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Microbial Population and Enzymatic Activities in Soil Contaminated With Heavy Metals as Influenced by Arbuscular Mycorrhizal Fungi (AMF) Species

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Abstract
Heavy metal contamination in soil poses a significant threat to ecosystem health and the sustainability of agricultural systems. This study examined the effects of three arbuscular mycorrhizal fungal (AMF) species—*Funneliformis mosseae* (Fm), *Glomus etunicatum* (Ge), and *Rhizophagus irregularis* (Ri)—on soil microbial populations and enzyme activities in soils contaminated with copper (Cu) at 100 and 300 mg/kg, lead (Pb) at 100 and 300 mg/kg, and zinc (Zn) at 300 and 600 mg/kg concentrations in a simulated environment. Both AMF species and heavy metal contamination significantly influenced bacterial population counts. At 100 mg/kg Cu, Ri increased bacterial activity to 2.00 x 10^8 CFU/g soil, Fm to 5.00 x 10^7 CFU/g soil, and Ge to 4.00 x 10^7 CFU/g soil. At 300 mg/kg Cu, Fm increased bacterial counts to 4.00 x 10^8 CFU/g soil, Ge to 6.00 x 10^7 CFU/g soil, while Ri had no detectable population. Lead contamination resulted in higher bacterial counts, with Ge having the highest counts at both contamination levels (4.00 x 10^8 and 4.00 x 10^7 CFU/g soil). Under Zn contamination at 300 mg/kg and 600 mg/kg, bacterial counts were 2.00 x 10^9 and 7.00 x 10^8 CFU/g soil, respectively. Fungal populations responded variably to AMF species and heavy metal contamination. Enzyme activities also varied based on AMF species and heavy metal contamination. Ge and Fm consistently exhibited higher enzyme activities than Ri under Cu and Pb contamination. Acid phosphatase activity was highest with Fm at 100 mg/kg Cu (12.29 µg p-nitrophenol/g soil/hour) and at 300 mg/kg Cu with Ri (11.76 µg p-nitrophenol/g soil/hour); Ge had the highest activity at Pb (100 mg/kg) with 11.74 µg p-nitrophenol/g soil/hour; and Fm had the highest activity at Pb (300 mg/kg) with 11.61 µg p-nitrophenol/g soil/hour. These results indicate that the three AMF species have significant potential for bioremediating soils contaminated with Pb, Cu, and Zn.

Keywords: Heavy metal contamination, arbuscular mycorrhizal fungi (AMF), soil microbial populations, enzyme activities, bioremediation, ecosystem sustainability.

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Introduction

Soil is fundamental for sustaining human life, ecological quality, and overall human health (Jiang and Li, 2020; Ji and Sang, 2022). Within soil ecosystems, microbial communities play a pivotal role as the most active agents in nutrient transformation, substance turnover, organic matter decomposition, and nutrient release (Jiang and Li, 2020; Ji and Sang, 2022). These microorganisms also contribute to soil mineral decomposition and nutrient assimilation, positively impacting plant growth by supplying diverse nutrients through the plant root system (Birch and Bachofen, 1990). Moreover, soil microbes can mobilize and immobilize metal cations, thereby influencing their availability to plants (Birch and Bachofen, 1990).

However, soil contamination with heavy metals has emerged as a global problem, posing significant threats to agro-environments, ecosystems, and human health (Alekseenko *et al*., 2018; Wang *et al*., 2018a; Kamran *et al*., 2019a; Wang *et al*., 2019a). While heavy metal accumulation in soil can occur naturally, human activities such as the use of fertilizers, sewage sludge, wastewater irrigation, and industrial processes have amplified the concentration of these toxic elements (Raza *et al*., 2020). This anthropogenic influence alters soil properties, potentially harming soil microorganisms and increasing the risk of heavy metals entering the food chain, ultimately endangering human health (Chaudri *et al*., 1993; Huang *et al*., 2018; Gong and Tian, 2019).

To mitigate the adverse effects of heavy metal contamination and preserve soil microbial communities, exploring the potential of arbuscular mycorrhizal fungi (AMF) has gained considerable attention. AMF, as a crucial group of microbes in the rhizosphere, establish symbiotic relationships with higher plants and significantly contribute to ecosystem dynamics (Sheng and Xia, 2006). These fungi form intracellular associations with plant roots and facilitate nutrient availability, including N, P, K, Ca, S, Zn, Co, Ni, and Cu, through extensive hyphal networks (Ilyas, 2021). Furthermore, AMF interactions with plants enhance soil resources and improve resilience to environmental constraints (Brundrett, 2004; Brundrett, 2009; Smith and Read, 2010a; Lenoir *et al*., 2016; Torres *et al*., 2018; Balestrini *et al*., 2018; Shi *et al*., 2019).

Although previous studies have recognized the beneficial role of AMF in alleviating heavy metal phytotoxicity, research gaps remain. Specifically, there is a need to understand the mechanisms by which AMF detoxify heavy metal contamination and the extent of their involvement in metal adsorption, immobilization, and distribution (Ferreira Vilela and Barbosa, 2019; De Andrade *et al*., 2008). Additionally, exploring the resilience of soil microbial communities

in the presence of heavy metals and their potential to promote sustainable agriculture requires further investigation. In light of these research gaps, this study investigated the effects of three arbuscular mycorrhizal fungal (AMF) species – *Funneliformis mosseae*, *Glomus etunicatum*, and *Rhizophagus irregularis* – on soil microbial populations and enzyme activities in soils contaminated with copper (Cu), lead (Pb), and zinc (Zn).

Materials and Methods

The experiment was conducted in 2021 at the screen house of the College of Plant Science and Crop Production, Federal University of Agriculture, Abeokuta, Ogun State (FUNAAB), Nigeria (7º 15′ N, 3º 28′ E). The study was carried out under natural climatic conditions with temperatures ranging from 23-25°C and an average light period of 12 hours per day. The soil used for the experiment was collected from the top layer (0-20 cm) of a farm area within the Directorate of University Farms (DUFARMS) at FUNAAB. The collected soil samples were sandy loam, which were air-dried, screened through a 2 mm sieve, and sterilized in a high-capacity oven at 115°C for 45 minutes.

The basic physical and chemical properties of the soil were as follows: pH 6.7, 0.15% nitrogen (N), 10.12 mg/kg available phosphorus (P), 0.47 cmol/kg potassium (K), 0.60% organic carbon (O.C), 0.28 cmol/kg sodium (Na), 0.24 cmol/kg calcium (Ca), 0.31 cmol/kg magnesium (Mg), 0.88 cmol/kg iron (Fe), 1.05 cmol/kg copper (Cu), and 5.90 mg/kg zinc (Zn).

The treatments consisted of soils contaminated with copper (Cu) at concentrations of 100 and 300 mg/kg, lead (Pb) at concentrations of 100 and 300 mg/kg, and zinc (Zn) at concentrations of 300 and 600 mg/kg, as well as a control treatment with non-contaminated soil. The treatments were laid out in a completely randomized design with three replications. The soil was air-dried and spiked with heavy metal salts of $CuSO_4 \cdot 5H_2O$, $PbSO_4$, and $ZnSO_4 \cdot 7H_2O$ as sources of copper (Cu), lead (Pb), and zinc (Zn), respectively. The pots were filled with 7 kg of soil each and left for 15 days to allow the heavy metals to bond with the soil medium. The soil was then saturated with water to ensure the even distribution of heavy metals throughout the soil mass.

AMF inoculation

Arbuscular Mycorrhizal fungi inoculum was a mixture of soil, spores, mycelium and colonized root fragments. Inoculated treatments were applied with 20 g of inoculum (~50 spores/g of soil) per pot. The AMF inoculum (*Funneliformis mosseae (Fm), Glomus etunicatum (Ge) and Rhizophagus irregularis (Ri)*) was obtained from the International Institute

of Tropical Agriculture, Ibadan, Nigeria. Sunflower was planted as the host plant.

Microbial population

A serial dilution method was employed on the samples obtained by collecting soils at the roots rhizosphere. Tenfold serial dilutions were made up to 10^{-6}. An amount of 0.1 ml from the diluted samples (dilution10^{-4} for fungi and 10^{-6} for bacteria) was spread on the respective culture media plates using a glass spreader. These plates were then incubated at ambient temperature for 24 hours for bacteria and 4-5 days for fungi. Soil extract agar (pH 7) was used for bacteria, colonies were counted and CFU/g was calculated for bacteria. Soil extract agar (pH 5), Potato dextrose agar (pH 5.0) and Malt extract agar (pH 5.0) were used for the isolation of fungi. The isolates were maintained on respective media slants and were identified (Barnett & Hunter, 1998; Barnett, 1960; Thom and Raper, 1945).

Enzyme activities

Urease activity was measured calorimetrically at 578 nm using the indophenol-based colorimetric method, following Guan (1986) and Guan *et al.* (1986). The assay used citrate solution (pH 6.7), sodium phenol solution, sodium hypochlorite solution, 10% urea solution, and a standard nitrogen solution as reagents.

Protease activity was determined according to Macura and Vágnerová (1969). Air-dried and sieved soil was mixed with toluene and azo-casein solution (pH 8.3) and incubated at 37°C for 24 hours. Post-incubation, sodium hydrogen carbonate and trichloroacetic acid were added, followed by filtration. The absorbance of the clear filtrate was measured at 430 nm.

Phosphatase activity was measured using the method of Tabatabai and Bremner (1969). Air-dried and screened soil was mixed with modified universal buffer (pH 6.5), toluene, and disodium p-nitrophenyl phosphate tetrahydrate, and incubated at 37°C for 1 hour. After adding calcium chloride and sodium hydroxide, the mixture was filtered, and the absorbance of the filtrate was measured at 400 nm. The amount of p-nitrophenol was calculated against the standard.

Statistical analysis

The data collected were subjected to Analysis of Variance (ANOVA) using GENSTAT, 12th edition, and the significant treatment means were separated using least significant differences (LSD) at a 5% probability level.

Results

Effect of Arbuscular mycorrhizal fungi species on bacterial population (cfu/g) in heavy metals contaminated soil

The effect of arbuscular mycorrhizal fungi species inoculation and heavy metal concentration on the bacterial

population is shown in Figure 1. The results showed that the interactions between AMF species inoculation and heavy metal contamination significantly ($p < 0.01$) enhanced the population of bacteria in Pb, Cu, and Zn-contaminated soil. Soils with no heavy metal contamination but inoculated with *Glomus etunicatum* had a significantly higher bacterial count (7×10^7 cfu/g) compared with soil inoculated with *Funneliformis mosseae*, which gave a lower number (1×10^7 cfu/g). Soil inoculated with *Rhizophagus irregularis* and polluted with Cu (100 mg/kg) significantly increased the bacterial count by 2×10^8 compared to that inoculated with *Glomus etunicatum*, which increased bacterial activity by 4×10^7. *Rhizophagus irregularis* inoculated into Cu-polluted soil (100 mg/kg) increased bacterial activity by 2×10^8 compared to *Glomus etunicatum* inoculation, which increased bacterial activity by 4×10^7. Cu-polluted soil at 300 mg/kg inoculated with *Rhizophagus irregularis* recorded zero population, while Cu-polluted soil at 300 mg/kg inoculated with *Funneliformis mosseae* increased the bacterial population by giving the highest (4×10^8) CFU/g. The soil polluted with Pb (100 mg/kg) and inoculated with *Glomus etunicatum* had the highest population of bacterial (1×10^8 CFU/g) compared to when the contaminated soil was inoculated with *Rhizophagus irregularis*, which had the lower population (9×10^6) CFU/g. Similar trends were observed in soil polluted with Pb (300 mg/kg). Zn polluted soil (300 mg kg^{-1}, 600 mg kg^{-1}) with *Glomus etunicatum* inoculation recorded the highest population of bacteria (2×10^9, 7.00×10^8) cfu/g, respectively. Lower bacterial populations were observed when soil was polluted with Zn was at 300 mg/kg and inoculated with *Funneliformis mosseae* at 600 mg kg^{-1} with *Rhizophagus irregularis* (1.00×10^6, 8.00×10^7).

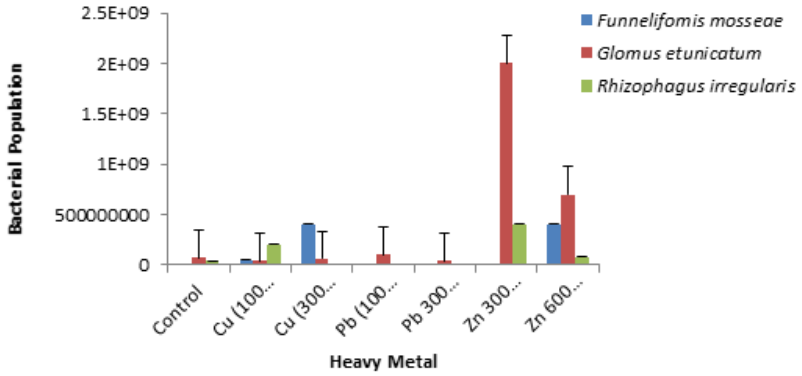

Figure 1: Interaction between heavy metals contaminated soil and Arbuscular mycorrhizal fungi inoculation on soil bacterial population (CFU/g)

Effect of heavy metal contamination on soil fungi populations as influenced by different Arbuscular mycorrhizal fungi (AMF) species

Figure 2 shows the interaction between AM fungi inoculation and heavy metal contamination in soil fungi populations. Fungi populations were affected by different AMF species inoculation with diverse levels of heavy metal contamination. In general, soil contaminated with Cu (300 mg/kg) and inoculated with Glomus *etunicatum* had the highest soil fungi population count (4.9 x 10^6 cfu/g), while soil contaminated with Zn (600 mg/kg) and inoculated with *Funneliformis mosseae* had the lowest (1.1 x 10^3). Uncontaminated sunflower-grown soil inoculated with *Funneliformis mosseae* had significantly higher fungi pollution (158,400 CFU/g), while soil inoculated with *Rhizophagus irregularis* produced a lower fungi population. Soil contaminated with Cu (100 mg/kg) and innoculated with *Rhizophagus irregularis* had a significantly higher fungi population compared to Cu-contaminated soils inoculated with *Glomus etunicatum.* Sunflower-grown soil contaminated with Pb (100 mg/kg) and inoculated with *Glomus etunicatum*, Pb (300 mg/kg) contaminated soil inoculated with *Funneliformis mosseae*, and Zn (300 mg/kg) contaminated soil inoculated with *Rhizophagus irregularis* had significantly higher soil populations, each with 3.0 x 10^5 cfu/g.

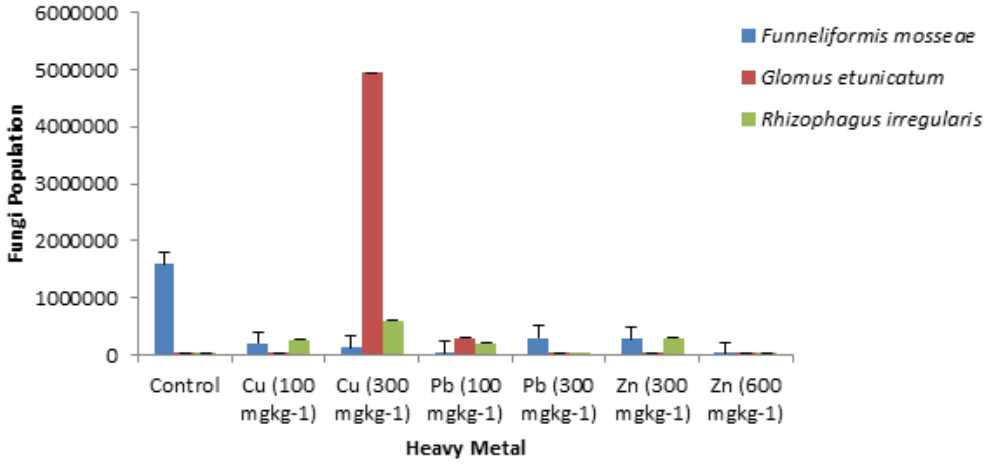

Figure 2: Effect of heavy metals contamination on soil fungi populations as influenced by different Arbuscular mycorrhizal fungi (AMF) species

Influence of Arbuscular mycorrhizal fungi (AMF) on protease activity in soil contaminated with heavy metals

The effects of AMF inoculation in soil planted with sunflowers and contaminated with different levels of heavy metals on protease activity is reported in Figure 3. Soil with no heavy metal contamination but inoculated with Fm was significantly higher in protease (6.95) compared to soil inoculated with Ri, which had the lowest. A similar trend was observed in Cu-polluted soil at 100 mg/kg. Cu contaminated at 300 mg/kg inoculated with Ge species considerably improved the activities of protease enzymes (7.94). A similar trend was recorded in Pb (100 mg/kg) polluted soil. The highest protease activities (8.04) were recorded in Pb-polluted (300 mg/kg) soil inoculated with Ri, while Gm-inoculated soils recorded the lowest activities (5.98). Zn (300 mg/kg) contaminated soil inoculated with Ge had significantly higher protease activity (7.04) compared to Zn (600 mg/kg) polluted soil inoculated with Fm, which had higher protease activities (7.12) and was not significantly different from Ri inoculated soil (7.05), while Gm gave the lowest.

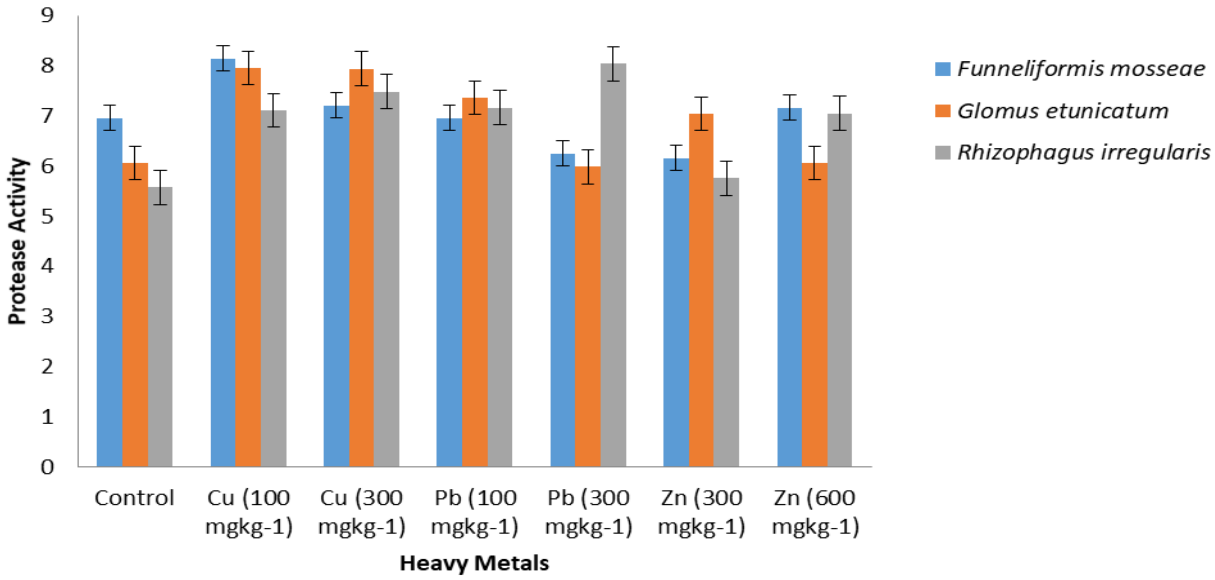

Figure 3: Effects of AMF inoculation in soil planted with sunflowers and contaminated with different levels of heavy metals on protease

Influence of Arbuscular mycorrhizal fungi (AMF) on urease activity in soil contaminated with heavy metals

The results show that the urease activities were significantly affected by different AMF species and heavy metal contamination (Figure 4). The urease activity was considerably higher in uncontaminated soil inoculated with Ri (26.94) compared to Ge-inoculated soil, which had the lowest. Cu-polluted soils (100 mg/kg) inoculated with Ge had significantly higher urease activity (32.13) in the soil compared to Cu-polluted soils at 300 mg/kg inoculated with Ri, which had considerably higher urease (28.27). At a Pb contamination level of 100 mg/kg, urease activities in soils inoculated with Ge were considerably greater (31.57) but lower (14.73) when inoculated with Fm. In soils polluted with Pb at 300 mg/kg, inoculation with Fm increased urease activities (29.71), whereas soil inoculated with Ge decreased urease activities (14.46). Moreover, Zn pollution at 300 mg/kg and inoculated with Ge had significantly higher urease activities (34.17) than inoculation with Ri, which had lower urease activities. Zinc contaminated and Ri-inoculated soil had substantially lower urease activities at 600 mg/kg (21.24), but Fm-inoculated soil had significantly greater urease (29.88) but was not significantly different from Ge-inoculated soil (28.83).

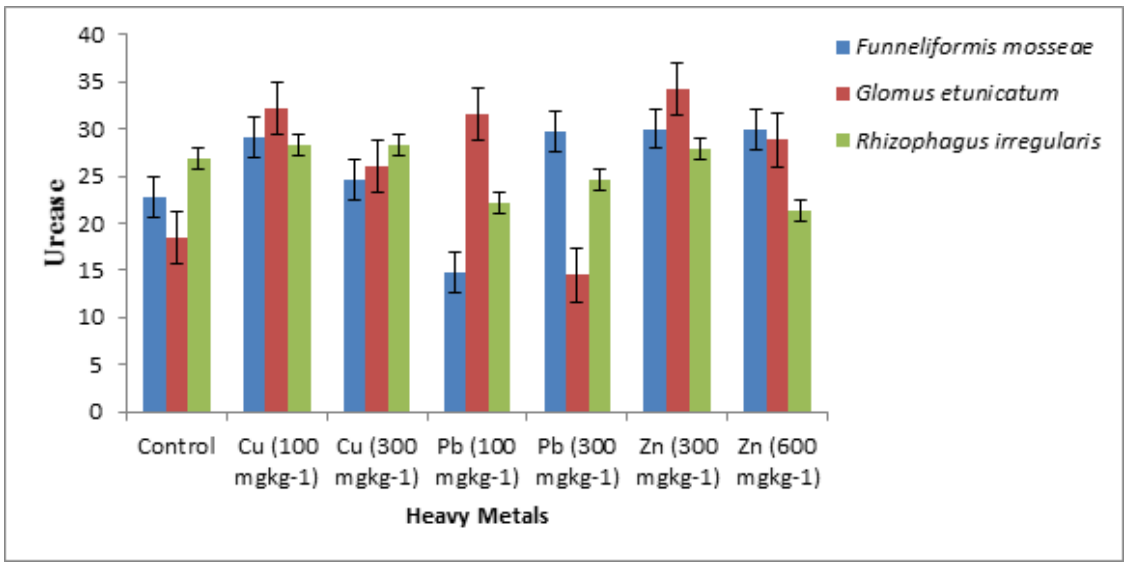

Figure 4: Effects of Arbuscular mycorrhizal fungi (AMF) on Urease activities in soil contaminated with heavy metals

Influence of Arbuscular mycorrhizal fungi (AMF) on acid phosphatase activity in soil contaminated with heavy metals

Figure 5 shows the acid phosphatase activity levels for three different AMF species (*Funneliformis mosseae, Glomus etunicatum, and Rhizophagus irregularis*) at diverse levels of heavy metal contaminations (100 mg/kg and 300 mg/kg for Cu, Pb, and 300 mg/kg and 600 mg/kg Zn). The results show that acid phosphatase was affected by different AMF species and heavy metals contamination. In un-contaminated soil, among the three AMF species, Ri-inoculated soil had significantly lower acid phosphatase (8.69 µg p-nitrophenol/g soil/hour). At a contamination level of 100 mg/kg Cu, *Fm-inoculated* soil had considerably higher acid phosphatase activity at 12.29 µg p-nitrophenol/g soil/hour, Ri-inoculated soil had 9.03 µg p-nitrophenol/g soil/hour while *Ge* had a level of 8.69 µg p-nitrophenol/g soil/hour and considerably lower. At a contamination level of 300 mg/kg Cu, Ri-inoculated soil had a significantly higher acid phosphatase (11.76 µg p-nitrophenol/g soil/hour), followed by Ge-inoculated soil (10.82 µg p-nitrophenol/g soil/hour) compared to Fm- inoculated soil that had lowest (8.37 µg p-nitrophenol/g soil/hour). Lead (Pb) soils contamination at 100 mg/kg with *Ge* inoculation resulted in the highest acid phosphatase activity levels among all three AMF species (11.74 µg p-nitrophenol/g soil/hour). This was followed by Ri-inoculation *(*9.91 µg p-nitrophenol/g soil/hour) while Fm-inoculated soil that had lowest (8.47 µg p-nitrophenol/g soil/hour). At a contamination level of 300 mg/kg Pb, Fm-inoculated soil had a significantly higher acid phosphatase (11.61 µg p-nitrophenol/g soil/hour), followed by Ge-inoculated soil *(9.76* µg p-nitrophenol/g soil/hour) while Ri-inoculated soil had lowest (8.57 µg p-nitrophenol/g soil/hour). However, for Zn contami-nation at both levels (300 mg/kg and 600 mg/kg), all three AMF species showed similar acid phosphatase activity levels.

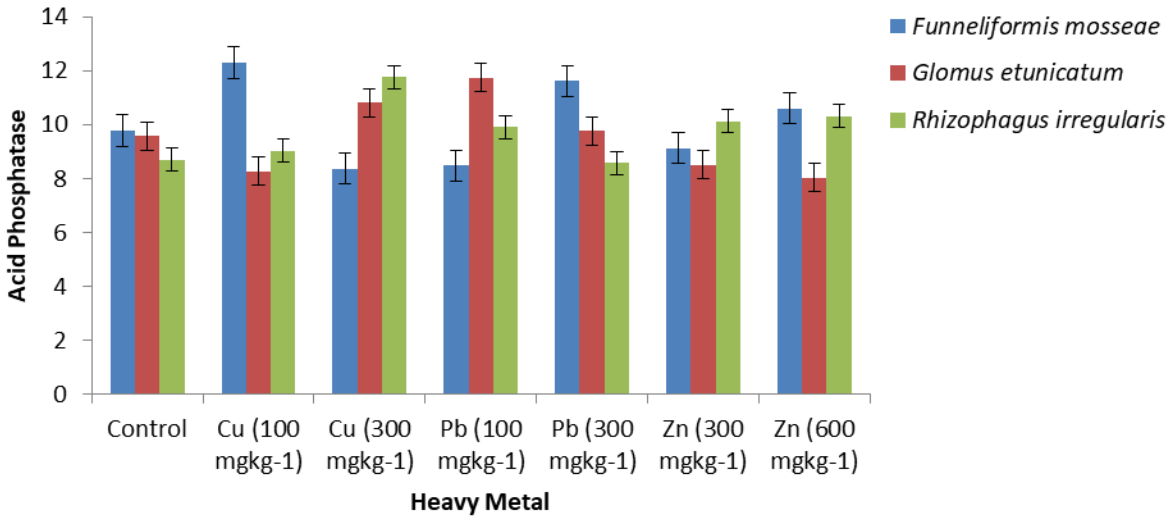

Figure 5: Effects of Arbuscular mycorrhizal fungi (AMF) on Acid phosphatase in soil contaminated with heavy metals.

Influence of Arbuscular mycorrhizal fungi (AMF) on alkaline phosphatase activity in soil contaminated with heavy metals

Figure 6 shows the alkaline phosphatase activity levels for three AMF species at different levels of heavy metal contamination (100 mg/kg and 300 mg/kg for Cu, Pb, 300 mg/kg and 600 mg/kg Zn). The results demonstrate that alkaline phosphatase is influenced by heavy metal contamination and AMF species.

In uncontaminated soils, Ri exhibited significantly higher alkaline phosphatase activity (143.36 µg p-nitrophenol/g soil/hour) compared to Fm (121.58 µg p-nitrophenol/g soil/hour) and Ge (120.40 µg p-nitrophenol/g soil/hour). At a contamination level of 100 mg/kg Cu, Fm-inoculated soil showed significantly higher alkaline phosphatase activity (219.18 µg p-nitrophenol/g soil/hour) compared to Ri (126.74 µg p-nitrophenol/g soil/hour) and Ge (99.94 µg p-nitrophenol/g soil/hour). For contamination at 300 mg/kg Cu, Ri-inoculated soil exhibited significantly higher alkaline phosphatase activity (124.45 µg p-nitrophenol/g soil/hour) than Ge-inoculated soil (102.7 µg p-nitrophenol/g soil/hour). Under Pb contamination at 100 mg/kg, Ri-inoculated soil consistently displayed the

highest alkaline phosphatase activity (115.61 µg p-nitrophenol/g soil/hour), which was significantly higher than Fm and Ge. At a contamination level of 300 mg/kg Pb, Fm-inoculated soil showed significantly higher alkaline phosphatase activity (125.19 µg p-nitrophenol/g soil/hour) compared to Ge-inoculated soil (119.31 µg p-nitrophenol/g soil/hour). However, for Zn contamination at both 300 mg/kg and 600 mg/kg levels, no significant differences were observed among Ri, Fm, and Ge in terms of alkaline phosphatase activity.

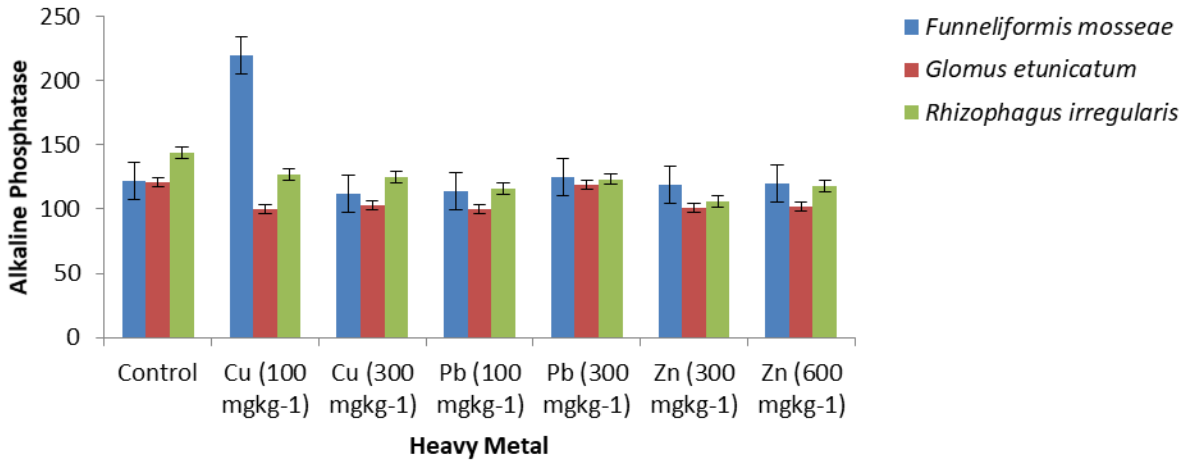

Figure 6: Effects of Arbuscular mycorrhizal fungi (AMF) on alkaline phosphatase in soil contaminated with heavy metals

Discussion

Arbuscular mycorrhizal fungi (AMF) play a significant role in enhancing soil enzyme activities, which are critical for nutrient cycling and ecosystem functioning. In heavy metal-contaminated soils, the side effects of heavy metals on soil enzyme activities can be significant. Understanding the impacts of AMF on soil enzyme activity in soil contaminated with heavy metals is therefore critical for establishing effective strategies for minimising the detrimental effects of heavy metal pollution on soil microbial communities and ecosystem functioning.

The results show that different AMF species had varying effects on bacterial populations, and the effectiveness of AMF species differed depending on the type and level of heavy metal contamination. Previous studies have also investigated the impacts of AMF on soil

microbial communities in soil contaminated with heavy metals. For example, Wu *et al*. (2018) found that inoculation with AMF increased soil microbial biomass and diversity in soils contaminated with cadmium. Similarly, a study by Wang *et al*. (2019) found that inoculation with AMF increased soil microbial biomass and activity in soils contaminated with cadmium. *Funneliformis mosseae* was more effective at promoting bacterial populations in soils contaminated with Cu at 100 mg/kg and 300 mg/kg. *Rhizophagus irregularis* was detected to be less effective at promoting bacterial populations in these same soils. These findings are consistent with previous studies that have found positive effects of AMF on soil microbial communities in heavy metal-contaminated soils (Wu *et al*., 2018; Wang *et al*., 2019). However, the effectiveness of different AMF species may vary depending on the specific heavy metal contaminants present in the soil.

Several other studies have investigated the effects of AMF on soil microbial communities in heavy metal-contaminated soils. For example, a study by Zhang *et al*. (2019) found that inoculation with AMF increased soil microbial biomass and activity in soils contaminated with lead. According to the findings, this is due to AMF's propensity to boost plant growth and nutrient intake, which promotes soil microbial activity. Similarly, Li *et al*. (2018) discovered that AMF inoculation improved soil microbial biomass and diversity in copper-contaminated soils. The authors suggest that this may be due to the ability of AMF to improve soil structure and nutrient availability, which can create more favourable conditions for soil microorganisms. In contrast, a study by Wang *et al*. (2018) found that AMF inoculation had no significant effect on soil microbial biomass or diversity in soils contaminated with cadmium. The study suggests that this may be due to the high toxicity of cadmium, which can limit the growth and activity of both plants and microorganisms. The findings suggest that the effectiveness of AMF in promoting bacterial populations in heavy metal-contaminated soils may be dependent on several factors, including the specific heavy metal contaminant present in the soil, the type of AMF species used for inoculation, and the overall health and condition of the soil.

The results of this study are consistent with previous research that has shown that AMF can have both positive and negative effects on soil fungi populations, depending on the specific AMF species used and the type and level of heavy metal contamination present in the soil. For instance, Wu *et al*. (2016) found that inoculation with *Funneliformis mosseae* increased fungal biomass in soils contaminated with cadmium while *Glomus versiforme*-inoculation had no effect. Similarly, a study by Wang *et al*.

(2018) found that soil inoculated with *Rhizophagus irregularis* increased fungal biomass in soils contaminated with lead, while *Funneliformis mosseae* had no effect. However, other studies have reported adverse effects of AMF on soil fungi populations in heavy metal-contaminated soils (Zhang *et al*. 2017). Another study by Zhang *et al*. (2017) found that inoculation with *Glomus intraradices* decreased fungal biomass in soils contaminated with copper. These suggest that different AMF species may have varying abilities to mitigate the adverse effects of heavy metals on soil fungus populations. Therefore, it is significant to carefully select appropriate AMF species based on the specific heavy metal contamination present in a given soil.

The present studies also provide relevant information on the effects of Arbuscular mycorrhizal fungi (AMF) species on protease and urease activity in heavy metal-contaminated soils. The results of these studies are consistent with previous research that has shown that AMF can have both positive and negative effects on soil enzyme activities, depending on the specific AMF species used and the type and level of heavy metal contamination present in the soil. Wu *et al*. (2016) found that soil inoculated with *Funneliformis mosseae* increased protease activity in soils contaminated with cadmium, while *Glomus versiforme* had no effect. Similarly, a study by Wang *et al*. (2018) found that inoculation with *Rhizophagus irregularis* increased urease activity in soils contaminated with lead, while *Funneliformis mosseae* had no effect. Zhang *et al*. (2017) found that inoculation with *Glomus intraradices* decreased protease activity in soils contaminated with copper. These highlight the importance of considering both the specific AMF species used and the type and level of heavy metal contamination present in the soil when assessing the effects of AMF on soil enzyme activities. For example, under Cu 100 mg/kg contamination levels, *Funneliformis mosseae* showed an increase in protease activity compared to non-inoculated controls, and soil inoculated with *Rhizophagus irregularis* showed a decrease in protease activity compared to non-inoculated controls. In addition to these findings, previous research has similarly shown that AMF can have essential impacts on other aspects of soil microbial communities and ecosystem functioning beyond enzyme activities. In addition, Li *et al*. (2019) found that inoculation with AMF increased soil microbial biomass and diversity in soils contaminated with cadmium.

It is also worth noting that while AMFs can have beneficial effects on soil enzyme activities in some cases, they may not always be effective at mitigating the adverse effects of heavy metal contamination on soil microbial

communities and ecosystem functioning. For example, a study by Chen *et al*. (2019) found that inoculation with AMF had no significant effect on microbial biomass or diversity in soils contaminated with cadmium.

The present study also provides valuable insights into the effects of different AMF species on acid phosphatase and alkaline phosphatase activity in heavy metal-contaminated soils. For example, Wu *et al*. (2016) found that inoculation with *Funneliformis mosseae* increased acid phosphatase activity in soils contaminated with cadmium, while soil inoculated with *Rhizophagus intraradices* had no effect. Similarly, a study by Wang *et al*. (2018) found that inoculation with *Glomus mosseae* increased alkaline phosphatase activity in soils contaminated with lead, while *Rhizophagus clarus*-inoculated soil had no effect. Furthermore, Wu *et al*. (2016) found that inoculation with *Funneliformis mosseae* increased acid phosphatase activity in soils contaminated with cadmium by promoting the growth of cadmium-tolerant bacteria, which in turn enhanced soil enzyme activities. Similarly, a study by Wang *et al*. (2018) found that inoculation with *Glomus mosseae* increased alkaline phosphatase activity in soils contaminated with lead by increasing the availability of phosphorus and other nutrients through enhanced root growth and nutrient uptake.

Generally, these findings suggest that inoculation with appropriate AMF species may be an effective strategy for promoting bacterial populations and mitigating the adverse effects of heavy metal contamination on soil enzyme activities and other aspects of soil microbial communities and ecosystem functioning in soils contaminated with heavy metals. However, further research is needed to determine the most effective AMF species for different types and levels of heavy metal contamination.

Conclusion

In conclusion, our study underscores the complex interactions between AMF, soil enzymes, and heavy metal contaminants in soil ecosystems. While AMF show promise in enhancing bacterial populations and soil enzyme activities under certain conditions, their effectiveness can vary significantly depending on the specific AMF species and the types and levels of heavy metals present in the soil. Future research should focus on elucidating the underlying mechanisms governing AMF-mediated responses to heavy metal stress and identifying optimal AMF species for different metal-contaminated soils. Such insights are crucial for developing targeted strategies to mitigate the adverse impacts of heavy metal pollution on soil microbial communities and ecosystem functioning.

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