
Experimental study for recovery of heavy metals from contaminated soil using *arbuscular mycorrhizal* fungi

Bulent Budak

Faculty of Agriculture,
Odemis Vocational Training School,
Ege University,
Odemis, 35750, Izmir, Turkey
Email: bulent.budak@ege.edu.tr

Turgut T. Onay

Institute of Environmental Sciences,
Bogazici University,
Bebek 3432 Istanbul, Turkey
Email: onayturg@bogazici.edu.tr

Aysegul Yagmur Goren

Izmir Institute of Technology, Environmental Science and Engineering,
Urla, Izmir, Turkey
and
Faculty of Engineering and Applied Science,
Ontario Tech University,
2000 Simcoe Street North, Oshawa, Ontario L1H 7K4, Canada
Email: yagmurgoren@iyte.edu.tr

Ali Khalvati*

Viona Consulting Inc.,
Agro-Environmental Federal Company,
Associated with Ontario Tech University,
Thornhill, L3Y5Y1, Toronto, Canada
Email: alikhavati@vionaconsulting.ca
*Corresponding author

Abstract: Soil micro-organisms like *arbuscular mycorrhizal* fungi can provide beneficial symbiosis to their host plant and have been adopted to recover metal-polluted soils. This study investigates the removal of heavy metals from soil using phytoremediation in the presence of fungi. The results indicate that the sunflower plant illustrates the highest copper accumulation, with 18.55 mg/kg. In contrast, sunflower and sorghum controls (non-microorganisms) showed weak capability to transfer copper through plant biomass with 0.91 and 0.97 mg/kg, respectively. Both plants showed that phytoremediation can be a promising approach to providing sustainable solutions for soil heavy metal contamination in the presence of fungi.

Keywords: phytoremediation; rare earth element recovery; living organic; sorghum; rhizosphere.

Reference to this paper should be made as follows: Budak, B., Onay, T.T., Goren, A.Y. and Khalvati, A. (2025) 'Experimental study for recovery of heavy metals from contaminated soil using *arbuscular mycorrhizal fungi*', *Int. J. Global Warming*, Vol. 35, No. 1, pp.1–15.

Biographical notes: Bulent Budak is an Assistant Professor Dr. at the Ege University. He received his Doctorate's in Agriculture Science from the Ege University, Faculty of Agriculture, İzmir, Turkey. He is interested in agricultural applications, biofertilisers, phytoremediation processes, soil contamination, and monitoring.

Turgut T. Onay is a Professor at the Boğaziçi University. He received his Doctorate's in Environmental Engineering from the University of Pittsburgh, Department of Environmental Engineering, USA. He is interested in waste to energy: biogas and renewable energy, environmental impact assessment, leachate treatment, and soil-groundwater remediation.

Aysegul Yagmur Goren is a research assistant at the Izmir Institute of Technology in Turkey. She received her Doctorate in Environmental Engineering from the Izmir Institute of Technology, Izmir, Turkey. Her current field placement is as a researcher at the Ontario Tech University in Canada. She is interested in electrochemical wastewater treatment, bio-electrochemical, bio-hydrogen production, and risk assessment.

Ali Khalvati is the Director of Research and Development at the Viona Consulting Inc. in Canada. He received his Doctorate's in Plant Science from the Technical University of Munich, Department of Plant Sciences, Germany. He is interested in agricultural plant science, soil science, irrigation and water management, and drought.

1 Introduction

Industrial wastewater contains a complex mixture of contaminants, which are harmful and cause significant risk to humans upon unsuitable disposal into soil. Massive industrial pollutants have leaked into the environment, and neighbouring inhabitants have used groundwater supplies throughout the last few decades. Few comprehensive studies have been done compared to organic pollutants; evaluations of the anthropogenic origins, environmental behaviour, and dangers to the human and ecological health associated with rare earth elements (REEs) are lacking (Gwenzi et al., 2018). The three primary heavy metals that pose the most severe risks to public health are cadmium, lead, and mercury (Cabrera-Rodríguez et al., 2018). Due to their specific features, REEs have been utilised in several industrial, medical, and agricultural applications. The production of high-tech electronics requires using scarce and expensive resources. It is crucial to improve the collection, treatment, and recycling of electronics at the end of their lifecycle to improve the environmental management of waste-water electrical and electronic equipment (WWEEE), including REEs, which support a circular economy and increase resource efficiency. Moreover, REE contamination could severely impact on human health and the

soil ecosystem due to their toxicity, non-biodegradability, and bioaccumulation in the food chain (Goren et al., 2022). These metals may also cause health problems such as cancer, anaemia, cardiovascular diseases, and damage to the nervous system (Tariq et al., 2024).

Developed to recover metals, traditional methods such as precipitation, filtration, extraction, ion exchange, and supercritical extraction is neither ecologically nor economically appealing, and these methods typically require considerable amounts of effort and time. An innovative biotechnological method for economically extracting REEs from aqueous solutions is biosorption. Biosorption, while environmentally friendly and cost-effective, faces scalability challenges compared to conventional methods like evaporation, ion exchange, chemical precipitation, and membrane filtration. It is slower, reliant on plant growth cycles, and requires large areas, making it less efficient for highly contaminated or space-limited sites. In contrast, conventional methods offer faster and more efficient metal recovery, making them more suitable for industrial-scale applications. Although biosorption is ideal for low to moderately contaminated sites due to its sustainability and minimal environmental impact, it is less scalable than these established techniques for rapid or large-scale recovery operations. Nevertheless, it has been suggested that various biomaterials, including resin, bacteria, fungus, algae, and activated carbon, might be used as adsorbents to recover costly metals like REEs. Therefore, there is a need to develop new and innovative technologies to recover metals using beneficial aspects of phytoremediation as a biological recovery technique; meanwhile, microorganisms can be a good source for metal recovery from polluted lands (Mikołajczak et al., 2017). The arbuscular mycorrhizal fungus (AMFs) are known to enhance plant nutrition, development, and survival since they better use the nutrients in the soil (Khalvati et al., 2005, 2010). More precisely, it has been demonstrated that heavy metal toxicity can have an impact on AMF; yet, heavy metal-contaminated areas frequently host AMF colonisation in mycotrophic plants (Khalvati and Dincer, 2013). Spore counts and in situ root colonisation estimates have been measured in several papers related to this. In areas contaminated with heavy metals, some people have reported metal-tolerant AMF (Nafady and Elgharably, 2018).

The diversity and tolerance of AMF in heavy metal (HM)-contaminated lands have drawn attention in recent years. Researchers are attempting to understand the mechanisms underlying AMF's adaptability and tolerance to HMs in the land, as this may help manage the soil microorganisms on these lands for phytoremediation and bioremediation programs. Some potential plants were investigated to find the ability for HM tolerance and a fast growth rate with significant biomass to test the potential material for phytoremediation that can live in extensively polluted fields with various HMs. The sunflower (*Helianthus annuus* L.), used for food, oil, and fuel bioenergy, grows in various regions worldwide (Forte and Mutiti, 2017). It can accumulate relatively large concentrations of Cd, Cu, Pb, Cr, Zn, and Ni. All of these methods, such as mycorrhizal remediation technology, have an impact on lowering a plant's exposure to potentially harmful substances. In contaminated soils with Cu and As, Gonzalez-Chavez et al. (2002) investigated the form and location of Cu accumulation in the extra-radical mycelium of three AMFs removed from the identical polluted area. More research is necessary to fully comprehend the role of AMF in plant tolerance and its ecological relevance in soils due to the nature of accumulation and the mechanisms at play. Moreover, most of the traditional REE removal methods have several limitations related to their cost and

harmful environmental impacts. Moreover, these procedures are usually time-consuming and labour-intensive. Therefore, there is a need to provide better use of technologies to recover REEs. One of the cost-efficient biological methods is phytoremediation, which can be considered an emerging and environmentally friendly technology and can also apply to the recovery of REEs. However, there is a condition to understand the microbial interactions controlling this process, especially between the plant and the sorption of the metals.

Furthermore, in this study, the hypothesis was aimed to investigate the absorption, translocation, efflux, and metabolites of selected HMs and REEs as inorganic xenobiotics in the plant-root-rhizosphere system, their influence on different species of AMF and its development and performance (in vitro study). The ecology of such a mycorrhizosphere network of plant-microbe-soil interactions must be defined not only to understand the functions of the rhizosphere but also to exploit the potential of rhizoremediation. Therefore, the results presented in this article emphasised the ecological network in a myco-bacterrhizosphere and described the variety of combinations of plants, microbes, and soil, especially the close collaboration between various AMF soil bacterial species and plant roots in the rhizosphere. Essential basic processes in the root zone and the role microorganism symbiosis plays under the influence of industrial pollutants with metals such as HMs and REEs were evaluated. The objectives of this paper were to investigate AMF's contribution to recovering REEs to evaluate the actual role of plant-microbe interaction to REEs uptake, sequestration, and partitioning in plant cell tissues in order to recover REEs from the soil irrigated by the wastewater. Therefore, the hypothesis is to test and highlight the ecological impact of essential processes of uptake and detoxifying REEs in the root zone through the microorganism symbiosis.

2 Materials and methods

In this section, the experimental setup for the growth of plants and phytoremediation experiments is explained in more detail. Moreover, the analytical measurement methods are described.

2.1 The experimental model setup

The test setup was designed with control groups that included sorghum and sunflower seeds without AMF and concentrations of 100, 500, and 1,000 ppm of Cu to determine the germination potential of sorghum and sunflower seeds containing AMF (*Glomus mosseae*), which are obtained from Ege University. We sourced sunflower and sorghum seeds from Turkish companies in Izmir and Edirne, and Petri dishes were cleaned and disinfected to be used in the germination test. The seeds were treated multiple times and washed with ethanol and pure water. The following protocol was used to sterilise the sunflower and sorghum seeds: seeds were first treated with a 70% ethanol solution for 1 min and then washed for three minutes with distilled water. Moreover, the AMF was added to the system by dissolving 30.002 g of glucose hydrate ($C_6H_{14}O_7$) in 100 mL of pure water. After that, the produced solution was supplemented with 1.8 g of AMF spores. Each Petri dish, except for the controls, received 5 mL of this combination to track how *Glomus mossaea* affected germination, and the AMF spore content in each petri dish was initially 100 mg.

Trap culture experiments (lab-scale) were set up in the Institute of Environmental Science's greenhouse with the rhizosphere. Shoots are removed at the crown, and roots are chopped into small fragments, mixed thoroughly with the associated soil, and 1:1 (v/v) with autoclaved coarse sand. Sand mixtures were used for the trap culture with well-known mycotrophic plants, (i.e., clover, maize, etc.) grown under controlled conditions in a greenhouse in İstanbul, Turkey. Stream sand was sanitised in a drying oven at 151°C for 20 hours. The *Glomus mosseae* and *Glomus intraradices* were grown in dark-green plastic pots that had a capacity of 0.6 L and a weight of 40 g. Initially, 500 g of sterilised stream sand, 100 mL of distilled water, and sorghum seeds were placed in 15 pots with a volume of 0.6 L for every species, and pots were maintained at a temperature of 21 °C and humidity of 20%. Furthermore, to keep the sorghum seeds and AMF spores functioning properly and to ensure that the plants received enough nutrients, the number of plants in each pot was cut in half by pruning the deficient plants. After 21 days, every plant was trimmed, and black plastic bags were placed over every pot to accelerate plant breakdown and start the AMF spore generation process (Figure 1).

Figure 1 Pots for the decomposition of plants and initiate the AMF spore production (see online version for colours)



2.2 The analytical procedures

The HM concentrations were measured using inductively coupled plasma optical emission spectroscopy (ICP-OES). This solution was applied to the plants during the pot experiments. Table 1 shows the concentrations of several rare metals obtained from a standard solution. For phytoremediation tests, 3.7 L plastic pots with a dark green tint were utilised. Two hyperaccumulator plants were used to identify the mechanisms of HMs via the phytoextraction pathway. Moreover, to assess the degree of mycorrhization in root samples stored at 4°C in a 70% ethanol solution before root staining. The following steps were used to complete the root mycorrhizal determination test utilising the staining process method: each root sample was meticulously wrapped in tulle and incubated for 4 h at 60°C in a water bath containing 10% KOH solution (w/v). The roots were thoroughly washed with a 1% HCl solution for 3 min, and an AxioCam MR5 camera (5-megapixel resolution) and an EC Plan-Neofluar 10x/0.3 objective were mounted on an Axio Observer.Z1 microscope to observe the surface morphology of the roots.

Table 1 Elements apply to the plants

<i>Elements</i>	<i>Min [mg/kg]</i>	<i>Max [mg/kg]</i>	<i>Average [mg/kg]</i>
La	18.95	24.08	21.28
Nd	116.9	136.7	129.2
Ce	16.2	17.6	17.02
Pd	67.5	79.5	73.9
Pt	16.0	77.5	44.2
Au	136.5	188.5	168.2
Mo	183.5	928.0	350.5
Co	162.5	198.5	173.4
Cd	7.0	7.5	7.2
Mn	279.0	294.0	288.7
Cr	979.5	1.04	1000.9
Ag	606.0	744.0	695.4
Fe	18.24	21.7	19.43
Al	14.15	16.2	15.09
Pb	22.25	24.8	23.23
Cu	151.1	204.7	166.7
Ni	4.52	5.19	4.93
Zn	7.92	11.11	9.31
Si	11.02	12.43	11.59

Glomalin was measured in the system to determine soil quality by plant-microbe interactions and association. The reason was that as much as elements were up-taking by plants, the glomalin, which was nominated as soil organic matter, increased in the soil. glomalin and total glomalin (T-GRSP) are operationally defined as Bradford-reactive soil proteins following extraction in mycorrhizal (Gianinazzi et al., 1979). GRSP was measured with a common method, which is developed by Tisserant et al. (1993). Moreover, soluble proteins were extracted from barley root. Photometric measurements of enzyme activities were accomplished with the model substrates 1-chloro-2,4 dinitrobenzene (CDNB), 1,2-dichloro-4-nitrobenzene (DCNB) and 1,2-dichloro-4-nitrobenzoyl-chloride (p-NBoC). The assays were conducted in 0.1 M KPP buffer, pH 6.4 (pH 7.5 in the case of DCNB) with 1 mM glutathione and 1 mM substrate concentration. Using Bradford's technique, the quantity of soluble protein in the samples was calculated.

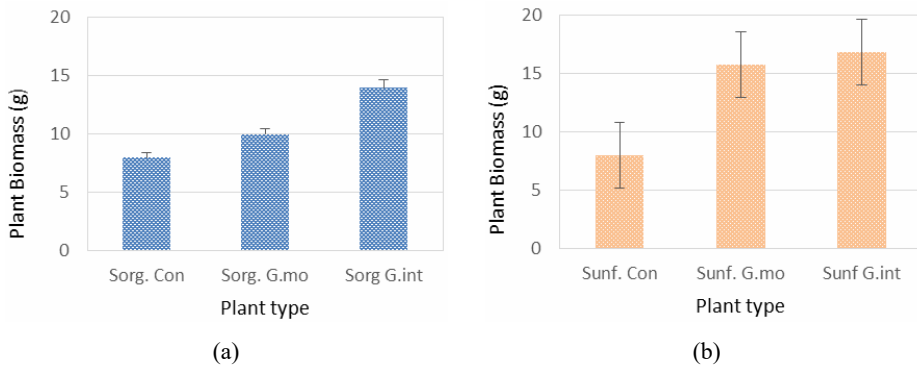
3 Results and discussion

The plant growth and biomass production results are evaluated, considering HM impacts on specified plant species in this section. In addition, the HM adsorption with the impacts of enzymes and glomalin are comprehensively discussed.

3.1 Plant growth and biomass production

Sunflower and sorghum plants were harvested for eight weeks after seed cultivation plantation of the seeds. During harvesting, roots and stems were collected separately. Fresh weights of the harvested sorghums and sunflowers within the first year after growing with the REEs are shown in Figures 2(a) and 2(b), respectively. The abbreviations in the figures are listed as follows: Sorg: Con: Sorghum control (no association), Sorg. G. moss: sorghum associated with *Glomus mossaeae*, Sorg. G. int: Sorghum associated with *Glomus intraradices*, Sun. Con: Sunflowers control (no association), Sun. G. mos: Sunflowers associated with *Glomus mossaeae*, Sun. G. int: Sunflowers associated with *Glomus intraradices*.

Figure 2 Fresh weight of the plants for (a) sorghum, (b) sunflower (see online version for colours)



3.2 Accumulation of metals from WEEE in plants

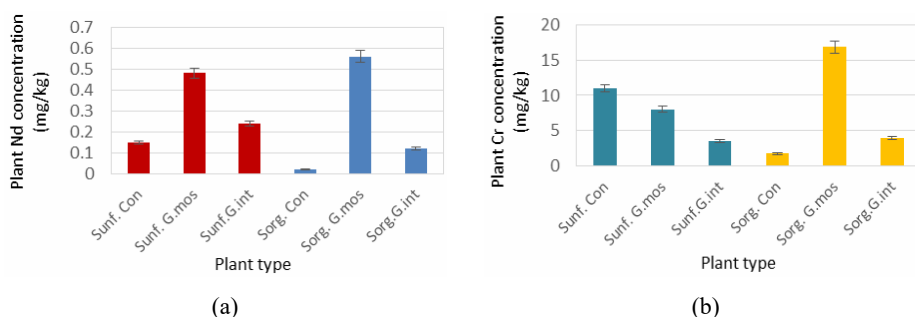
The artificial solutions, including REEs and HMs, were implemented three weeks after germination of sunflower and sorghum plants with 50 mL volume in total by three times. Plants were harvested after eight weeks. REEs and HMs uptake of the plants differs due to various factors. These factors can be sorted as:

- 1 the effect of the elements on the system
- 2 the capacity of the plants to immobilise and absorb the related element
- 3 AMF's ability to make symbiosis with the plant in polluted lands caused by WEEE.

Since the experimental set-up was the same for each sample, factors such as soil type, temperature, and moisture could not influence this analysis. As seen in Figures 3–5, REEs and HMs uptake of the plants differs due to various factors. Since the experimental set-up was the same for each sample, factors such as soil type, temperature, and moisture could not influence this analysis during the experimental period (three months and ten days in the first year). In the second year of the experiment (2021), data indicates a higher accumulation rate of Neodymium (Nd) in both plants associated with two mycorrhizal fungi (*G. intraradices* and *G. mosseae*) in comparison to control plants (non-microorganisms) [Figure 3(a)]. However, sorghum and sunflower plants performed

better Nd absorption to the roots and shoots when grown by *G. mosseae*, where sorghum was significantly better than sunflower. Both plants associated with *G. intraradices* also showed a significant accumulation of Nd compared to control plants (non-microorganisms). Interaction effects between plants and microorganisms (mycorrhizal) show that Chromium (Cr) was accumulated in both plants associated with two mycorrhizal fungi (*G. intraradices* and *G. mosseae*) in comparison to control plants (non-microorganisms) [Figure 3(b)]. Higher Cr absorption was achieved by sorghum and sunflower plant roots and shoots when grown with *G. mosseae*. Similar to Nd scenario, sorghum was significantly better than sunflower. Both plants associated with *G. intraradices* also showed a significant accumulation of Cr compared to control plants (non-microorganisms).

Figure 3 HM content in the shoot of both sunflower and sorghum plants associated with or without microorganisms for (a) neodymium, (b) chromium metals (see online version for colours)



Our findings in the second year of the experiment indicated the meaningful difference between microbial-associated and non-symbiosis plants regarding Cerium (Ce) absorption from the soil. In this respect, sorghum and sunflowers revealed significant accumulation of Ce in their shoot and root cells [Figure 4(a)]. Both microorganism species (*G. intraradices* and *G. mosseae*) performed higher amounts of Ce in sorghum with the presence of *G. intraradices* and in sunflowers when *G. mosseae* inoculates in the rhizosphere by 4.43 and 2.01 mg/kg respectively in total shoot dry weight. These values are the average of data obtained during 3 months of experiment for two years. Moreover, one of the most exciting phenomena in our experiment was the interaction between plant-microbes and copper (Cu) accumulation. Both microorganisms have shown tremendous impact on the Cu absorption and accumulation in the sorghum and sunflower plants. *G. mosseae* accomplished better Cu translocation in the shoot than *G. intraradices* in those plants. However, the highest Cu accumulation illustrated by the sunflower plant by 18.55 mg/kg, whereas sunflower and sorghum controls (non-microorganisms) showed very weak capability to transfer Cu through plant biomass (0.91 and 0.97 mg/kg, respectively) [Figure 4(b)].

Both plants revealed significant performance in terms of degradation of the lead (Pb) from the soil contaminated with different REEs from WWWW. Surprisingly, translocation of Pb in plants dry matter appeared in one of the microorganism's species (*G. mosseae*) compared to the other (*G. intraradices*). Plants without being the host of those two microorganisms were shown very slight, negligible Pb values in their shoot dry matter [Figure 5(a)]. Instead, varieties of the value of zinc element (Zn) absorption were

shown in both plants (sorghum and sunflowers). In this test, *G. mosseae* was also able to achieve better uptake of Zn for both plants compared to *G. intraradices* and non-microorganisms. Our experiment showed that the highest values for element uptake were indicated in Zn far beyond other REEs for both plants in association with two different microorganisms (Figure 5). Nickel (Ni) uptake was also analysed in our experiment, where sorghum and sunflower plants demonstrated fantastic performance in terms of accumulation of Ni in dry matters. Both microorganisms showed a significant impact on boosting Ni to sunflowers shoot by 20.00 and 19.74 mg/kg in *G. intraradices* and *G. mosseae*, respectively. Non-microorganisms showed the lowest Ni uptake from the soil in sorghum and sunflowers.

Figure 4 HM content in the shoot of both sunflower and sorghum plants associated with or without microorganisms for (a) cerium, (b) copper metals (see online version for colours)

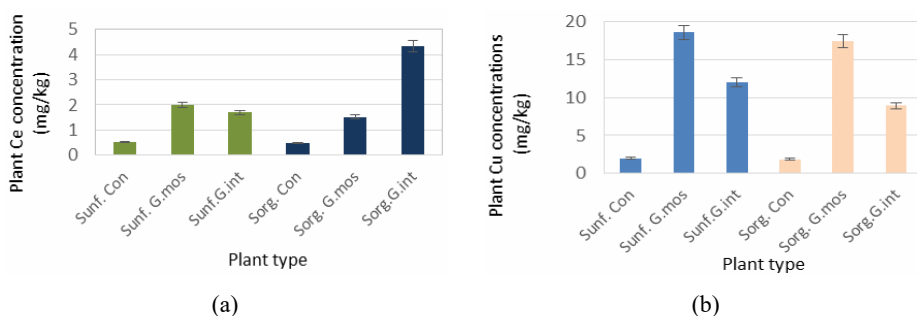
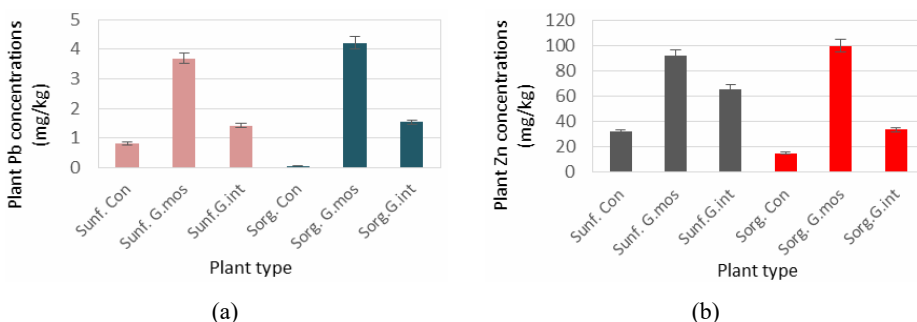


Figure 5 HM content in the shoot of both sunflower and sorghum plants associated with or without microorganisms for (a) lead and (b) zinc metals (see online version for colours)



These results proved that the AMF exhibits some degree of selectivity in their interaction with different metal ions, but this selectivity is not absolute and depends on various factors such as fungal species, metal type, concentration, and environmental conditions. AMF generally shows a higher affinity for essential micronutrient metals like zinc (Zn) and copper (Cu), as these metals are involved in plant and fungal metabolic processes, including enzyme function and structural stability. In contrast, toxic metals like cadmium (Cd) and lead (Pb) are less favorably absorbed but can still be sequestered by AMF as a defense mechanism to protect the fungus and the plant from toxicity. The selectivity can

also be influenced by soil chemistry, including pH and the presence of other competing ions, which affect metal bioavailability and uptake efficiency. While AMF may exhibit some preference for biologically essential metals, their ability to form complexes with a broad range of metals enables them to play a versatile role in metal homeostasis and detoxification in contaminated soils.

Overall, AMF play a crucial role in plant-microbe-metal interactions, particularly in soil contaminated with HMs. These fungi can absorb and sequester metals within their hyphae and vesicles, primarily through complexation with fungal proteins, organic acids, and polyphosphates. Once absorbed, metals may be compartmentalised in vacuoles or bound to fungal cell walls, reducing their mobility and toxicity to the plant. This metal sequestration is generally considered a protective mechanism for both the fungus and the host plant, allowing AMF to continue their symbiotic functions such as nutrient uptake (especially phosphorus) and plant growth promotion. However, metal sequestration is not entirely irreversible. Under certain environmental conditions, such as changes in soil pH, redox potential, or the presence of chelating agents, the fungi may release the absorbed metals back into the soil. This release is often slow, depending on the metal type, fungal species, and soil chemistry. The retention of metals within the fungal tissues can last for the entire life cycle of the AMF, but once the fungi die and decompose, the stored metals are gradually returned to the soil. Therefore, while AMF can temporarily immobilise metals, they do not provide a permanent solution for soil metal contamination, and HM-raised edibles cannot be consumed by humans because of their toxicity. Instead, they contribute to the stabilisation and reduction of bioavailable metal fractions in soil, with potential for bioremediation applications, and they can be utilised as biomass for bioenergy production.

3.3 *Enzymatic assay*

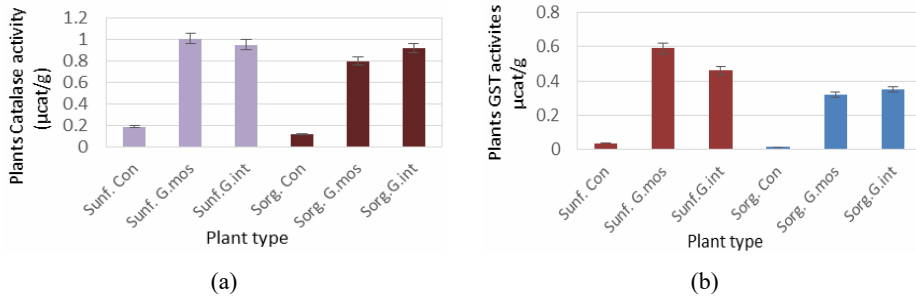
Both plant detoxification and stress enzymatic reactions were investigated through integration with two different microorganisms in this experiment. Catalase and GST activities were monitored to determine plant-microbes interaction capabilities to impose resistance and conjugate HMs negative impacts on plant growth and promotion. Phyto-enzymes were measured at the end of eight weeks of treatments. Therefore, two catalase and GST activities were compared in sorghum and sunflowers based on their association with or without microorganisms. Catalase and GST enzymes showed significant accumulation in the roots of both plants' microbes' networks. As Figure 6 indicates, plants inoculated with *G.mosseae* enhanced detoxification enzymes in exposure to REEs solution up to 20 times more than non-mycorrhizal plants.

3.4 *Glomalin survey*

Glomalin is a specific protein (or group of proteins), so there is an issue of terminological confusion. It is well known that the adverse effects of mining on natural ecosystems are mainly due to the discharge of HMs, which are toxic to higher organisms and microbes (Belford, 2004). This toxicity can cause losses in the diversity and functionality of native plant species, leading to extensive damage to local vegetation, a strong change in soil characteristics, and limiting vegetation establishment (González and Ite, 1992). However, exposure to HMs may cause the selection and development of tolerant populations, both plants and soil microorganisms, which could be useful in the restoration/remediation of

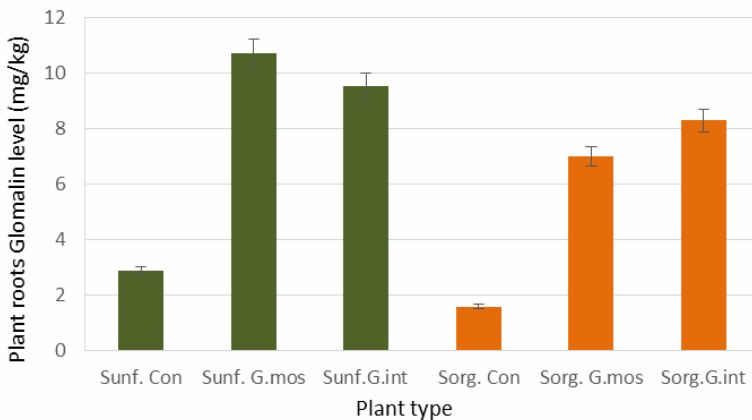
ecosystems affected by this pollution (Adriano, 2001). In our experiment, we planned to discover the soil protein that could be released by AMF in terms of Cu sequestration activity.

Figure 6 The activities of (a) catalase, (b) GST in the roots of sorghum and sunflowers associated with mycorrhizal and non-mycorrhizal under conditions of REEs (see online version for colours)



Moreover, Figure 7 displays glomalin accumulations in the sorghum roots and sunflowers with two mycorrhizal fungus associations under metal conditions. Glomalin appears when the rhizosphere sequesters metals; therefore, increasing glomalin amounts reveals the resistance attitude of plants in symbiosis with AMF.

Figure 7 Glomalin accumulation in the plants (sorghum and sunflower) associated with microorganisms (see online version for colours)



3.5 The High-performance mycorrhizal species propagules

In comparing various technologies for spore production, such as pots cultures as we used in last year's experiment. The new brand bioreactor, named airlift mycorrhizal bioreactor, appears to be a simple and efficient solution for laboratory-scale production levels. The fungal material is free of impurities and is simple to collect. More than ten times as many spores were produced in this straightforward technique as were seen in the pot cultures utilising liquid media that were the subject of this investigation. However, even modest

systems are insufficient for industrial-scale manufacturing to fulfil global market demand at competitive pricing. The work's outcomes demonstrated that this co-culture technique could also be used in submerged culture settings with a standard airlift bioreactor setup, resulting in a sizable output of fungal propagules. This is the first time a second-generation mycorrhizal propagule manufacturing procedure has been disclosed. The airlift bioreactor provides significant scale-up potential, which may one day be considered for large-scale industrial production. However, there is still more to be done to address the cultural circumstances.

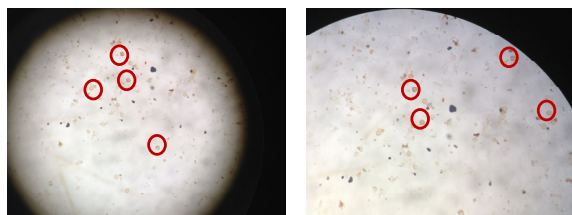
All harvested spores in airlift bioreactors and pots had germination rates of 60% and 28%, respectively, and were of the same quality. The notable distinction in output levels between these two culture systems, however, may be attributed to the differences in their culture circumstances, which were primarily characterised by variations in hydrodynamic conditions, root-to-root contact level, and gas phase renewal rate. Since fungi often thrive in static environments like soil, the airlift system's constant liquid movement may have inhibited the growth of the fungus. Additionally, under conditions of high inoculums cultivation, the roots could have generated excessive exudates, which are thought to negatively impact spore development (Maitra et al., 2021). Similarities between aseptic culture conditions and the symbionts' natural niche, such as soil water content, may also be important parameters. We used the airlift bioreactor to propagate the most effective mycorrhizal fungi associated with host plants in the contribution of REE uptake over the two-year investigation. Our data analysis and final observation led us to decide that *G. mosseae* has demonstrated a better impact on plants, sorghum, and sunflowers than *G. intraradices*. Therefore, using the new brand bioreactor enables us to provide the mass production of this selected mycorrhizal fungus professionally. The bioreactor was purchased from Canada to produce mycorrhizal fungus purely and rapidly.

3.6 Out-put test of microorganism provided by the bioreactor

Two microorganisms (*G. mosseae* and *G. intraradices*) had been previously inoculated at the beginning of experiment in order to test their impact on elements uptake and investigate these microorganisms' accumulation. Eventually, one microorganism (*G. mosseae*) was selected among two microorganisms based on better performance on uptake and translocating elements in both plants shoot and dry matters. Therefore, *G. mosseae* was extracted from the pots in the new brand bioreactor. As the bioreactor initially needs to be optimised and adjust to the growth medium as well as lab condition. Therefore, several weeks were spent to establish and optimise bioreactor in the lab.

One month after the initial optimisation, we successfully achieved the first pure *G. mosseae* sample (Figure 8). The samples were scientifically and professionally checked by Bashan Institute of Sciences (BIS), Alabama, USA.

Figure 8 Spores of *G. mosseae* produced by airlift bioreactor (see online version for colours)



Overall, plants showed reduced germination and growing phase when exposed to high concentrations of rare elements. Both plants are negatively affected by irrigation with high REE levels. This is in agreement with several studies (Coccina et al., 2019; Neidhardt, 2021). Uptake of rare elements was enhanced and translocated in different parts of plants in association with AMF. Moreover, several types of research have been done based on improving plants' resistance and strength to rare metals by using AMF species. Our results are similar to those of previous studies (Maitra et al., 2021; Yeghicheyan et al., 2019). Plant-microbe interaction application for phytoremediation is a long-term remediation effort, and it requires screening of many suitable plants and beneficial specific microbes, such as mycorrhizal fungi, to reduce metal concentrations to acceptable levels. Sunflower plants (*Helianthus annuus*) proved to have 35%–58% better absorbance in the shoots for HMs such as lead (Pb), zinc (Zn), copper (Cu), and nickel (Ni), and *Glomus mosseae* showed better potential to be more operative with mentioned contaminants' presence. On the other hand, Sorghum bicolor performed better in translocating REEs to plant shoots. *Glomus intraradices* for sorghum plants promoted Neodymium (Nd), and Cerium (Ce) absorbance more than *Glomus mosseae*. The interaction of Sorghum bicolor with *Glomus intraradices* also enabled the system for better immobilisation of concerned pollutants via rhizostabilisation. Plants rhizosphere was involved in accumulating each REEs individually between 15%–45% higher than non-microorganism plants in the condition REEs concentrations. Initial findings conclude that further research on REEs' phytoextraction and rhizostabilisation in order to reduce their toxicity and bioavailability in the soil is needed; while symbiosis between host plant here Sorghum bicolor and AMF *Glomus intraradices* is more effective than symbiosis of *H. annuus* species and AMF species *Glomus mosseae*.

4 Conclusions

This study evaluates the potential of the sunflower and sorghum plants in the presence of AMF for treatment of HM-contaminated soil using phytoremediation method. Results revealed that the hyper-accumulator plants and microbe interactions reduce metals and accumulate in the shoots of the plants. For instance, both microorganisms showed a significant impact on boosting nickel to sunflowers shoot by 20.00 and 19.74 mg/kg in *G. intraradices* and *G. mosseae*, respectively. Furthermore, sunflower plants proved to have 35%–58% absorbance in the shoots for HMs. On the other hand, sorghum and sunflower plants performed better Nd absorption to the roots and shoots when grown by *G. mosseae*, where sorghum was significantly better than sunflower. Overall, this study indicates that beneficial soil-born microbes such as AMF can enhance HM mobility and recovery when soil is highly contaminated with immobilised hazardous elements leached from WEEE.

References

- Adriano, D.C. (2001) 'Bioavailability of trace metals', in *Trace Elements in Terrestrial Environments*, Springer, New York, NY.
- Belford, E. (2004) *Purification and Characterization of Xenobiotic Detoxification Enzymes in Pachyrhizus yam Bean and their Role in Agrochemical Metabolism*, PhD thesis, Technische Universität München.
- Cabrera-Rodríguez, R., Luzardo, O.P., González-Antuña, A., Boada, L.D., Almeida-González, M., Camacho, M., Zumbado, M., Acosta-Dacal, A.C., Rial-Berriel, C. and Henríquez-Hernández, L.A. (2018) 'Occurrence of 44 elements in human cord blood and their association with growth indicators in newborns', *Environ. Int.*, Vol. 116, pp.43–51.
- Coccina, A., Cavagnaro, T.R., Pellegrino, E., Ercoli, L., McLaughlin, M.J. and Watts-Williams, S.J. (2019) 'The mycorrhizal pathway of zinc uptake contributes to zinc accumulation in barley and wheat grain', *BMC Plant Biol.*, Vol. 19, No. 133, pp.1–14.
- Forte, J. and Mutiti, S. (2017) 'Phytoremediation potential of *helianthus annuus* and *hydrangea paniculata* in copper and lead-contaminated soil', *Water, Air, Soil Pollut.*, Vol. 228, No. 77, pp.1–11.
- Gianinazzi, S., Gianinazzi-Pearson, V. and Dexheimer, J. (1979) 'Enzymatic studies on the metabolism of vesicular-arbuscular mycorrhiza. III. Ultrastructural localization of acid and alkaline phosphatase in onion roots infected by *Glomus mosseae* (Nicol. & Gerd.)', *New Phytol.*, Vol. 82, No. 1, pp.127–132.
- González, S. and Ite, R. (1992) 'Acumulación metálica en suelos del área bajo influencia de las chimeneas industriales de Ventanas (Provincia de Valparaíso, V Región)', *Agric. Técnica*. Vol. 50, pp.214–219.
- Gonzalez-Chavez, C., D'haen, J., Vangronsveld, J. and Dodd, J.C. (2002) 'Copper sorption and accumulation by the extraradical mycelium of different *Glomus* spp. (*arbuscular mycorrhizal* fungi) isolated from the same polluted soil', *Plant Soil.*, March, Vol. 240, pp.287–297.
- Goren, A.Y., Genisoglu, M., Kazanci, Y. and Sofuoglu, S.C. (2022) 'Countrywide spatial variation of potentially toxic element contamination in soils of turkey and assessment of population health risks for nondietary ingestion', *ACS Omega*, Vol. 7, No. 41, pp.36457–36467.
- Gwenzi, W., Mangori, L., Danha, C., Chaukura, N., Dunjana, N. and Sanganyado, E. (2018) 'Sources, behaviour, and environmental and human health risks of high-technology rare earth elements as emerging contaminants', *Sci. Total Environ.*, 15 September, Vol. 636, pp.299–313.
- Khalvati, M. and Dincer, I. (2013) 'Environmental impact of soil microorganisms on global change', in Dincer, I., Colpan, C. and Kadioglu, F. (Eds.): *Causes, Impacts and Solutions to Global Warming*, Springer, New York, NY.
- Khalvati, M., Bartha, B., Dupigny, A. and Schröder, P. (2010) 'Arbuscular mycorrhizal association is beneficial for growth and detoxification of xenobiotics of barley under drought stress', *J. Soils Sediments.*, January, Vol. 10, pp.54–64.
- Khalvati, M.A., Hu, Y., Mozafar, A. and Schmidhalter, U. (2005) 'Quantification of water uptake by *arbuscular mycorrhizal* hyphae and its significance for leaf growth, water relations, and gas exchange of barley subjected to drought stress', *Plant Biol.*, Vol. 7, No. 6, pp.706–712.
- Maitra, P., Al-Rashid, J., Barman, N.C., Khan, M.N.M., Mandal, D., Rasul, N.M., Chowdhury, A., El-Sappah, A.H. and Li, J. (2021) 'Sand particle size and phosphorus amount affect *Rhizophagus irregularis* spore production using in vitro propagated spore as a starter inoculum in rhizosphere of maize (*Zea mays*) plantlets', *J. Fungi.*, Vol. 7, No. 10, p.846.
- Mikołajczak, P., Borowiak, K. and Niedzielski, P. (2017) 'Phytoextraction of rare earth elements in herbaceous plant species growing close to roads', *Environ. Sci. Pollut. Res.*, June, Vol. 24, pp.14091–14103.
- Nafady, N.A. and Elgharably, A. (2018) 'Mycorrhizal symbiosis and phosphorus fertilization effects on *Zea mays* growth and heavy metals uptake', *Int. J. Phytoremediation.*, Vol. 20, No. 9, pp.869–875.

- Neidhardt, H. (2021) 'Arbuscular mycorrhizal fungi alleviate negative effects of arsenic-induced stress on crop plants: a meta-analysis', *Plants, People, Planet.*, Vol. 3, No. 5, pp.523–535.
- Tariq, M., Iqbal, B., Khan, I., Khan, A.R., Jho, E.H., Salam, A., Zhou, H., Zhao, X., Li, G., Du, D. (2024) 'Microplastic contamination in the agricultural soil – mitigation strategies, heavy metals contamination, and impact on human health: a review', *Plant Cell Rep.*, Vol. 43, p.65.
- Tisserant, B., Gianinazzi-Pearson, V., Gianinazzi, S. and Gollotte, A. (1993) 'In planta histochemical staining of fungal alkaline phosphatase activity for analysis of efficient arbuscular mycorrhizal infections', *Mycol. Res.*, Vol. 97, No. 2, pp.245–250.
- Yeghicheyan, D., Aubert, D., Bouhnik-le Coz, M., Chmeleff, J., Delpoux, S., Djouaev, I., Granier, G., Lacan, F., Piro, J. and Rousseau, T. (2019) 'A new interlaboratory characterisation of silicon, rare earth elements and twenty-two other trace element concentrations in the natural river water certified reference material SLRS-6 (NRC-CNRC)', *Geostand. Geoanalytical Res.*, Vol. 43, No. 3, pp.475–496.