



Neighbor Presence of Plant Growth-promoting Rhizobacteria (PGPR) and Arbuscular Mycorrhizal Fungi (AMF) Can Increase Sorghum Phytoremediation Efficiency in a Soil Treated with Pb Polluted Cow Manure

Amir Hossein Baghaie^{a*} | Aminollah Aghilizefreei^b

a. Department of Soil Science, Arak Branch, Islamic Azad University, Arak, Iran.

b. Department of Chemical Engineering, Isfahan University of Technology, Isfahan, Iran.

*Corresponding author at: Department of Soil Science, Arak Branch, Islamic Azad University, Arak, Iran.

Postal code: 38361-1-9131.

E-mail address: a-baghaie@iau-arak.ac.ir

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ABSTRACT

Background: The present study aimed to evaluate the effects of PGPR and AMF on the changes in sorghum phytoremediation efficiency in the soil amended with lead (Pb)-polluted cow manure.

Methods: Treatments consisted of applying two rates of Pb [0 (Pb₀) and 800 (Pb₈₀₀) mg/kg] polluted cow manure [0 (C₀) and 30 (C₃₀) t/ha], two levels of AMF [without (AMF-) and with (AMF+)] and PGPR (without (PGPR-) and with (PGPR+) inoculation. *Pseudomonas* sp. R9 was considered as a PGPR bacteria. The plant used in this experiment was sorghum. Plant Pb concentration was measured using atomic absorption spectroscopy.

Results: The highest plant Pb concentration belonged to the cultivated plant in the soil treated with 30 t/ha Pb-polluted cow manure, while the lowest that was observed in the soil without amending cow manure in the absence of PGPR or AMF. The presence of AMF significantly increased the Pb translocation value and sorghum phytoremediation efficiency by 8% and 13.4%, respectively.

Conclusion: According to the results, the inoculated plant with PGPR and AMF had positive effects on increasing Pb phytoremediation efficiency that, which is notable in environmental studies. However, the effects of soil chemical properties on Pb phytoremediation efficiency cannot be overlooked.

1. Introduction

The appearance of various modern industries, including chemical fertilizer and pesticide industries, and the uncontrolled use of these agrochemicals have largely contributed to extensive heavy metal contamination in agricultural soils, thereby causing severe environmental pollution and threatening human health. Heavy metals such as lead (Pb) and cadmium (Cd) are considered to be particularly problematic in this regard since they are persistent in nature, non-biodegradable, and could bio-

accumulate in living organisms and plants [1]. These elements enter the environment mainly through anthropogenic sources, such as smelting processes, mining, fuel production, and industrial effluents [2]. Therefore, use of proper approaches to the reduction of heavy metal availability is essential. Conventional remediation approaches for contaminated soils, sediments, and groundwater are based on the technologies that have been developed over the past two decades [3], including a wide variety of physical, chemical, and thermal treatment alternatives and their combinations, as well as engineering



strategies to accelerate or reduce mass transport in the contaminated matrix. The success of these highly engineered technologies is attributable to their relative insensitivity to heterogeneity in the contaminant matrix, their effectiveness over a wide range of oxygen concentrations, pH, pressure, temperature, and osmotic potentials, and their ability to produce relatively rapid contaminant mitigation rates [4]. Several techniques are currently available for the removal of heavy metals, such as ion exchange, solvent extraction, oxidation reduction, reverse osmosis, phytoremediation, membrane separation, and precipitation. Among these methods, phytoremediation is the most cost-efficient and involves the 'green technology' that only exploits living plants for the removal of heavy metals from soil and water [5]. In this method, metals are taken up into shoots and harvested, so that they could be removed from the polluted area [6].

The applications of phytoremediation could be classified based on the processes impacting the mitigation of contaminants in the plant-soil system; these classifications include degradation, extraction, immobilization, containment or a combination of these processes. Phytoremediation is defined as the use of plants to destroy, sequester, and remove toxicants from the environment. It is notable that phytoremediation is associated with some limitations, such as the emergence of rhizospheric microorganisms as an acceptable agronomic remediation technology [7].

The interaction between plants and microbes in the rhizosphere plays a key role in enhancing the efficacy of phytoremediation through a process known as bio-assisted phytoremediation. The microorganisms that are present in soil and around plant roots are referred to as plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF), which use various mechanisms to promote plant growth and minimize stress [8]. Furthermore, PGPR and AMF contribute to plant growth enhancement and the bioremediation of contaminated soil through the sequestering or degradation of heavy metals and other toxicants. Therefore, bioremediation is a viable option to destroy or render various contaminants harmless based on natural biological activity [9].

PGPR and AMF assist phytoremediation directly or indirectly through several mechanisms, such as increased nutrient uptake, suppression of pathogens by producing antibiotics, siderophores or bacterial/fungal antagonistic substances (e.g., hydrogen cyanide), phytohormone production (e.g., indoleacetic acid), and nitrogen fixation [10]. Cow manure is also used as an organic amendment to affect plant growth and increase phytoremediation efficiency [11].

The present study aimed to evaluate the effects of PGPR and AMF on increasing sorghum phytoremediation efficiency in the soil treated with Pb polluted cow manure.

2. Materials and Methods

To investigate the effect of PGPR and AMF on sorghum phytoremediation efficiency in the soil treated with Pb-polluted cow manure, non-Pb-polluted soil with low organ carbon was collected from the surface layer of soil (0-15 cm) in Shahr-e Kord, Iran. Table 1 shows the selected physico-chemical properties of the soil sample.

The cow manure used in this experiment was eight-month decomposed and contained high concentrations of plant nutrients and organic matters, which could be used on farmlands as a fertilizer. Table 1 shows the selected chemical properties of the organic amendments.

This research was conducted as a factorial experiment in the layout of randomized complete block design in the three replicates. Treatments consisted of applying two levels of Pb (0 (Pb₀) and 800 (Pb₈₀₀) mg/kg) polluted cow manure [0 (C₀) and 30 (C₃₀) t/ha], two levels of AMF [without (AMF-) and with (AMF+)] and PGPR [without (PGPR-) and with (PGPR+)] inoculation. It is notable that *Pseudomonas* sp R9 was considered as a PGPR bacteria.

The cow manure used in this experiment was spiked with Pb(NO₃)₂ as a Pb salt at the rates of 0 and 800 mg Pb/kg and incubated for two weeks. Following that, the soil sample was amended with Pb-polluted cow manure and incubated for two weeks, and placed in five-kilogram pots.

To obtain the AMF inoculum, soil samples were collected from around the roots of sorghum plants grown in the studied soil before starting of the experiment. To this aim after growth duration the roots of plants together with the soils around it has taken and crashed completely to use as AMF inoculum. We used this method to have indigenous soil AMF as it has been reported that indigenous AMF species can improve better plant growth and nutrient uptake than non-indigenous species due to their better adaption to the soil condition [12]. Indigenous AMF ecotypes result from long period adaptation to soils with extreme characteristics [13,14]. Following that the experimental pots filled with 5 Kg of the treated soil and then half of that inoculated with AMF. For AM fungus inoculations 20 g of inoculum was placed in a layer at a depth of 3 cm from the soil surface.

The bacteria used in this study were previously isolated from sorghum rhizosphere, identified, and tested. Thereafter, the seeds of sorghum (Pegah cultivar) were surface-sterilized in 70% ethanol for one minute followed by dipping seeds in 5% sodium hypochlorite solution for 10 min and rinsed several times with sterilized distilled water, inoculated by immersion in the appropriate PGPR suspension (at 10⁹ CFU mL⁻¹) for 2 h on a rotary shaker at 80 rpm, air dried [15], and immediately were transferred to the treated soil. Ten seeds were sown in each pot and thinned to five plants per pot after the germination of the first leaf. After 60 days, plants were harvested and the plants Pb concentration was measured using atomic absorption spectroscopy (AAS). DTPA-extractable Pb in soil (soil Pb availability) was measured according to method described by the Lindsay and Norvell (1978) [16]. Dry ash extraction method was used to determine plant heavy metal concentration. Accordingly, two g of each plant sample was put in a porcelain crucible.

The samples were placed in an oven for 2 hours at 550°C. After that, 5 ml of HCl 2N was added to the samples. Then, the samples were filtered and the plant heavy metals concentration was determined using the atomic absorption spectrophotometer (AAS) [17].

The soil pH in various treatments was measured in the suspension of 1:5 (w/v), and soil phosphorous concentration was determined using the method proposed by Mkhabela et al. (2005)[18].

Table 1: Selected properties of soil and cow manure used in this study

Characteristic	Unit	Amount	
		Soil	Cow Manure
Soil texture	-----	loamy	----
pH	-----	7.1	7.2
EC	dS/m	0.7	6.8
Soil Pb availability	mg kg ⁻¹	1.2	0.3
Soil Cd availability	mg kg ⁻¹	0.1	0.2
Soil As availability	mg kg ⁻¹	0.2	0.1
Organic carbon	%	0.3	30.8
CaCO ₃	%	8%	---

Pb translocation factor (TF) was calculated using the following formula [19]:

$$TF = H_{shoot}/H_{root}$$

Where H_{shoot} and H_{root} are heavy metal concentrations in plant shoot and root.

The soil microbial respiration was measured as evolved CO₂. For this purpose, samples of each treatment were incubated for three days at 26 °C in 250-ml glass containers closed with rubber stoppers. The evolving CO₂ was trapped in NaOH solution and the excess in alkali was then titrated with HCl [20].

2.1. Statistical Analysis

Statistical analyses were calculated according to the ANOVA procedure. The differences between means were evaluated using the least significant difference (LSD) test. The P = 0.05 value was considered to determine the significant difference.

3. Result and Discussion

According to the obtained results, PGPR could increase soil nutrient availability by lowering the soil pH (Figure 1-a), and the soil phosphorous availability increased 1.8 times (Figure 1-b) in the presences of PGPR. However, the presence of AMF had no significant effect on the soil pH (Figure 1-b). On the other hand, the interactive effects of PGPR and AMF could significantly increase the soil nutrient availability (e.g., phosphorous) (Figure 1-b), thereby improving the plant biomass. According to the findings of the current research, the highest plant phosphorous concentration belonged to the plant cultivated in the soil containing both AMF and PGPR (Table 2).

According to the results of the current research, the presence of PGPR had more significant effects compared to AMF (Figure 1-b) as PGPR more significantly increased the soil phosphorous (38%) compared to AMF. In a study in this regard, Lin et al. assessed the effects of PGPR on corn growth with fertility sources, concluding that PGPR had significant effects on corn plant growth; this is consistent with our findings. However, the role of soil properties and vegetative growth stages in PGPR efficiency cannot be overlooked [21].

In another research, Namli et al. (2017) investigated the effects of phosphorus solubilizing bacteria on some soil properties, wheat yield, and nutrient contents, concluding that the application of PGPR with lower amounts of chemical fertilizers could reduce the use of chemical fertilizers, while it also has the potential to enhance soil health in the long run [22].

Table 2: Effects of PGPR, AMF, and applying Pb-polluted cow manure on plant phosphorous concentration (%)

Pb Concentration	Cow Manure (t/ha)							
	PGPR ⁺				PGPR ⁻			
	AMF ⁺		AMF ⁻		AMF ⁺		AMF ⁻	
	0	30	0	30	0	30	0	30
0	0.43 ^g	0.88 ^a	0.35 ^h	0.72 ^b	0.30 ⁱ	0.65 ^d	0.27 ^j	0.61 ^{e*}
800	0.35 ^h	0.72 ^b	0.26 ^j	0.68 ^c	0.23 ^k	0.60 ^e	0.18 ^k	0.51 ^f

*Data with the similar letters are not significant (P= 0.05).

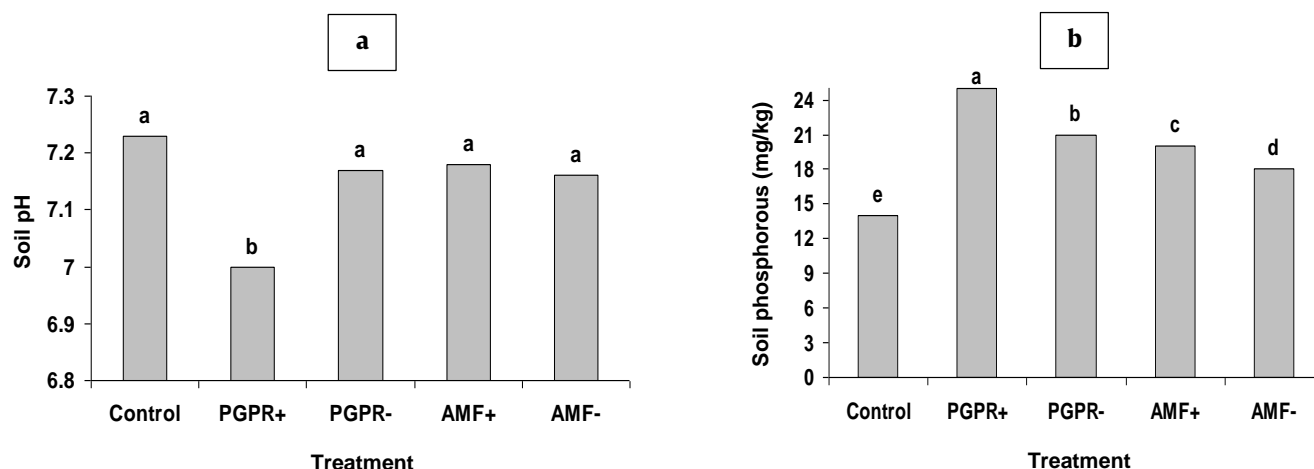


Figure 1: Effect of AMF and PGPR on soil pH (a) and soil phosphorous (b), columns with the similar letters are not significant (P= 0.05)

Table 3: Effect of PGPR, AMF and applying Pb-polluted cow manure on plant Pb concentration and Pb TF value

Pb Concentration	Cow Manure (t/ha)							
	PGPR ⁺				PGPR ⁻			
	AMF ⁺		AMF ⁻		AMF ⁺		AMF ⁻	
	0	30	0	30	0	30	0	30
Soil Pb concentration								
0	ND*	ND	ND	ND	ND	ND	ND	ND
800	35.9 ^b	37.3 ^a	30.1 ^d	32.4 ^c	28.5 ^e	30.6 ^d	20.3 ^g	24.2 ^{f**}
Root Pb concentration								
0	ND*	ND	ND	ND	ND	ND	ND	ND
800	66.1 ^b	69.1 ^a	59.3 ^d	62.1 ^c	52.6 ^f	55.3 ^e	46.8 ^h	50.5 ^{g**}
Shoot Pb concentration								
0	ND*	ND	ND	ND	ND	ND	ND	ND
800	46.9 ^b	55.2 ^a	39.7 ^d	45.9 ^c	28.9 ^f	35.9 ^e	23.4 ^h	27.7 ^g
Pb TF value								
0	NC*	NC	NC	NC	NC	NC	NC	NC
800	0.716 ^b	0.803 ^a	0.672 ^d	0.749 ^c	0.551 ^f	0.658 ^e	0.502 ^g	0.554 ^f

*ND: not detectable by atomic absorption spectroscopy (limit of detection is equal to 0.1 ppm), *NC: not calculated, ** data with the similar letters in each parameter are not significant ($P=0.05$).

In the present study, the highest soil Pb availability was observed in the soil amended with 30 t/ha Pb enriched cow manure in the presence of PGPR and AMF, while the lowest that has belonged to the soil without amended without receiving cow manure in the absence of PGPR and AMF.

In addition, soil Pb availability significantly increased in the soil amended with 30 t/ha of the cow manure in the presences of PGPR (23.1%) (Table 3); this could be attributed to the effects of PGPR on the soil pH. Therefore, it could be concluded that the presence of PGPR significantly lowered the soil pH (0.2 unit) as opposed to the control soil. Previous studies have also denoted increased soil Pb availability due to the reduction of soil pH [16, 23]. It is also notable that the primary concentrations of the heavy metals in the cow manure were lower than the standard values suggested by the US Environmental Protection Agency [24].

In a study in this regard, Abdelkrim et al. (2018) evaluated the effects of Pb-resistant PGPR inoculation on plant growth and Pb uptake by *Lathyrus sativus*, concluding that PGPR inoculation had significant potential to improve the phytoremediation of Pb-polluted soils; this finding is in congruence with the results of the present study. Furthermore, the mentioned study indicated the Pb uptake was affected by the incubation time, demonstrating that Pb accumulation was associated with cell growth and enhancement of the cell biomass [25]. According to these results, the potential to enhance Pb²⁺ retention in the selected PGPR was not only due to cell-surface binding, but it also was due to intracellular accumulation [25]. Similar results have also been reported by Jebara et al. (2015) [26], denoting that PGPR microorganisms may reduce soil pH and increase soil Pb availability through releasing organic acids (e.g., carboxylic acid) [27].

According to the current research, the simple effects of the presence of AMF and PGPR on the root Pb concentration was significant. Moreover, the root Pb concentration was significantly higher in the presence of PGPR compared to AMF (Figure 2-a). However, the highest root Pb concentration belonged to the soil amended with Pb-enriched cow manure in the presence of PGPR and AMF, while the lowest concentration was observed in the soil without Pb-enriched cow manure in the absence of PGPR and AMF (Table 3).

According to the results of the present study, applying 30t/ha Pb-enriched cow manure significantly increased the root Pb concentration by 12.4 % in the presence of AMF and

PGPR. However, our findings indicated that plant Pb concentration was affected by the symbiosis type, as the root Pb concentration was higher in the presence of PGPR compared to the AMF inoculation. The Pb resistance of the plants was also higher in the presence of PGPR compared to the AMF-inoculated plants as the highest plant biomass (data was not shown) was observed in the plant inoculated with PGPR as opposed to AMF. Their synergistic effects positively influenced the plant biomass, thereby increasing the plant Pb uptake.

In a similar research, Sadaghiani et al. (2016) assessed the effects of PGPR and AMF on the growth and some physiological parameters of *Onopordum acanthium* in a Cd-contaminated soil, concluding that PGPR and AMF inoculation could be sustained and promoted plant growth in phytoremediation processes [28]; this is in line with the results of the present study. In general, PGPR could increase plant growth in contaminated soils through improving nutrient availability (especially soil phosphorus) and producing growth hormones and root exudate [29]. A similar trend was also observed for the shoot concentration of Pb in the current research (Table 3).

In the present study, the highest Pb TF has belonged to the soil amended with 30 t/ha Pb-enriched cow manure in the presence of AMF and PGPR, while the lowest value was observed in the soil without the cow manure in the absence of PGPR or AMF inoculation (Table 3). On the other hand, the Pb TF value significantly increased in the presence of PGPR as opposed to AMF inoculation, which could be attributed to the greater role of PGPR inoculation in plant resistance to Pb stress. In this regard, Zaefarian et al. (2012) investigated the effectiveness of PGPR in the facilitation of Pb and nutrient uptake by *littleseed canarygrass*, reporting that the Pb TF value was >1 in the inoculated treatments with in the in the soil Pb concentration range of 200-400 mg/kg. However, the findings of the mentioned research indicated that in the Pb-polluted soil (> 800 mg/kg), the TF value was less than one even in the inoculated treatments [30]. Despite the contradictory reports regarding the effects of Pb toxicity on the plants Pb TF value [31,32], most of the findings have indicated that PGPR and AMF inoculation could reduce abiotic stress, such as heavy metal toxicity [33].

In the current research, applying 30 t/ha cow manure increased the Pb TF value (0.1 unit) in the presence of AMF

and PGPR. Therefore, it could be concluded that the cow manure could increase the plant resistance to Pb-induced stress through increasing the plant nutrient uptake, which in turn increased the Pb TF value. On the same note, the presence of AMF or PGPR could also increase the soil nutrient availability, thereby enhancing the plant biomass (data was not shown). Some studies have demonstrated that soil contamination with heavy metals prevents plant growth by decreasing nutrient uptake (e.g., nitrogen and iron).

According to the study by Zaefarian et al. (2012) high concentrations of Pb in soil could significantly reduce the nitrogen content of plant roots [30]. In addition, rhizobacteria were reported to produce various growth-promoting hormones (e.g., auxins, gibberellins, and B vitamins), which stimulated root exudate production [30]. Similarly, Abou-Shanab et al. (2006) reported that bacterial inoculants could affect the nickel uptake by *Alyssum murale* from the soils containing low, moderate, and high levels of nickel [34].

In the present study, the highest plant phosphorous concentration belonged to the plants cultivated in the soil treated with 30 t/ha of the non-polluted cow manure in the presence of AMF and PGPR (Table 2), while the lowest concentration was observed in the soil without the cow manure in the presence of PGPR or AMF. On the other hand, applying 30 t/ha of the non-polluted cow manure significantly increased the plant phosphorous concentration in the soil non-inoculated and inoculated with PGPR and AMF (29.3% and 24.1%, respectively). Soil amending with organic residues (e.g., cow manure) could influence phosphorus dynamics through competition between low-molecular-weight organic acids and phosphates for sorption sites, which often favors the adsorption of organic acids and delays phosphorus adsorption [35]. However, Pb contaminated cow manure could increase plant nutrient availability.

According to the current research, increasing the Pb concentration in the cow manure from zero to 800 mg/kg caused the plant phosphorous concentration to decrease by 18.4% in the absence of PGPR and AMF. The previously reported interactive effects of Pb and soil phosphorus are consistent with our findings [36].

In the present study, the highest soil microbial respiration was observed in the soil amended with 30 t/ha of the non-polluted cow manure in the presence of PGPR and AMF,

while the lowest rate belonged to the soil without the cow manure in the absence of PGPR or AMF (Table 4).

In another research, Yolcu et al. (2011) investigated the effects of PGPR and organic amendments on the yield and quality propititates of Italian ryegrass in semi-arid conditions, concluding that the presence of PGPR and organic amendments could affect the plant biomass owing to their interactive effects [37]. However, Pb pollution may decrease the soil microbial respiration as applying 30 t/ha of the Pb-polluted cow manure reduced the soil microbial respiration by 9.1% in the present study.

In a similar study, Shi et al. (2017) assessed the effects of Cd pollution on soil microbial activities, reporting that heavy metals adversely affected soil microbial respiration. Although the mentioned study indicated that soil heavy metals variably affected soil microbial activity, the obtained results indicated that at lower Cd concentrations, soil microbes became resistant to heavy metals [38]; this is in line with our findings. According to the results of the present study, although increased soil contamination with Pb reduced microbial population, soil microbial respiration remained higher compared to the control soil samples.

According to the current research, applying the organic amendment significantly increased the soil microbial respiration, as well as the plant Pb TF value. Therefore, it could be concluded that the applied cow manure significantly improved the soil and plant nutrient availability, thereby increasing the plant biomass (data was not shown). On the other hand, plant root exudates (e.g., amino acids) may enhance the soil microbial activity, thereby increasing plant resistance to the stress induced by heavy metals. However, the synergetic effects of PGPR and AMF on the improvement of such resistance cannot be overlooked. Therefore, it could be inferred that increased plant resistance to abiotic stress due to higher soil microbial activity could also enhance phytoremediation efficiency. It is notable that heavy metal concentrations could determine the soil microbial activity as Osborne et al. (2010) reported that PGPR strains were able to survive up to 300 mg/l of Cd [39].

In the current research, the simple effects of the presence of PGPR and AMF were significant, as the plant Fe concentration significantly increased in the soil containing PGPR and AMF (15% and 11.2%, respectively) (Figure 2-b).

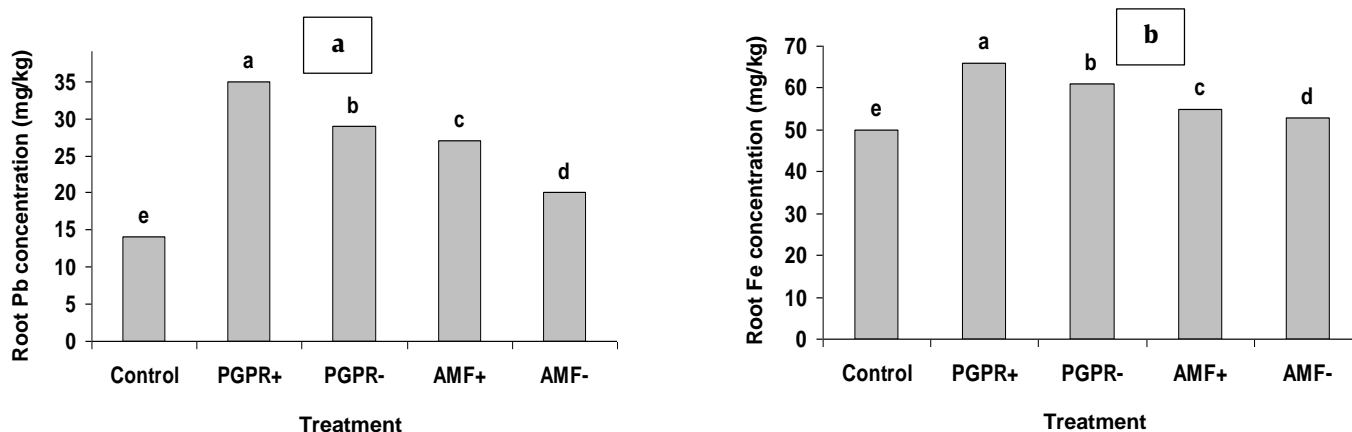


Figure 2: The simple effects of AMF and PGPR on root Pb (a) and root Fe concentration (b)

Table 4: Effect of PGPR, AMF and applying Pb-polluted cow manure on soil microbial respiration (mg C- CO₂/kg soil)

Pb concentration	Cow Manure (t/ha)							
	PGPR ⁺				PGPR ⁻			
	AMF ⁺		AMF ⁻		AMF ⁺		AMF ⁻	
	0	30	0	30	0	30	0	30
0	3.5 ^h	8.4 ^a	3.3 ⁱ	7.9 ^c	3.2 ^j	7.5 ^e	3.0 ^l	7.0 ^f
800	3.3 ⁱ	8.1 ^b	3.1 ^k	7.5 ^e	2.9 ^m	7.7 ^d	2.8 ⁿ	6.4 ^g

*Data with the similar letters are not significant ($P = 0.05$)

Table 5: Effect of PGPR, AMF and applying Pb-polluted cow manure on plant Fe concentration (mg/kg)

Pb concentration	Cow Manure (t/ha)							
	PGPR ⁺				PGPR ⁻			
	AMF ⁺		AMF ⁻		AMF ⁺		AMF ⁻	
	0	30	0	30	0	30	0	30
0	12.4 ^h	40.1 ^a	11.3 ^j	35.7 ^c	10.5 ^l	33.1 ^d	9.8 ^m	32.1 ^e
800	11.9 ⁱ	37.2 ^b	10.8 ^k	33.2 ^d	9.7 ^m	30.1 ^f	9.1 ⁿ	25.4 ^g

*Data with the similar letters are not significant ($P = 0.05$)

The highest Fe concentration belonged to the plants cultivated in the soil amended with 30 t/ha of the non-polluted cow manure containing AMF and PGPR, while the lowest concentration belonged to the soil without the cow manure in the absence of AMF or PGPR (Table 5).

According to the findings of the current research, soil pollution with Pb had significant effects on the plant Fe concentration as the increasing of the soil Pb concentration decreased the plant Fe concentration. In another study, Tabarteh et al. (2017) investigated the effects of enriched cow manure with converter sludge on the bio-availability of Fe in a Pb-polluted soil, concluding that increased soil Pb pollution could significantly decrease soil and plant Fe availability [40].

Previous studies have denoted the interactive effects of heavy metals on nutrients such as Fe or Zn [23], which is consistent with our findings. It is notable that although PGPR and AMF reduced the plant Pb concentration by increasing its Fe availability (interactive effect), the role of soil microorganism activities in increasing plant Pb resistance and phytoremediation efficiency cannot be overlooked. The significant improvement in the plant phytoremediation efficiency in the inoculated confirmed to the non-inoculated samples confirmed this finding.

4. Conclusion

According to the results, the presence of PGPR and AMF significantly increased the plant Pb efficiency. The highest plant Pb TF value was observed in the soil treated with 30 t/ha of the Pb-polluted cow manure containing AMF and PGPR. Similar results were also observed in terms of soil microbial respiration, indicating that the chemical properties of soil played a pivotal role in the changes in the plant resistance to abiotic stress (e.g., heavy metals). On the other hand, the presence of PGPR and AMF significantly increased the plant nutrient availability, thereby affecting the plant growth. Therefore, it could be concluded that increased plant growth could also enhance plant Pb phytoremediation, which is a positive impact on environmental pollution. However, the role of other physicochemical properties of soil and plant physiology in phytoremediation efficiency cannot be overlooked. In conclusion, it is recommended that the specific role of other microorganism activities in plant heavy metal phytoremediation efficiency be assessed in further

investigations, in which the findings of the current research could also be beneficial.

Authors' Contributions

This article was carried out by all the authors. A.H.B., and A.A., designed the manuscript and contributed to carry out data collection and data analysis and A.H.B., and A.A., wrote the manuscript.

Conflict of Interest

The Authors declare that there is no conflict of interest.

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References

- Hou S, Zheng N, Tang L, Ji X, Li Y, Hua X. Pollution Characteristics, Sources, and Health Risk Assessment of Human Exposure to Cu, Zn, Cd and Pb Pollution in Urban Street Dust Across China between 2009 and 2018. *Environ Int.* 2019; 128: 430-7.
- Kasemodel MC, Sakamoto IK, Varesche MB, Rodrigues VG. Potentially Toxic Metal Contamination and Microbial Community Analysis in an Abandoned Pb and Zn Mining Waste Deposit. *Sci Total Environ.* 2019; 675: 367-79.
- Odoh CK, Zabbey N, Sam K, Eze CN. Status, Progress and Challenges of Phytoremediation - An African Scenario. *J Environ Manage.* 2019; 237: 365-78.
- Cameselle C, Gouveia S. Phytoremediation of Mixed Contaminated Soil Enhanced with Electric Current. *J Hazard Mater.* 2019; 361: 95-102.
- Dong Q, Fei L, Wang C, Hu S, Wang Z. Cadmium Excretion Via Leaf Hydathodes in Tall Fescue and Its Phytoremediation Potential. *Environ Pollut.* 2019; 252: 1406-11.
- Xiao R, Ali A, Wang P, Li R, Tian X, Zhang Z. Comparison of the Feasibility of Different Washing Solutions for Combined Soil Washing and Phytoremediation for the Detoxification of Cadmium (Cd) and Zinc (Zn) in Contaminated Soil. *Chemosphere.* 2019; 230: 510-8.
- Wang B, Wang Q, Liu W, Liu X, Hou J, Teng Y, et al. Biosurfactant-Producing Microorganism *Pseudomonas* sp. SB Assists the Phytoremediation of DDT-Contaminated Soil by Two Grass Species. *Chemosphere.* 2017; 182: 137-42.
- Hou J, Liu W, Wang B, Wang Q, Luo Y, Franks AE. PGPR Enhanced Phytoremediation of Petroleum Contaminated Soil and Rhizosphere Microbial Community Response. *Chemosphere.* 2015; 138: 592-8.
- Quintella CM, Mata AMT, Lima LCP. Overview of Bioremediation with Technology Assessment and Emphasis on Fungal Bioremediation of Oil Contaminated Soils. *J Environ Manage.* 2019; 241:156-66.

10. Gupta P, Rani R, Chandra A, Varjani SJ, Kumar V. Effectiveness of Plant Growth-Promoting Rhizobacteria in Phytoremediation of Chromium Stressed Soils. *InWaste Bioremediation*. 2018; 301-12.
11. Nguyen BT, Trinh NN, Le CMT, Nguyen TT, Tran TV, Thai BV, et al. The Interactive Effects of Biochar and Cow Manure on Rice Growth and Selected Properties of Salt-Affected Soil. *Archive Agr Soil Sci*. 2018; 64(12): 1744-58.
12. Aghili F, Gamper HA, Eikenberg J, Khoshgoftarmanesh AH, Afyuni M, Schulin R, et al. Green Manure Addition to Soil Increases Grain Zinc Concentration in Bread Wheat. *PLoS One*. 2014; 9(7): e101487.
13. Bae Y, Fukushima S, Harada A, Kataoka K. Design of Environment-Sensitive Supramolecular Assemblies for Intracellular Drug Delivery: Polymeric Micelles that are Responsive to Intracellular pH Change. *Angew Chem Int Ed*. 2003; 42(38): 4640-3.
14. Sylvia DM, Williams SE. Vesicular-Arbuscular Mycorrhizae and Environmental Stress. *Mycorrhizae Sustain Agr*. 1992: 101-24.
15. Baris O, Sahin F, Turan M, Orhan F, Gulluce M. Use of Plant-Growth-Promoting Rhizobacteria (PGPR) Seed Inoculation as Alternative Fertilizer Inputs in Wheat and Barley Production. *Commun Soil Sci Plant Anal*. 2014; 45(18): 2457-67.
16. Lindsay WL, Norvell WA. Development of a DTPA Soil Test for Zinc, Iron, Manganese, and Copper. *Soil Sci Soc Am J*. 1978; 42: 421-8.
17. Hassani A, Nouri J, Mehregan I, Moattar F, Sadeghi Benis M. Phytoremediation of Soils Contaminated with Heavy Metals Resulting from Acidic Sludge of Eshtehard Industrial Town Using Native Pasture Plants. *J Environ Earth Sci*. 2015; 5(2): 87-93.
18. Mkhabela M, Warman P. The Influence of Municipal Solid Waste Compost on Yield, Soil Phosphorus Availability and Uptake by Two Vegetable Crops Grown in a Pugwash Sandy Loam Soil in Nova Scotia. *Agr Ecosystem Environ*. 2005; 106(1): 57-67.
19. Anning AK, Akoto R. Assisted Phytoremediation of Heavy Metal Contaminated Soil from a Mined Site with *Typha Latifolia* and *Chrysopogon zizanioides*. *Ecotox Environ Safe*. 2018; 148: 97-104.
20. Besaltpour A, Hajabbasi M, Khoshgoftarmanesh A, Dorostkar V. Landfarming Process Effects on Biochemical Properties of Petroleum-Contaminated Soils. *Soil Sediment Contam*. 2011; 20(2): 234-48.
21. Lin Y, Watts DB, Kloepper JW, Torbert HA. Influence of Plant Growth-Promoting Rhizobacteria on Corn Growth under Different Fertility Sources. *Commun Soil Sci Plant Anal*. 2018; 49(10): 1239-55.
22. Namlı A, Mahmood A, Sevilir B, Özkır E. Effect of Phosphorus Solubilizing Bacteria on Some Soil Properties, Wheat Yield and Nutrient Contents. *Eur J Soil Sci*. 2017; 6(3): 249-58.
23. Baghaie AH, Daliri A. Effect of Applying Sunflower Residues as a Green Manure on Increasing Zn Concentration of Two Iranian Wheat Cultivars in a Pb and Cd Polluted Soil. *J Human Environ Health Promot*. 2019; 5(1): 9-14.
24. Baghaie AH, Fereydoni M. The Potential Risk of Heavy Metals on Human Health Due to the Daily Consumption of Vegetables. *Environ Health Eng Manage J*. 2019; 6(1): 11-6.
25. Abdelkrim S, Jebara SH, Saadani O, Chiboub M, Abid G, Jebara M. Effect of Pb-resistant Plant Growth-Promoting Rhizobacteria Inoculation on Growth and Lead Uptake by *Lathyrus sativus*. *J Basic Microbiol*. 2018; 58(7): 579-89.
26. Jebara SH, Saadani O, Fatnassi IC, Chiboub M, Abdelkrim S, Jebara M. Inoculation of *Lens culinaris* with Pb-Resistant bacteria Shows Potential for phytostabilization. *Environ Sci Pollut Res*. 2015; 22(4): 2537-45.
27. Sarikhani M, Malboobi M, Ebrahimi M. Phosphate Solubilizing Bacteria: Isolation of Bacteria and Phosphate Solubilizing Genes, Mechanism and Genetics of Phosphate solubilization. *Agr Biotechnol J*. 2014; 6(1): 77-110.
28. Sadaghiani MR, Kazemalilou HK-MB-S. Influence of PGPR Bacteria and Arbuscular Mycorrhizal Fungi on Growth and Some Physiological Parameters of *Onopordon acanthium* in a Cd-Contaminated Soil. *J Water Soil*. 2016; 30(2): 542-54.
29. Feng H, Zhang N, Du W, Zhang H, Liu Y, Fu R, et al. Identification of Chemotaxis Compounds in Root Exudates and Their Sensing Chemoreceptors in Plant-Growth-Promoting Rhizobacteria *Bacillus amyloliquefaciens* SQR9. *Mol Plant Microb Int*. 2018; 31(10): 995-1005.
30. Zaeferian F, Vahidzadeh S, Rahdari P, Rezvani M, Zadeh HG. Effectiveness of Plant Growth Promoting rhizobacteria in Facilitating Lead and Nutrient Uptake by Little Seed Canary Grass. *Braz J Bot*. 2012; 35(3): 241-8.
31. Chandrasekhar C, Ray JG. Lead Accumulation, Growth Responses and Biochemical Changes of Three Plant Species Exposed to Soil Amended with Different Concentrations of Lead Nitrate. *Ecotox Environ Safe*. 2019; 171: 26-36.
32. Wang M, Chen S, Han Y, Chen L, Wang D. Responses of Soil Aggregates and Bacterial Communities to Soil-Pb Immobilization Induced by biofertilizer. *Chemosphere*. 2019; 220: 828-36.
33. Khanna K, Jamwal VL, Sharma A, Gandhi SG, Ohri P, Bhardwaj R, et al. Supplementation with Plant Growth Promoting Rhizobacteria (PGPR) Alleviates Cadmium Toxicity in *Solanum lycopersicum* by Modulating the Expression of Secondary Metabolites. *Chemosphere*. 2019; 230: 628-39.
34. Abou-Shanab RAI, Angle JS, Chaney RL. Bacterial Inoculants Affecting Nickel Uptake by *Alyssum murale* From Low, Moderate and High Ni Soils. *Soil Biol Biochem*. 2006; 38(9): 2882-89.
35. Fathololomi S, Asghari S, Goli KE. Effects of Municipal Sewage Sludge on the Concentration of Macronutrients in Soil and Plant and Some Agronomic Traits of Wheat. *J Soil Manage Sustain*. 2015; 5(2): 49-70.
36. Cao RX, Ma LQ, Chen M, Singh SP, Harris WG. Phosphate Induced Metal Immobilization in a Contaminated Site. *Environ Pollut*. 2003; 122(1): 19-28.
37. Yolcu H, Turan M, Lithourgidis A, Çakmakçı R, Koc A. Effects of Plant Growth-Promoting Rhizobacteria and Manure on Yield and Quality Characteristics of Italian Ryegrass under Semi arid Conditions. *Aust J Crop Sci*. 2011; 5(13): 1730-6.
38. Shi W, Ma X. Effects of Heavy Metal Cd Pollution on Microbial Activities in Soil. *Ann Agric Environ Med*. 2017; 24(4): 722-5.
39. Osborne WJ, Saravanan V, Mukherjee A, Chandrasekaran N. Impact of *Vetiveria zizanioides* rhizosphere Bacterial Isolates on PGPR Traits and Cadmium Resistance. *J Ecobiotechnol*. 2010; 2(5): 286-90.
40. Tabarteh FN, Baghaie AH, Polous A. Effect of Enriched Cow Manure with Converter Sludge on Fe bio-availability in a Lead Polluted Soil. *J Water Soil Conserv*. 2017; 24(1): 205-20.

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