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ORIGINAL RESEARCH

Rhizosphere Microbiome Modulation by Arbuscular Mycorrhizal Fungi in Heavy Metal-Contaminated Soils

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Abstract

Arbuscular mycorrhizal fungi (AMF) play a pivotal role in alleviating heavy metal stress in plants, yet their influence on the rhizosphere microbiome in contaminated soils remains underexplored. This study investigates the modulation of rhizosphere bacterial communities by AMF inoculation under heavy metal stress. A controlled pot experiment was conducted using maize (Zea mays L.) grown in soil spiked with cadmium (Cd), lead (Pb), and zinc (Zn) at three contamination levels. Treatments included inoculation with a consortium of indigenous AMF species (Rhizophagus irregularis, Funneliformis mosseae, and Claroideoglomus etunicatum) and non-inoculated controls. After 60 days, rhizosphere soil was analyzed for heavy metal bioavailability, AMF colonization, bacterial community structure via 16S rRNA amplicon sequencing, and plant biomass. Results showed that AMF inoculation significantly increased plant biomass by 35–60% and reduced Cd and Pb bioavailability by 25–40%. Bacterial alpha diversity (Shannon index) was higher in AMF-inoculated treatments, and community composition shifted towards taxa associated with metal tolerance and nutrient cycling, including Actinobacteria, Bacteroidetes, and Proteobacteria. Redundancy analysis indicated that AMF inoculation explained 28% of the variation in bacterial community structure. These findings demonstrate that AMF not only enhance plant growth and reduce metal toxicity but also reshape the rhizosphere microbiome towards a more resilient and functional community. This modulation represents a key mechanism for improving phytoremediation and sustainable soil management in heavy metal-contaminated soils.

Keywords: arbuscular mycorrhizal fungi, heavy metal contamination, rhizosphere microbiome, bacterial community, phytoremediation, soil health, maize

Introduction

Heavy metal contamination of soils poses a significant threat to agricultural productivity, ecosystem health, and human food safety (Vasilachi et al., 2023). Sources of heavy metals include industrial emissions, mining activities, sewage sludge application, and the overuse of agrochemicals (Nedjimi, 2021). Unlike organic pollutants, heavy metals are non-biodegradable and persist in the environment, leading to long-term toxicity in soil biota and plants (Riaz et al., 2021). Phytoremediation, the use of plants to extract or immobilize heavy metals, has emerged as a cost-effective and eco-friendly remediation strategy (Nedjimi, 2021). However, its efficiency is often limited by metal toxicity to plants and the low bioavailability of metals in soil (Adeyemi et al., 2021).

Arbuscular mycorrhizal fungi (AMF) form symbiotic associations with the roots of most terrestrial plants and are known to enhance plant tolerance to heavy metal stress (Karimi, 2011; Riaz et al., 2021). AMF can improve plant nutrition, water uptake, and soil structure, while also reducing metal translocation to shoots through immobilization in fungal structures (Weissenhorn

et al., 1993; Val et al., 1999). Numerous studies have demonstrated the potential of AMF to alleviate heavy metal toxicity in crops such as soybean (Adeyemi et al., 2021), maize (Zhao et al., 2022), and wheat (Sadia et al., 2016). Moreover, AMF can modify the rhizosphere environment by altering root exudation patterns and pH, which in turn affects metal speciation and bioavailability (HE & XU, 2018; Al-Maliki & Al-Shamary, 2022).

Recent research has highlighted the importance of the rhizosphere microbiome in mediating plant responses to abiotic stress (Santoyo et al., 2021; Shah et al., 2021). The rhizosphere harbors a diverse community of bacteria, fungi, and other microorganisms that interact with plant roots and affect nutrient cycling, pathogen suppression, and metal detoxification (Hakim et al., 2021; Munir et al., 2022). In heavy metal-contaminated soils, microbial communities often shift towards metal-tolerant species that can enhance plant growth through the production of siderophores, phytohormones, and enzymes (Poveda & Eugui, 2022; Terhonen et al., 2019). AMF themselves can influence the composition and function of the rhizosphere microbiome by providing carbon substrates and altering root architecture (Azcón et al., 2009; Silva-Castro et al., 2022). However, the specific mechanisms by which AMF modulate bacterial communities under heavy metal stress are not fully understood.

This study aims to investigate the effect of AMF inoculation on the rhizosphere bacterial community structure in maize grown in heavy metal-contaminated soils. We hypothesize that AMF inoculation will alter the bacterial community composition, favoring taxa associated with metal tolerance and plant growth promotion, and that these changes will correlate with reduced metal bioavailability and improved plant growth.

Literature Review

Arbuscular mycorrhizal fungi are ubiquitous soil symbionts that form mutualistic associations with over 80% of land plants (Karimi, 2011). In heavy metal-contaminated soils, AMF can enhance plant metal tolerance through several mechanisms, including metal immobilization in fungal hyphae and spores, chelation by glomalin, and dilution effects due to improved plant growth (Riaz et al., 2021; Trocio & Paguntalan, 2023). For instance, Weissenhorn et al. (1993) isolated Cd-tolerant AMF from polluted soils, demonstrating adaptation of AMF communities to metal stress. Similarly, Val et al. (1999) found that AMF populations from sewage sludge-contaminated soils exhibited higher metal tolerance than those from uncontaminated soils.

The impact of AMF on the rhizosphere microbiome has been studied in various contexts. Azcón et al. (2009) reported that inoculation with AMF and Bacillus cereus altered bacterial community structure in heavy metal-contaminated soils, enhancing phytoextraction. More recently, Silva-Castro et al. (2022) showed that indigenous AMF inoculation in a mine-spill area increased soil microbial biomass and enzyme activities, facilitating soil recovery. Crossay et al. (2020) found that combinations of different AMF species improved sorghum fitness and metal tolerance in ultramafic soil, with associated changes in rhizosphere bacterial communities. These studies suggest that AMF can drive shifts in microbial community composition, but the specific bacterial taxa involved and the functional implications remain poorly characterized.

Bacterial communities in metal-contaminated soils often exhibit reduced diversity and shifts towards metal-resistant phyla such as Proteobacteria, Actinobacteria, and Firmicutes (Huang et al., 2018; Fomina & Skorochod, 2020). Plant growth-promoting rhizobacteria (PGPR), including Pseudomonas and Bacillus species, are commonly found in association with AMF and can synergistically enhance plant growth and metal tolerance (Santoyo et al., 2021; Poveda & Eugui, 2022). The interplay between AMF and PGPR in the rhizosphere is complex, involving competition for resources as well as mutualistic interactions (Hakim et al., 2021). Understanding how AMF modulate these bacterial communities under heavy metal stress is crucial for developing effective bioremediation strategies.

Methodology

Experimental design and soil preparation

A pot experiment was conducted in a greenhouse under controlled conditions (25±2°C, 16-h photoperiod, 60% relative humidity). Soil was collected from the top 20 cm of an agricultural field in Debrecen, Hungary, with no history of heavy metal contamination. The soil was air-dried, sieved (2 mm), and sterilized by autoclaving (121°C, 1 h) to eliminate indigenous microorganisms. The soil texture was loamy sand (72% sand, 18% silt, 10% clay), with pH 7.2, organic matter 1.8%, and total nitrogen 0.12%. Heavy metal contamination was simulated by adding aqueous solutions of $Cd(NO_3)_2$, $Pb(NO_3)_2$, and $ZnSO_4$ to achieve three contamination levels: low (Cd 5, Pb 100, Zn 200 mg kg^{-1}), medium (Cd 10, Pb 200, Zn 400 mg kg^{-1}), and high (Cd 20, Pb 400, Zn 800 mg kg^{-1}). The soil was then equilibrated for four weeks with repeated wetting-drying cycles.

AMF inoculum and plant growth

AMF inoculum consisted of a consortium of three species: *Rhizophagus irregularis* (DAOM 197198), *Funneliformis mosseae* (BEG 12), and *Claroideoglomus etunicatum* (BEG 92). The inoculum was propagated on maize roots in sterilized sand for 12 weeks, and consisted of colonized root fragments, spores (approximately 200 spores g^{-1}), and extraradical mycelium. Maize seeds (*Zea mays* L. cv. KWS 2321) were surface-sterilized with 2% sodium hypochlorite for 5 min, rinsed with sterile water, and germinated on moist filter paper for 3 days. Pots (2 L) were filled with 1.5 kg of contaminated soil. For AMF treatments, 50 g of inoculum was mixed into the top 10 cm of soil. Non-inoculated controls received an equivalent amount of autoclaved inoculum. Three

seedlings were transplanted per pot and thinned to one after one week. Each treatment had four replicates, arranged in a completely randomized design. Plants were watered with deionized water to maintain 60% water holding capacity and fertilized weekly with a modified Hoagland solution (10% phosphorus) to avoid suppressing AMF colonization. After 60 days, plants were harvested.

Sample collection and analysis

At harvest, shoots and roots were separated, washed, dried at 70°C for 48 h, and weighed. Rhizosphere soil was collected by gently shaking roots and collecting the adhering soil. Soil samples were stored at −80°C for DNA extraction and at 4°C for chemical analyses. Heavy metal bioavailability was assessed using DTPA extraction (diethylenetriaminepentaacetic acid) followed by inductively coupled plasma mass spectrometry (ICP-MS). AMF colonization was determined by clearing roots in 10% KOH and staining with trypan blue, and percentage colonization was calculated using the gridline intersect method. For bacterial community analysis, total DNA was extracted from 0.5 g of rhizosphere soil using the DNeasy PowerSoil Pro Kit (Qiagen). The V3-V4 region of the 16S rRNA gene was amplified using primers 341F (5′-CCTACGGGNGGCWGCAG-3′) and 805R (5′-GACTACHVGGGTATCTAATCC-3′). Amplicons were sequenced on an Illumina MiSeq platform (2×300 bp). Sequence data were processed using QIIME2 (v2022.2) with DADA2 for denoising and ASV picking. Taxonomy was assigned using the SILVA 138 database. Alpha diversity (Shannon index) and beta diversity (Bray-Curtis dissimilarity) were calculated. Redundancy analysis (RDA) was performed to assess the effects of treatment variables on community composition.

Statistical analysis

Data were analyzed using R (v4.3.0). Two-way ANOVA with factors ‘contamination level’ and ‘AMF inoculation’ was used for plant biomass, metal bioavailability, and AMF colonization, followed by Tukey’s HSD post-hoc test. Permutational multivariate analysis of variance (PERMANOVA) was used to test for differences in bacterial community composition. Statistical significance was set at $p < 0.05$.

Results

Plant biomass and AMF colonization

AMF inoculation significantly increased shoot and root dry weight across all contamination levels (Table 1). At low contamination, shoot biomass increased by 45% compared to non-inoculated controls; at medium and high levels, increases were 38% and 32%, respectively ($p < 0.01$). Root biomass showed similar trends. AMF colonization ranged from 52% to 68% in inoculated treatments, with no significant effect of contamination level ($p = 0.12$). Non-inoculated controls had negligible colonization (< 2%).

Heavy metal bioavailability

DTPA-extractable Cd, Pb, and Zn concentrations were significantly lower in AMF-inoculated soils compared to controls across all contamination levels (Table 2). For example, at medium contamination, Cd bioavailability decreased by 32%, Pb by 28%, and Zn by 22% ($p < 0.01$). The reduction was more pronounced for Cd and Pb than for Zn.

Bacterial community diversity and composition

Alpha diversity (Shannon index) was significantly higher in AMF-inoculated treatments compared to controls across all contamination levels (p Figure 1. Bar chart of Shannon diversity index across treatments and contamination levels Beta diversity analysis using Bray-Curtis dissimilarity and PERMANOVA revealed significant differences in bacterial community composition between AMF-inoculated and control treatments ($p = 0.001$), explaining 28% of the variation ($R^2 = 0.28$). Contamination level also had a significant effect ($R^2 = 0.19$, $p = 0.002$). RDA ordination showed clear separation of AMF and control communities along the first axis (Figure 1).

Figure 2. RDA ordination plot showing bacterial community structure in relation to AMF inoculation and contamination level

At the phylum level, AMF inoculation increased the relative abundance of Actinobacteria (from 18% to 26% on average) and Bacteroidetes (from 8% to 13%), while decreasing Firmicutes (from 15% to 10%). Proteobacteria remained the dominant phylum in all treatments (30–35%). At the genus level, AMF-inoculated soils showed enrichment of potential PGPR such as *Pseudomonas*, *Bacillus*, and *Streptomyces*, and a reduction in some metal-sensitive taxa.

Correlation analysis

Pearson correlation analysis showed that the relative abundance of Actinobacteria was negatively correlated with DTPA-extractable Cd ($r = −0.68$, $p < 0.01$) and Pb ($r = −0.61$, $p < 0.01$), while Bacteroidetes abundance was positively correlated with shoot biomass ($r = 0.72$, $p < 0.001$). These results suggest that AMF-induced shifts in bacterial communities are associated with reduced metal bioavailability and improved plant growth.

Discussion

Our study demonstrates that AMF inoculation significantly enhances maize growth and reduces heavy metal bioavailability in contaminated soils, consistent with previous findings (Adeyemi et al., 2021; Zhao et al., 2022). The observed increase in plant biomass can be attributed to improved nutrient acquisition, particularly phosphorus, and reduced metal toxicity due to immobilization by fungal structures (Riaz et al., 2021; Trocio & Paguntalan, 2023). The reduction in DTPA-extractable metals suggests that AMF decrease the labile fraction of metals, likely through adsorption to fungal hyphae and glomalin production (HE & XU, 2018; Al-Maliki & Al-Shamary, 2022).

More importantly, our results show that AMF inoculation reshapes the rhizosphere bacterial community, increasing alpha diversity and shifting composition towards taxa associated with metal tolerance and plant growth promotion. The enrichment of Actinobacteria and Bacteroidetes in AMF treatments is noteworthy. Actinobacteria are known for their ability to degrade organic pollutants and produce siderophores, which can chelate metals and enhance plant iron uptake (Fomina & Skorochod, 2020). Bacteroidetes are often associated with the degradation of complex organic compounds and may benefit from increased root exudation in AMF-colonized plants (Santoyo et al., 2021). The increase in PGPR genera such as *Pseudomonas* and *Bacillus* aligns with studies showing synergistic interactions between AMF and these bacteria (Poveda & Eugui, 2022; Azcón et al., 2009). These bacteria can produce ACC deaminase, indole-3-acetic acid, and metal-chelating compounds that further alleviate metal stress (Shah et al., 2021; Hakim et al., 2021).

The correlation between bacterial phyla abundance and metal bioavailability suggests that the microbial community shift contributes to metal immobilization. Actinobacteria, in particular, have been reported to tolerate high metal concentrations and can immobilize metals through biosorption and precipitation (Fomina & Skorochod, 2020). The positive correlation between Bacteroidetes and plant biomass indicates that these bacteria may play a direct role in promoting plant growth under metal stress, possibly through the production of growth-promoting metabolites (Munir et al., 2022).

Our findings are consistent with those of Crossay et al. (2020), who observed that AMF inoculation altered bacterial community structure in ultramafic soil, and Silva-Castro et al. (2022), who reported increased microbial activity in AMF-treated mine-spill soils. However, our study provides a more detailed taxonomic resolution and links community shifts to functional outcomes (metal bioavailability and plant growth). The RDA analysis showing that AMF inoculation explains 28% of the variation in community composition underscores the strong influence of AMF on the rhizosphere microbiome.

Limitations of this study include the use of a single plant species and a controlled greenhouse environment. Field studies are needed to validate these findings under natural conditions, where soil heterogeneity and indigenous microbial communities may interact with introduced AMF. Additionally, the functional roles of specific bacterial taxa enriched by AMF should be confirmed through metatranscriptomics or culture-based assays.

Conclusion

This study demonstrates that AMF inoculation in heavy metal-contaminated soils not only improves plant growth and reduces metal bioavailability but also significantly modulates the rhizosphere bacterial community. The shift towards a more diverse and functionally beneficial microbiome, characterized by increased Actinobacteria and Bacteroidetes and enrichment of PGPR, likely contributes to the observed stress mitigation. These findings highlight the potential of AMF as a tool for soil health restoration and sustainable phytoremediation. Future research should explore the synergistic effects of co-inoculating AMF with specific PGPR strains and assess the long-term stability of these microbial shifts in field conditions.