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Arbuscular Mycorrhizal Fungi and Microbial Activity in Mine Tailings: Potential to Promote Plant Growth Under Stress Conditions
Hongos Micorrízicos Arbusculares y Actividad Microbiana en Jales Mineros: Potencial para Promover el Crecimiento de Plantas Bajo Condiciones de Estrés

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SUMMARY

Mining facilities release considerable amounts of toxic elements into the environment, which persist for long periods of time, their remediation is necessary for human health. Arbuscular mycorrhizal symbiosis improves plant resistance to biotic and abiotic stress, and aids in the adsorption of nutrients under heavy metal stress. The present study aims to determine the mycotrophic plants established in mine tailings, their relationship with microbial activity, and with the physicochemical characteristics of mine tailings. A physical, chemical, and plant characterization of the mine tailings was carried out, and the percentage of mycorrhizal colonization, microbial activity, and concentrations of lead, cadmium, nickel, and zinc were evaluated. The results showed that the mine tailings had neutral pH, very low organic matter content, medium nitrogen content, high phosphorus and potassium content, low calcium availability, and medium magnesium availability. Eleven species were identified from nine families, four species were shrubs, three species were trees, three species were herbaceous, and one species was cactus; 10 of them are perennial. Colonization percentages ranged from 11 to 91%, and microbial activity values ranged from 6 to 42 µg p-nitrophenol g^{-1} dry soil. Concentrations of 10.63 mg kg^{-1} for lead, 0.58 mg kg^{-1} for cadmium, 0.20 mg kg^{-1} for nickel, and 36.21 mg kg^{-1} for zinc were found. The data obtained showed that the plants were colonized by arbuscular mycorrhizal fungi (AMF) and the rhizosphere zone had a wide range of microbial activity that could allow them to tolerate the stress conditions present in mine tailings.

Index words: *microorganisms, mines, plant cover, symbiosis, toxic elements.*

RESUMEN

Los centros de extracción minera liberan al medio ambiente cantidades considerables de elementos tóxicos, que persisten durante largos periodos de tiempo, por lo que su remediación es necesaria para la salud humana. La simbiosis micorrízica arbuscular mejora la resistencia de las plantas al estrés biótico y abiótico, y ayuda a la adsorción de nutrientes bajo estrés por metales pesados. El presente estudio pretende determinar las plantas micotróficas establecidas en los jales de mina, su relación con la actividad microbiana y con las características fisicoquímicas de jales. Se realizó una caracterización física, química y vegetal de los jales de mina y se evaluaron el porcentaje de colonización micorrícica, la actividad microbiana y las concentraciones de plomo, cadmio, níquel y zinc. Los resultados mostraron que los jales presentaban un pH neutro, muy baja de materia orgánica, un contenido medio de nitrógeno, un alto contenido de fósforo y potasio, una baja disponibilidad de calcio y una disponibilidad media de magnesio. Se identificaron once especies, de nueve

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familias, cuatro especies eran arbustos, tres especies eran árboles, tres especies eran herbáceas y una especie era un cactus; 10 de ellas son perennes. Los porcentajes de colonización oscilaron entre el 11 y el 91%, y los valores de actividad microbiana entre 6 y 42 µg de p-nitrofenol g^{-1} de suelo seco. Se encontraron concentraciones de plomo de 10.63 mg kg^{-1}, de cadmio de 0.58 mg kg^{-1}, de níquel de 0.20 mg kg^{-1} y zinc de 36.21 mg kg^{-1}. Los datos obtenidos mostraron que las plantas estaban colonizadas por hongos micorrízicos arbusculares (HMA) y que la zona de la rizosfera presentaba diversos niveles de actividad microbiana, condiciones que podría permitir una mayor tolerancia a situaciones de estrés presente en los jales.

Palabras clave: *microorganismos, minas, cobertura vegetal, simbiosis, elementos tóxicos.*

INTRODUCTION

Mine tailings, or mine waste, are solid wastes that arise as a result of mineral processing, constituting remnants of crushed rocks, water and chemicals used in the extraction and mineral beneficiation processes. This accumulation results in the formation of conglomerates that can spread over large areas of the ground. These wastes not only negatively impact plant growth due to contamination and physical and microbiological alterations in the area but also present significant health risks to surrounding communities. This is due to water, soil, and air pollution, as well as exposure to toxic substances, as evidenced by recent research (Worlanyo and Jiangfeng, 2021).

The application of vegetative cover, or ground cover, is an alternative method used to mitigate the effects of particle dispersion and runoff in tailings (Sun *et al.*, 2018). However, implementing vegetative cover on mine tailings is not a straightforward task. It is necessary to condition these materials using, for example, organic matter such as vermicompost, to improve the physical, chemical, and biological properties present in the tailings. This process aims to optimize the conditions for the growth and development of the plants that will be established to cover the surface of the tailings (Bautista-Gabriel *et al*., 2016). The presence of microorganisms is a crucial factor in the successful establishment of plants, as they develop new mechanisms to adapt to the extreme physical and chemical properties prevalent in mine tailings. Through beneficial interactions, these microorganisms facilitate plant survival (Thavamani *et al*., 2017). For example, acid drainage is enhanced (Ledin and Pedersen, 1996). Determining microbial activity in mine tailings enables the identification of beneficial microorganisms that contribute to plant establishment. Within this microbiota, arbuscular mycorrhizae stand out as a crucial element, as they enable plants to resist and develop under conditions of water stress, nutritional deficiencies, and climatic variations. Additionally, it plays a fundamental role in the immobilization of potentially toxic elements, thus contributing to site recovery (Tamayo, Zapata, and Osorio, 2016).

For this reason, the following hypothesis was put forward: even in adverse conditions and contaminated by heavy metals from the tailings, there are plant species and microorganisms that manage to establish themselves in these seemingly inert places, and that can help establish vegetation. Therefore, the objective of this study is to determine potentially efficient plants established by ecological succession as vegetal cover in mine tailings, and to evaluate their ability to associate with mycorrhizal fungi and host microorganisms involved in the biogeochemical processes of these tailings, through the quantification of microbial activity, colonization percentage and the concentrations of lead, cadmium, nickel and zinc.

MATERIALS AND METHODS

Location and Description of the Experimental Site

The study was carried out in a mine tailing located in the mountain range of the metallogenic region in the Mexican Neovolcanic Belt, where historically the primary extraction was silver in colonial times. The geographical coordinates of the site are 20° 06′ 00″ N, 98° 43′ 00″ W, and 20° 06′ 06.64 N, 98° 42′ 00.53 W. The average rainfall varies from 400 to 900 mm, the climate is semi-dry temperate, and winds range from 65 to 75 kph (INEGI, 2010).

Three sampling points were identified within the mine tailings, sites in which a greater vegetation cover was observed, each point had an average area of 20 m^2. The complete plant (root, stem, leaves and inflorescence, when were present) and rhizosphere mine tailings were collected. The distance between sampling points was 1 km (Figure 1). The botanical identification of the plants was conducted at the Biology Laboratory of the Division of Forest Sciences at Chapingo Autonomous University.

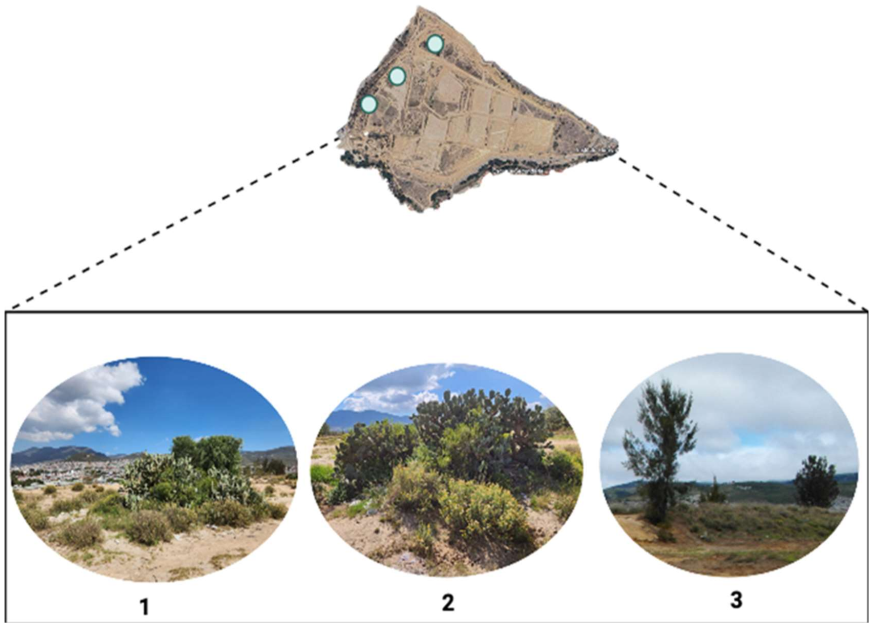

Figure 1. Location of mine tailings, where the presence of arbuscular Mycorrhizal and microbial activity in plants was evaluated displaying the three sampling sites.

For microbiological analysis, the rhizosphere samples and roots were transported to the laboratory, keeping them refrigerated with ice in a cooler. Subsequently, a composite sample of mine tailings from each plant was prepared and kept frozen in Ziploc bags. The roots were washed, cut into 1 cm fragments, and preserved in FAA (formaldehyde-Aceto-Alcohol solution).

Sampling and Physical and Chemical Analysis of Mine Tailings

Random sampling was used, since all sampled units may have the same probability of being sampled. Mine tailings were sampled in three vegetation islands (Figure 1), which were considered as sampling units. In each island, 11 subsamples were collected using the zigzag method, at a depth of 5 to 10 cm, covering the entire surface. These subsamples were mixed using the quartering method until a composite sample of approximately one kilogram was obtained, following the indications of NOM-021-RECNAT-2000 (SEMARNAT, 2002). It is important to clarify that an experimental design was not implemented at the site, since the objective of the study was to characterize the physicochemical and microbiological properties of the mine tailings present in the vegetation islands.

The composite samples obtained were analyzed according to the procedures established in NOM-021-RECNAT-2000 to determine the bulk density, pH, percentage of organic matter, concentrations of inorganic nitrogen, phosphorus, potassium, calcium, magnesium, and potentially toxic elements.

The physicochemical analysis of mine tailings was conducted to characterize the environment in which mycotrophic plants and microorganisms are established. These data are fundamental to understand the specific conditions that determine the survival and adaptability of plant and microbial species in this extreme environment.

Determination of the Percentage of Mycorrhizal Colonization and Microbial Activity in the Rhizosphere of Plants Growing in the Mine Tailings

The percentage of mycorrhizal colonization in plant roots was determined following the Phillips, and Hayman (1970) thinning and staining technique. As a first step, considering that the roots of the collected plants were highly pigmented, the staining technique was modified and changes of 10% potassium hydroxide were made every 72 hours, for 30 days only at room temperature. After that period, the roots were washed with distilled water to remove the potassium hydroxide, and 10% hydrogen peroxide was added for three minutes to clarify the roots. Then, it was rinsed with distilled water and covered with 10% hydrochloric acid for 5 minutes. After this time, the acid was removed, eliminated and without rinsing, 0.05% trypan blue was added for 24 hours to achieve its staining. Finally, the excess of colorant was eliminated. Roots colonized with AMF were observed for 100 root pieces using a light microscope Leica with a magnification of 40x. The percentage of mycorrhizal colonization was calculated based on the following formula by Giovanetti and Mosse (1980):

Percentage root colonized = (Number root colonized/Number root observed) ×100 (1)

Alkaline Phosphate Activity

Microbial activity in the mine samples was quantified using p-nitrophenyl phosphate as a substrate, following the alkaline phosphatase activity test proposed by Tabatabai and Bremner (1969). The quantification was based on the absorbance measured at 410 nm. For this purpose, a Mub stock solution was used, which was adjusted to pH 11 with 0.1 M NaOH. It was then calibrated to 1000 mL and labeled as "working solution". Subsequently, 0.928 g of p-nitrophenyl phosphate was weighed and dissolved in 100 mL of the "working solution", which was labeled as "MuB substrate solution". After being kept dry and frozen, the rhizospheric samples from mine tailings were removed from the vial and pulverized. Subsequently, 0.25 g of the pulverized samples was transferred into a clean tube. To this tube, 50 µL of toluene, 1 mL of MuB working solution, and 1 mL of MuB substrate solution were added. The mixture was then incubated for 1 hour at 37 °C in a water bath. After the incubation period, 250 µL of 0.5 M $CaCl_2$ and 1 mL of 0.1 M NaOH were added to the tube. The resulting solution was filtered using a 0.20 µm membrane, and the absorbance was measured at 410 nm using a spectrophotometer.

It is crucial to note that a calibration curve was constructed using p-nitrophenyl. A regression model was employed to ascertain the concentration of phosphatase activity in micrograms of p-nitrophenyl equivalents per gram of dry soil. Each sample from the mine tailings underwent triplicate analysis to ensure the accuracy of the results.

Statistical Analysis

The data did not satisfy the assumption of normality. The non-parametric Kruskal-Wallis test, followed by multiple comparisons with Bonferroni-Dunn correction, was used to determine whether there were significant differences among plants with respect to the percentage of mycorrhizal colonization and alkaline phosphate activity. Differences were considered significant at $P < 0.05$. Data analysis was performed with R statistical software, version 4.3.2 (R Core Team, 2023). Graphs were generated using the 'ggplot2' and 'ggpubr' packages.

RESULTS AND DISCUSSION

Identification of Mycotrophic Plants

Eleven species were identified from nine families: four were shrubs, three trees, three herbaceous, and one cactus. Ten of these species were perennial, meaning they maintain their foliage or structures throughout the year, while only one was annual, having a limited life cycle. These species were grouped as vegetation islands on mine tailings (López and Juambelz, 2015; CONABIO, 2016a; Noriega-Luna *et al*., 2017). Of the eleven species, *Isocoma* sp., *Flourensia cernua*, *Eucalyptus camaldulensis*, *Foeniculum vulgare,* and *Casuarina equisetifolia* were not found in scientific articles as plants that establish in mine tailings. However, for the remaining species, there are reports of their presence in these tailings (Hernández-Acosta, Mondragón, Cristóbal, Rubiños, and Robledo, 2009; Benitez, Sole, and Cisteró, 2010[1]; Elizondo, Márquez, Marín, and Gutiérrez, 2016). All selected plants belong to genera that, according to previous studies, have demonstrated the ability to phytoremediate, phytostabilize, tolerate heavy metals, or present antipathogenic properties (Table 1).

Physical and Chemical Characteristics of The Mine Tailings

The mine tailings had average values for the following variables: bulk density of 1.21 g cm^{-3}, which is typical of loam soils. They had a neutral pH (6.8), a very low concentration of organic matter (0.29%), a low concentration of inorganic N (20 mg kg^{-1}), and high concentrations of P (11.94 mg kg^{-1}) and Ca (3599 mg kg^{-1}). The average K (189 mg kg^{-1}) and Mg (196 mg kg^{-1}) were noted. According to national and international standards for soils, the Maximum Permissible Limits (MPL) for extractable Pb (7.95 mg kg^{-1}), Cd (0.65 mg kg^{-1}), Ni (0.52 mg kg^{-1}), and Zn (39.93 mg kg^{-1}) were not exceeded (Kabata-Pendias, 2000).

[1] Benitez, J. N., Sole, M. V., & Cisteró, X. F. (2010). Análisis de la vegetación de *Schinus molle* L. (el pirul), en saber si existe una relación de contaminación de arsénico entre la planta y el suelo en Zimapán, Hidalgo; México. En *XV Congreso Peruano de Geología, Cusco. Resúmenes Extendidos* (pp. 175-179). Cusco, Peru: Sociedad Geológica del Perú.

Table 1. Plants found in vegetation islands and their effects on mine tailings and contaminated soils.

Plant	Strate	Effect	Cited
Schinus molle		Can translocate Pb and Cu	Lazo, and Lazo (2020)
Boutelova curtiendula		Separates the mine tailings and the surrounding area	Salas-Luévano, Mauricio, González, Vega, and Salas (2017)
Isocoma sp.		They are considered tolerant to mine conditions, mainly in the boundary layer that separates the mine waste and the surrounding area	Harvey (2021[1])
Bouvardia ternifolia		Separates the mine tailings and the surrounding area	Harvey (2021[1])
Opuntia engelmannii		It can be used to remove heavy metals from water	Kumar and Sharma (2020)
Foeniculum vulgare		May probably be employed as a potent phytoremediation of heavy metals from polluted soils	Ziaratin, and Hosseini (2014)
Eruca sp.		It associates bacteria growing in the rhizosphere to help growth and tolerance, PGP bacteria enhance plant growth and increase bioavailability of trace metals.	Ashraf *et al*. (2017)
Casuarina equisetifolia		Potential adsorbent for sequestering Cu (II), Pb (II) and Ni (II) from aqueous solution on soils.	Rao and Khatoon (2017)
Baccharis sp.		Spontaneous growth in tailing area, properties of phytoextraction of Pb and phytostabilization	Afonso *et al*. (2022)
Flourensia cernua		Present antifungal properties against plant pathogens	Zavala-Hurtado, and Jiménez (2020)
Eucalyptus camaldulensis		Manganese accumulation	De Oliveira, and de Andrade (2021)

[1] Harvey, S. R. (2021). Desert marigold arsenic uptake and phytoremediation potential on mine tailings in the eastern mojave desert. Master's thesis, Arizona University.

 (high stratum),  (middle stratum),  (lower stratum).

Arbuscular Mycorrhizal Root Colonization (%)

There was no observed trend in response to the increase in phosphatase activity and mycorrhizal colonization in roots. Three species, *Euruca* sp., *B. temifolia*, and *Baccharis* sp. exhibited high colonization percentages exceeding 80%. *S. molle*, the species with the lowest phosphatase activity, had a colonization percentage of 53%. The species *Opuntia engelmannii*, *Eucalyptus camaldulensis*, *Foeniculum vulgare,* and *Isocoma* sp. showed colonization percentages ranging from 30% to 20%. *Casuarina equisetifolia* had colonization percentages below 8% (Figure 2a).

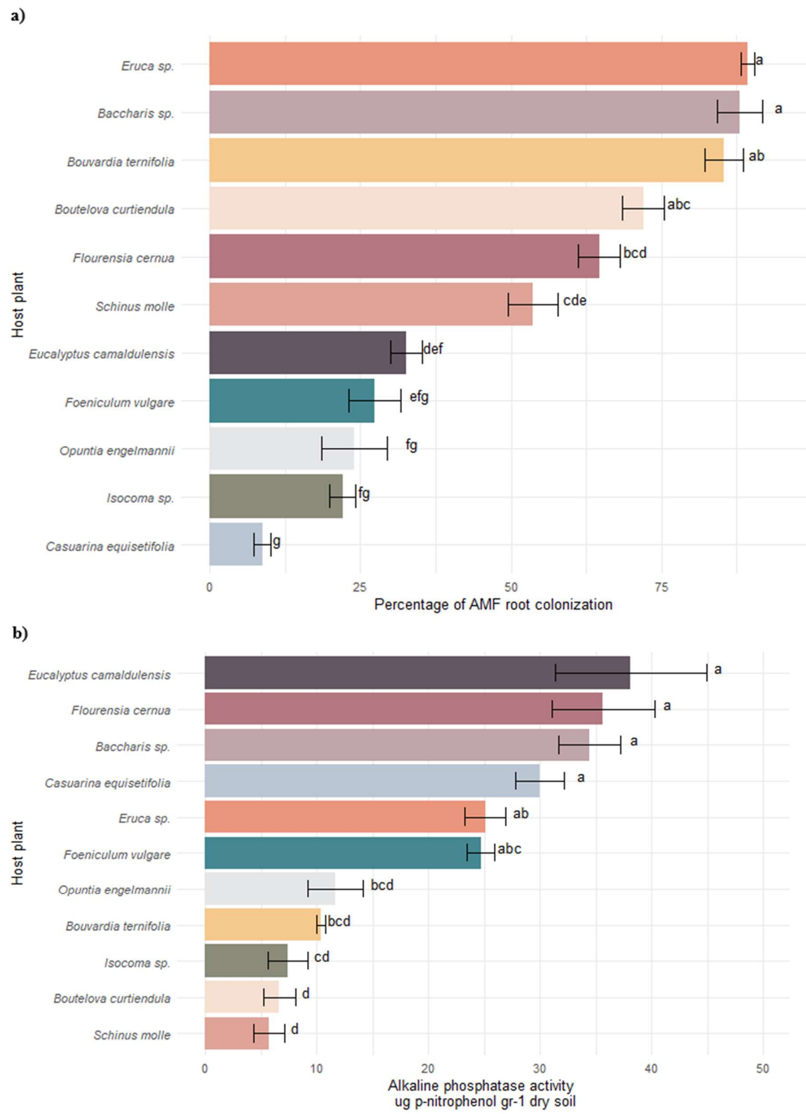

Figure 2. a) Percentage of mycorrhizal colonization in host plants established in mine tailings. b) Differences in microbial activity of the rhizosphere of plants established in mine tailings, as measured by the increase in alkaline phosphatase activity. In both cases, different letters indicate significant differences between plants (Kruskal-Wallis tests with Bonferroni corrections *P* < 0.05).

Alkaline Phosphate Activity

A significant increase in alkaline phosphatase activity was observed in the rhizosphere of *E. camaldulensis*, *F. cernua,* and *Baccharis* sp. compared to *O. engelmannii, B. ternifolia, Isocoma* sp., *B. curtiendula, and S. molle* (Figure 2b). *E. camaldulensis* has the highest microbial activity in roots (38.09 μg p-nitrophenol g^{-1} dry soil), representing a 7.6-fold increase compared to *S. molle,* a 5.57-fold increase compared to *B. curtiendula*, and a 5.2-fold increase compared to *Isocoma* sp. *Flourensia cernua* and *Baccharis* sp. showed high microbial activity (35.61 and 34.42 μg p-nitrophenol g^{-1} dry soil, respectively); however, they did not exhibit significant differences compared to *E. camaldulensis*.

Plants established in mine tailings with lower alkaline phosphatase activity were predominantly shrubs belonging to the following families: Cactaceae, Rubiaceae, Asteraceae, and Poaceae (CONABIO, 2016a). The plant species with the lowest microbial activity was *S. molle*, a perennial tree that thrives in disturbed areas, rocky terrain, and agricultural lands with slopes ranging from 20 to 40% (CONABIO, 2016b).

Discussion of the Experimental Results

The hypothesis proposed in the present work was confirmed; fungal structures were detected, demonstrating the presence of AMF, as well as microbial activity. However, no relationship was observed between colonization percentages and Alkaline phosphatase activity among the plants.

Of the eleven plants sampled, nine have already been reported, while two were not documented in the literature; however, they are located within the mycotrophic families Rubiaceae and Asteraceae (Sokornova, Malygin, Terentev, and Dolzhenko, 2022; Fernandez-Zarate *et al*., 2024). Regarding the presence of arbuscular mycorrhizae in plants, the present investigation observed that the *Eruca* species, which is not generally considered mycotrophic, showed a colonization percentage of 91%. This has been tested in other species of Brassicaceae (Demars and Boerner, 1996; Cosme, Fernández, Van der Heijden, and Pieterse, 2018). This could be attributed to its ability to adapt to the extreme conditions present in the mine tailings by associating with other plants that grew alongside them on the vegetation islands, which are mycotrophic. In this environment, *Eruca* sp. was able to establish connections with these plants and inoculate naturally (Figure 3).

Baccharis sp. is the only plant species that consistently shows high values of mycorrhizal colonization and microbial activity. This genus has a wide distribution from Canada and northwestern USA to Tierra del Fuego (Heiden, 2021); it is considered highly invasive (Lázaro-Lobo, Ervin, Caño, and Panetta, 2021) probably due to its high capacity to withstand adverse environments and it has also been proven to present high levels of mycorrhizal colonization (Murray, Schutte, Ganguli, and Lehnhoff, 2019). On the other hand, (Cornejo, Meier, Borie, Rillig, and Borie, 2008) demonstrated a higher content of total glomalin-related soil proteins (GRSP) in the rhizosphere of B. *linearis* compared to the soil in general in an area contaminated by metals (sludge), which was due to the presence of arbuscular mycorrhizal fungi.

In the revegetation of mine tailings, it is essential to consider the mycorrhizal fungi native to the site, as they establish a symbiotic relationship with the plants to be propagated. This is because both plants and mycorrhizae are adapted to the properties and climate of the mine tailings area. This adaptation is fundamental to promoting the increase of biodiversity in the vegetation islands and on the surface of the site. Regarding the vegetation islands, they play an important role in the mine tailings from an ecological point of view because they prevent dispersion (Duarte-Zaragoza, Pérez, Hernández, and Villanueva, 2020) and particle runoff. In the present investigation, it was found that in the vegetation islands, there is a "hot spot" of microbial communities, including mycorrhizal fungi, which play an important role in the mobilization of nutrients and their availability for plants that establish in the mine tailings.

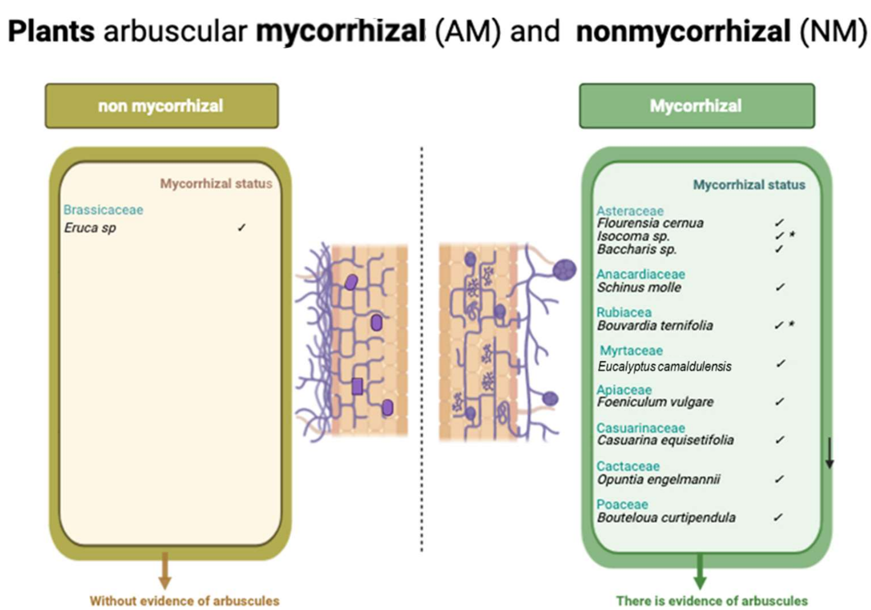

Figure 3. Graphical Analysis of Mycotrophic and Non-mycotrophic Plants in Mine Tailings: Identification of Species. ✓Presence of AMF structures. * Not documented in the literature.

In terms of microbial activity, it was shown that autochthonous microorganisms can develop favorably, mainly in the rhizosphere of *Baccharis* sp., *Eucalyptus camaldulensis,* and *Flourensia cernua*. No relationship was found between colonization percentage and microbial activity. However, *Baccharis* sp. exhibited a high colonization percentage and microbial activity, making it a promising alternative for phytoremediation programs.

A study of the biological activity of the microbial communities in the root zone of *Baccharis linearis*, naturally present in mine tailings, carried out by Aponte *et al*. (2023), showed that the five bacterial strains in the root zone showed plant-growth-promoting (PGP) traits, such as P solubilization and N acquisition, among others.

The presence of microorganisms serves as a sensitive bioindicator of soil quality. Parameters such as microbial biomass carbon and nitrogen rapidly respond to changes in soil management practices. Soil basal respiration, which measures the release of CO_2 during organic matter decomposition, varies depending on the levels of microbial stress. Microbial communities with plant growth-promoting (PGP) traits are more abundant in the root zone, benefiting from nutrients provided by plants. Although this study did not focus on nutrient incorporation by degrading plants, previous research (Brimecombe, De Leij, and Lynch, 2000; Singh and Mukerji, 2006) supports this relationship.

The functionality of living organisms relies on active interactions with microbiota, as evident in phytobionts that possess highly diverse microbial communities. Plants influence the distribution of rhizosphere microbes through root exudates, which stimulate the proliferation of beneficial microorganisms (Kurdish, and Chobotarov, 2024). These microorganisms enhance mineral availability, stimulate plant growth with biologically active compounds, and provide protection by improving the overall health of the rhizosphere.

Mycorrhizal associations, particularly with mycorrhizal trees, significantly contribute to restoration efforts by enhancing nutrient uptake and soil stability. Herbaceous plants emerge as resilient options for colonization due to their ability to adapt to harsh conditions. Nutrient incorporation by plants and the subsequent stimulation of microbial activity further underscores their ecological significance in mine tailings. Vegetation islands serve as hotspots for microbial communities, facilitating the mobilization of nutrients crucial for plant establishment.

The importance of native mycorrhizal fungi in revegetation strategies cannot be overstated, as they ensure symbiotic relationships well-suited to local soil and climate conditions, promoting biodiversity. While commercial inoculants offer convenience, they may lack specificity to mine tailings environments, potentially compromising their efficacy compared to native counterparts. Emphasizing the use of local materials as inoculants is advocated for its superior effects on both native and non-native plant species, enhancing the sustainability and success of revegetation.

It was observed that shrub and tree species help stabilize the mine tailings by primarily protecting them from wind. Carrillo-Saucedo, Puente, Montes, and Cruz (2022) cite that mycorrhizal trees play a crucial role in the restoration of degraded sites. It is known that their deep and extensive roots contribute to the biological diversity of the mine tailings. Rodríguez-González, Rangel, Velasco, Gómez, and Ruiz (2023) highlight the ecological importance of trees such as Juniperus, due to their abundance and distribution in mine tailings. On the other hand, herbaceous plants stand out as suitable to establish themselves as stabilizing plants in the mine tailings studied here, owing to their resistance to adverse conditions such as drought and salinity, as well as their rapid growth and development capacity (Conesa, Faz, and Arnaldos, 2007; Xie and van Zyl, 2023). In this regard, Hernández-Acosta, Acevedo, Robledo, and Castrellón (2024) have demonstrated the ability of some herbaceous *Cynodon dactylon* to reduce potentially toxic elements and the dispersion of particles in a mine tailing, highlighting its use as a vegetation cover to mitigate environmental impacts.

The plants adapted to the mine tailings, which presented favorable pH levels, high concentration of P (11.94 mg kg^{-1}) and Ca (3599 mg kg^{-1}), and average levels of K (189 mg kg^{-1}) and Mg (196 mg kg^{-1}). These physicochemical characteristics provide important information on the conditions that influence microbial activity and mycorrhizal colonization in the mine tailings. For example, neutral pH and high phosphorus levels may favor microbial activity, while low organic matter content hinders nutrient uptake by plants and microorganisms (Mishra, Jain, Jadeja, and Mishra, (2022). Locating these interactions (plant-microorganism) is fundamental to evaluating the potential for phytoremediation and revegetation of mine tailings.

Hernández-Acosta *et al*. (2009) reported medium alkaline pH values, medium nitrogen content, low phosphorus levels, high calcium, and very low to high Mg, as well as low to high K levels. Despite being the same mine tailings, changes in nutrient contents over a 15-year period were evident.

In the mine tailings, it was also observed that plants participate in the incorporation of nutrients as they degrade, stimulating microbial activity. This, in turn, further contributes to nutrient availability, hence trees such as *Prunus* spp., *Casuarina* spp., and *Eucalyptus* spp. manage to survive (Yin, Dijkstra, Wang, Zhu, and Cheng, 2018). In addition to these species, other plants of the families Cactaceae, Asteraceae, Poaceae, Leguminosae, Chenopodiaceae, Solanaceae and Cruciferae have been identified as growing in mine tailings (Hernández-Acosta *et al*., 2009), as well as the grasses *Lolium perenne* and *Poa pratensis*, all considered phytoremediators due to their capacity to adsorb potentially toxic elements such as Pb, Cd, Ni, Zn, Mn and Cu in their stems and leaves (Amezcua-Avila, Hernández-Acosta, and Díaz, 2020).

Regarding the presence of arbuscular mycorrhizal in plants, the present investigation observed that the *Eruca* species, which is generally not considered mycotrophic, showed a colonization percentage of 91%. This could be attributed to its ability to adapt to the extreme conditions present in the mine tailings by associating with other plants that grew alongside them in the vegetation islands and are mycotrophic. In this environment, *Eruca* was able to establish connections with these plants and naturally inoculate itself.

The choice between the use of native or commercial inoculants for the vegetative cover of the mine tailings will depend on several circumstances. For example, isolated native inoculants may offer significant advantages, as they are better adapted to the specific conditions of the mine tailings and can establish more effective symbioses with native plants. This can result in a more successful and sustainable plant cover, as it helps conserve genetic diversity in the mine tailings.

On the other hand, commercial inoculants, containing a variety of efficient mycorrhizal species, may be convenient due to their availability and the reduced time required for application compared to the process of isolating and preparing native inoculants. However, it is important to keep in mind that these inoculants are not specifically adapted to the conditions of the mine tailings and may not be as effective as native inoculants in promoting the establishment, growth, and development of the plants that are desired to be propagated in the mine tailings. In this regard, Emam (2016) notes that the use of local material as inoculants has a greater effect on both native and non-native plants than a commercial product.

CONCLUSIONS

Our results demonstrate the potential of the species present in the vegetation islands, all of which have a high dependence on arbuscular mycorrhizal fungi, which favors their survival. Microbial activity, influenced by intrinsic and extrinsic environmental factors, is a key indicator of the dynamics of the mine tailings and their health. Species such as *Eucalyptus camaldulensis*, *Baccharis* sp., and *Flourensia cernua*, which harbor beneficial microorganisms in their rhizosphere, promote significant microbial activity, reflecting optimal physical and chemical conditions for the development of other plants in succession that could continue the colonization of mine tailings. It is essential to continue research on the selection and identification of arbuscular mycorrhizal fungi in these environments, and to integrate both plants and fungi into phytostabilization programs to mitigate tailings contaminants.

ETHICS STATEMENT

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF SUPPORTING DATA

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

The authors declare that they have no competing interest.

FINANCING

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AUTHORS' CONTRIBUTIONS

Conceptualization: D.T.A., and E.H.A. Methodology: D.T.A., E.H.A., and Y.B.G. Software: Y.B.G. Validation: D.T.A., E.H.A., and Y.B.G. Formal analysis: D.T.A. Investigation: D.T.A., and E.H.A. Data curation: Y.B.G. Laboratory methods and analysis: Y.B.G. Statistical analysis: D.T.A. Writing-original draft preparation: D.T.A., and E.H.A. Writing-review and editing: D.T.A., E.H.A., and Y.B.G. Project administration: E.H.A. Funding acquisition: E.H.A.

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