport of heavy metals in plants (Boechat et al. 2017).

3.4 Phytoextraction Efficiency

The phytoextraction efficiency was estimated based on the net removal of metals from the soil (Table 4). The phytoextraction efficiency followed the metal concentration order in shoot biomass (Pb>Zn>Cd), a finding with direct implications for our understanding of phytoextraction processes. Cadmium removal by jack beans and mucuna was not influenced by inoculation when cultivated in soil with lower contamination levels, suggesting the need for targeted inoculation strategies. However, inoculation influenced metal phytoextraction in L3, which increased or decreased efficiency according to the inoculated strain, highlighting the potential for strain-specific interventions. Inoculation in jack beans with BR 3051 and BR 7606 strains did not affect Cd removal; interestingly, the other strains caused a lower Cd accumulation in the jack beans than the non-inoculated plants, indicating the need for further strain-specific research. All inoculants in the mucuna promoted greater net removal of Cd than control, and plants inoculated with the BR 3051 and BR 2811 strains showed significantly greater removals, suggesting the potential for enhanced Cd removal with specific inoculants.

The treatment without inoculation showed higher net removal of Pb by the jack bean plants in L2 (Table 4). Yet, the net removal of Pb in L3 was greater in plants with the BR 3051 inoculant. The BR 10,026 strain inoculation in the mucuna promoted the most significant net removal of Pb in L2. At the same time, inoculated plants differed significantly from non-inoculated plants in the L3 with higher net metal removals. Mucuna inoculations with the tested strains generally caused a Pb removal of up to 46% compared to non-inoculated plants.

Zinc removal increased as the soil contamination level increased (L3>L2>L1) (Table 4). Mucuna bean inoculation with the BR 10,026 strain significantly increased the potential of the legume-rhizobia system to remove Zn from L2, while the BR 2811 and BR 3051 strains were more efficient in L3. The Cd, Pb and Zn removal was proportional to increased soil contamination levels in the jack bean and mucuna (Table 5). However, it is essential to note that even with the greatest net removal, soils with very high metal contents can make phytoextraction unfeasible due to the time required to bring the metals to regulatory concentrations (Da Silva et al. 2017). Therefore, to optimize the process, we must consider the best relationship between net removal, metal concentration in the soil, the most suitable rhizobia strain and plant species. There were no significant differences in L1 soils about inoculations,

Table 5 Net removal (mg pot⁻¹) of of cd, pb and zn on shoots and roots of jack bean and mucuna plants, inoculated with selected rhizobia strains or non-inoculated, cultivated in soil samples with different levels of contamination collected in Santo Amaro, Bahia, Brazil (means followed by the same lower case letter, in the columns, and uppercase letters, in the lines, are not significantly different at 5% probability according to Tukey's test. L1- lowest contamination level; L2=intermediate contamination level; L3=highest contamination level)

Inoculant	Jack bean			Mucuna	Mucuna		
	L1	L2	L3	L1	L2	L3	
Non-inoculated	0.01 aB	0.04 aB	0.25 abA	0.01 aB	0.01 aB	0.09 abA	
BR 2811	0.01 aB	0.03 aB	0.18 bA	0.01 aB	0.01 aB	0.10 abA	
BR 3051	0.01 aB	0.01 aB	0.28 aA	0.01 aB	0.01 aB	0.10 abA	
BR 7606	0.01 aB	0.02 aB	0.19 bA	0.01 aB	0.01 aB	0.18 aA	
BR 10,026	0.01 aA	0.02 aA	0.01 cA	0.01 aB	0.01 aB	0.08 bA	
BR 10,247	0.01 aB	0.02 aB	0.22 abA	0.01 aB	0.01 aB	0.12 bA	
Non-inoculated	0.01 aB	0.02 aB	0.13 bcA	0.01 aB	0.02 bB	0.43 aA	
BR 2811	0.01 aB	0.01 aB	0.17 bcA	0.01 aB	0.01 bB	0.35 aA	
BR 3051	0.01 aB	0.01 aB	0.20 bA	0.01 aB	0.03 abB	0.41 aA	
BR 7606	0.01 aB	0.01 aB	0.40 aA	0.01 aB	0.01 abB	0.39 aA	
BR 10,026	0.01 aB	0.02 aB	0.10 cA	0.01 aB	0.03 aB	0.44 aA	
BR 10,247	0.02 aB	0.01 aB	0.16 bcA	0.01 aB	0.01 abB	0.32 aA	
Non-inoculated	0.15 aB	0.14 aB	0.49 Aa	0.29 aB	0.30 bB	0.48 bcA	
BR 2811	0.14 aB	0.12 aB	0.46 aA	0.25 aB	0.28 bB	0.72 aA	
BR 3051	00.8 aB	0.08 aB	0.58 aA	0.24 aB	0.29 bB	0.64 abA	
BR 7606	0.14 aB	0.11 aB	0.51 aA	0.26 aA	0.34 bA	0.37 cA	
BR 10,026	0.06 aB	0.15 aAB	0.24 bA	0.26 aB	0.46 aA	0.51 bcA	
BR 10,247	0.07 aB	0.08 aB	0.55 aA	0.24 aB	0.30 bB	0.75 aA	

which indicates that the bacteria studied can help phytoextraction in soils with higher levels of heavy metals.

4 Conclusions

The metal-removing capabilities and the ability to grow over high soil metal concentrations of the strains tested in our study offer an opportunity to use these organisms together with legume plants for metal remediation. Both legumes and inoculated strains were tolerant to soil contamination, but a specific legume-bacteria combination is recommended for increasing biomass and metal accumulation. For example, the BR 2811 strain increased the biomass of the jack bean plants while mucuna biomass was diminished regardless of strains or soil contamination level. Such an opposite effect was related to higher nodulation in jack beans plants. Significant interactions were found between the strains and the soil contamination levels for phytoextraction efficiency, which directly impacts our understanding of the phytoextraction processes enhanced by microorganisms. The Cd, Pb and Zn net removal was proportional to increased soil contamination levels. Legumes did not show potential for Pb phytoextraction, but they have potential for Zn phytoextraction and can be used for phytostabilization, especially of Cd, in the studied areas. The BR 2811 (*Bradyrhizobium elkanii*), BR 3501 (Ensifer sp.) and BR 7606 (*Rhizobium leguminosarum* bv. Trifolii) strains were the most promising to increase the phytoremediation potential of jack and mucuna beans.

Author Contributions All authors contributed to the study conception, writing of the first draft and design. Material preparation, data collection and analysis were performed by Jessyca Adriana Gomes Florêncio da Silva, Ana Dolores Santiago de Freitas Vinicius Santos Gomes da Silva, Pablo Acácio Santos Souza, Clistenes Williams Araujo do Nascimento, Nielson Machado Santos. The first draft of the manuscript was written by Jessyca Adriana Gomes Florêncio Silva and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability No datasets were generated or analysed during the current study.

Declarations

Competing Interests The authors declare no competing interests.

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