

Short Note [Nota Corta]

MESQUITE, PINE SAWDUST, AND ARBUSCULAR MYCORRHIZAL FUNGI: A STRATEGY FOR CYANIDE PHYTOREMEDIATION †

[MESQUITE, ASERRÍN DE PINO Y HONGOS MICORRÍZICOS ARBUSCULARES: UNA ESTRATEGIA PARA LA FITORREMEDIACIÓN **DE CIANURO**]

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SUMMARY

Background: Heavy metals and cyanide compounds are common pollutants derived from mining activities. Cyanide can transform into hydrogen cyanide, a highly toxic substance for humans, and inhibits the growth of plants and microorganisms. If mining areas used as waste repositories remain without vegetation, pollutants remain exposed and can spread to other areas through wind or water. There is limited information on solutions that use plants and microorganisms to remediate cyanide-contaminated sites. Objective: To propose a strategy to adapt Prosopis sp. (mesquite) in a mining waste contaminated with 155 mg kg⁻¹ of cyanide, using pine sawdust and an inoculant of arbuscular mycorrhizal fungi (AMF). Methodology: An experiment was conducted using 50 mL polypropylene tubes with the following treatments: mining waste (MT) and mining waste with 20% sawdust (MTS) as controls, along with the same treatments inoculated with mycorrhizae (MT-AMF and MTS-AMF). The experiment lasted for 60 days, pH, electrical conductivity (EC), and the number of bacteria in the substrate were measured, as well as the dry weight of the plants and the presence of mycorrhizal colonization. Results: Treatments that included pine sawdust and AMF showed healthy plants, decreased pH (from 7.82 to 7.15), reduced EC (from 6.25 to 4.71 mS cm⁻¹), and significantly increased the number of bacteria. Implications: The combination of pine sawdust and arbuscular mycorrhizal fungi may facilitate the adaptation of *Prosopis* sp. in cyanide-contaminated mining soils, suggesting its potential as a bioremediation strategy. Conclusion: Pine sawdust and AMF can aid in adapting Prosopis sp. and represent a promising alternative for the recovery of cyanide-contaminated areas.

Key words: Phytoremediation; cyanide; arbuscular mycorrhizal fungi.

RESUMEN

Antecedentes: Los metales pesados y los compuestos de cianuro son contaminantes comunes derivados de la actividad minera. El cianuro, en particular, puede transformarse en ácido cianhídrico, una sustancia altamente tóxica para los seres humanos y que inhibe el crecimiento de plantas y microorganismos. Si las áreas mineras que reciben residuos carecen de vegetación, los contaminantes quedan expuestos y pueden dispersarse hacia otras zonas a través del viento o el agua. Existe poca información sobre soluciones que emplean plantas y microorganismos para la remediación de sitios contaminados con cianuro. Objetivo: Proponer una estrategia para adaptar Prosopis sp. (mezquite) en un residuo minero contaminado con 155 mg kg⁻¹ de cianuro, utilizando aserrín de pino y un inoculante de hongos micorrízicos arbusculares (AMF). Metodología: Se desarrolló un experimento utilizando tubos de propileno de 50 mL, con los siguientes tratamientos: residuos mineros (MT) y residuos mineros con un 20 % de aserrín (MTS) como controles, además de los mismos tratamientos inoculados con micorrizas (MT-AMF y MTS-AMF). El experimento tuvo una duración de 60 días. Se determinó el pH, la conductividad eléctrica (CE) y el número de bacterias en el sustrato, así

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como el peso seco de las plantas y la presencia de colonización micorrízica. **Resultados:** Los tratamientos que incluían aserrín de pino y AMF mostraron plantas saludables, disminuyeron el pH (de 7.82 a 7.15), redujeron la CE (de 6.25 a 4.71 mS cm⁻¹) e incrementaron significativamente el número de bacterias. **Implicaciones:** La combinación de aserrín de pino y hongos micorrízicos arbusculares puede facilitar la adaptación de *Prosopis* sp. en suelos mineros contaminados con cianuro, lo que sugiere su potencial como estrategia de biorremediación. **Conclusión:** El uso de aserrín de pino y AMF puede ayudar en la adaptación de *Prosopis* sp. y representa una alternativa prometedora para la recuperación de áreas contaminadas por cianuro.

Palabras clave: Fitorremediación; cianuro; hongos micorrízicos arbusculares.

INTRODUCTION

Mining has been key in the development of several economies around the world (Worlanyo and Jiangfeng, 2021). In Mexico, mining activity is one of the largest sources of income (Téllez-Ramírez and Sánchez-Salazar, 2024). Approximately nine million people died in 2015 due to environmental pollution (Landrigan et al., 2017). Pollution and cyanide (CN-) poisoning problems are common in underdeveloped countries where artisanal gold mining prevails. This occurs because of the widely used hydrometallurgical process to recover gold (Au), silver (Ag), and platinum (Pt), among other metals. Despite the difficulties and risks of working with cyanide, cyanide leaching continues to offer a high yield at a low cost compared to other alternatives that have been developed (Tarkaman and Veiga, 2023, Zhang et al., 2022). Many cyanide wastes go into the environment through landfills or the toxic gasses they emit. Due to the toxicity of cyanide to humans, animals, and plants, it is of interest to seek methods for the degradation of cvanide to less toxic compounds without generating more pollution or more toxic compounds (Rangel-González et al., 2024). Therefore, bioremediation through mycorrhizal fungi, organic amendments, and plants is suggested. By leveraging the symbiotic relationship between arbuscular mycorrhizal fungi (AMF) and the roots of endemic plants in the region where this problem exists, the aim is to reduce the amount of cyanide in the soil through enzymemediated degradation produced by AMF and increase absorption surface by the fungal hyphae. Additionally, the addition of pine sawdust has been shown to reduce the bioavailability of cyanide (Carrillo et al., 2022). This research aimed to propose a strategy for adapting Prosopis sp. (mesquite) in mine tailings contaminated with 155 mg kg⁻¹ of cyanide.

MATERIALS AND METHODS

Mine Tailings

The mine tailing was previously characterized (Rangel, 2018). The waste has a pH of 8, clay texture, low organic matter content (0.34 %), total nitrogen (123.7 kg ha⁻¹), total phosphorus (47.4 kg ha⁻¹), total cyanide (155 mg kg⁻¹), Al (2 mg kg⁻¹), As (5 mg kg⁻¹),

Co (1 mg kg⁻¹), Fe (500 mg kg⁻¹), Mn (300 mg kg⁻¹), and Pb (10 mg kg⁻¹).

Pine Sawdust

Pine sawdust (particle size less than or equal to 2 mm) was used to improve the texture of the mine waste and to reduce the bioavailability of some potentially toxic elements (PTEs) and cyanide (Carrillo *et al.*, 2022).

Arbuscular Mycorrhizal Fungi (AMF)

The AMF inoculant (registered with the laboratory code AMF2) used in this experiment was isolated from alkaline desert soil (pH 8.2 and EC 6.76 mS cm⁻¹). Previously, this inoculant was demonstrated to confer salinity resistance to carrot and lettuce plants irrigated with a high saline concentration of NaCl (120 mM) (Félix et al., 2023). Two thousand propagules (spores, hyphae, and segments of mycorrhizal roots) mixed with sand as a support were inoculated into each treatment with AMF. Inoculum came from a trap culture, using *Lactuca sativa* as a trap plant, grown in sand as support material (Félix et al., 2023). The trap culture was harvested, and the AMF colonized roots were chopped and mixed homogeneously with the sand. In order to know the number of propagules per inoculum gram, 50 g of the inoculum (sand, roots segments, spores, and hyphae segments) were used. Propagules were isolated by wet sieving and decanting (Gerdemann and Nicolson, 1963), through a set of sieves. Material retained in the sieves was placed into a Petri dish containing tap water to count propagules. The number of propagules was registered by observing the whole content of the Petri dish using a stereoscope Zeiss Stemi DV4 and placing a grid (0.5 x 0.5 cm squares) under the Petri dish. This was carried out by quadruplicate to get the propagules average amount per gram of inoculum.

Greenhouse Experiment

A short-term experiment was carried out on a small scale to reduce the amount of toxic waste by each experimental unit after harvesting plants. Polypropylene tubes with a capacity of 50 ml (Figure 1) were used as experimental units, which were packed with the corresponding material for each treatment: mine tailings (MT); mine tailings with 20 % sawdust

(MTS); MT with AMF; and MTS with AMF. Two seeds of *Prosopis* sp. were planted in all treatments to ensure germination, and then one plant was left to continue with the experiment. The experimental units were watered with tap water and maintained in the greenhouse at 32 °C, under a light/dark cycle of 11/13 h, respectively. The plants were harvested after 60 days to reduce the root stress due to the lack of space in the experimental unit. The variables pH, electrical conductivity (EC), and the number of heterotrophic bacteria and filamentous fungi were measured in each treatment. Mycorrhizal colonization was observed in the plant roots. pH and EC were measured according to the method described by Hayes (2009). Moisture was determined by the gravimetric method. The number of heterotrophic bacteria and fungi was determined in nutrient agar and potato dextrose agar (PDA) culture media, respectively, according to Solís-Domínguez et al. (2012). For the fungal count, 200 mg L⁻¹ of antibiotic (streptomycin) was added to the PDA medium to inhibit bacterial growth. The presence of AMF was observed by trypan blue staining, following the method of Phillips and Hayman (1970); however, due to the limited amount of root material, the percentage of mycorrhizal colonization could not be determined.

RESULTS AND DISCUSSION

Prosopis sp. seeds failed to develop in unamended mine tailings. In the treatment where mine tailings were combined with 20 % pine sawdust, the seeds germinated, seedlings grew etiolated, and did not develop leaves, except for the first pair of leaves formed after germination. When mine tailings were inoculated with arbuscular mycorrhizal fungi, mesquite seeds germinated and significantly improved seedling growth. Finally, combining pine sawdust with arbuscular mycorrhizal fungi and tailings promoted more remarkable foliar development and a more vigorous appearance in the plants (Figure 1).

Mining tailings have a clayey texture that causes significant moisture loss and compaction. The addition of pine sawdust improved the structure of the tailings and increased their moisture retention capacity significantly ($p \le 0.05$), facilitating plant adaptation. Furthermore, when combining pine sawdust with arbuscular mycorrhizal fungi, the increase in moisture retention was even more pronounced (Figure 2).

Inoculation with arbuscular mycorrhizal fungi (AMF) significantly reduced the pH of mine tailings, from 7.82 to 7.15 (p \leq 0.05), both in treatments with and without pine sawdust, compared to untreated tailings (MT) (Figure 3). These results suggest that plants, through their roots, sought to neutralize the pH, a process facilitated by mycorrhizal inoculation and, to a lesser extent, by the effect of pine sawdust. On the other hand, electrical conductivity (EC) tended to decrease from 6.25 to 4.71 mS cm⁻¹; however, this reduction was not statistically significant due to the variability between the replicas of the mine tailings (Figure 4). The decrease in pH in the mesquite rhizosphere can be explained not only by the capability of plants to modify the pH in the root environment but also by interactions between plant and microorganisms (Solís-Domínguez et al., 2012). When plants take up more cations than anions, they compensate by releasing the excess positive charge as H⁺, thereby acidifying the rhizosphere (Hinsinger et al., 2003; Solís-Domínguez et al., 2012). Also, AMF induces rhizospheric pH changes depending on the symbiotic status of the root (Bago et al, 1997).

Bacteria and Fungi Count

Mine tailings, characterized by high toxicity levels, have a low microbial density. In this case, the mine tailings had 6×10^3 colony-forming units (CFU) g⁻¹ and 2×10^1 CFU g⁻¹ of bacteria and fungi, respectively. Inoculation with arbuscular mycorrhizal fungi (AMF) significantly increased the number of bacteria and filamentous fungi by up to two logarithmic units for bacteria and three logarithmic units for fungi ($p \leq$ 0.05), with recorded values of up to 1×10^5 CFU g⁻¹ and 1×10^4 CFU g⁻¹, respectively (Figure 5). AMF significantly increases microbial group biomass and modifies the community structure in mesquite rhizosphere and other plant species (Luo et al., 2022; Solís-Domínguez et al., 2011). It is well-known that AMF extraradical mycelium exudate metobolites, but also AMF modify the composition of plant root exudation, promoting the growth of these microorganisms groups (Luo et al., 2022; Marschner and Baumann, 2003).

Although it was impossible to determine the percentage of AMF colonization due to scarce root production, AMF structures were observed colonizing the roots (Figure 6).



Figure 1. *Prosopis* sp. plants growing in mine tailings with 155 mg kg⁻¹ of CN⁻. MT = mine tailings; MTS = mine tailings with 20 % pine sawdust; MT-AMF = mine tailings with arbuscular mycorrhizal fungi (AMF); MTS-AMF = mine tailings with 20 % pine sawdust and arbuscular mycorrhizal fungi (AMF).



Figure 2. Moisture retention in the different treatments at the end of the experiment. MT = mine tailings; MTS = mine tailings with 20 % pine sawdust; MT-AMF = mine tailings with arbuscular mycorrhizal fungi (AMF); MTS-AMF = mine tailings with 20 % pine sawdust and arbuscular mycorrhizal fungi (AMF). Bars represent the mean of three replicates ± the standard deviation.



Figure 3. pH in the different treatments at the end of the experiment. MT = mine tailings; MTS = mine tailings with 20 % pine sawdust; MT-AMF = mine tailings with arbuscular mycorrhizal fungi (AMF); MTS-AMF = mine tailings with 20 % pine sawdust and arbuscular mycorrhizal fungi (AMF). Bars represent the mean of three replicates ± the standard deviation.



Figure 4. Electrical conductivity (EC) in the different treatments at the end of the experiment. MT = mine tailings; MTS = mine tailings with 20 % pine sawdust; MT-AMF = mine tailings with arbuscular mycorrhizal fungi (AMF); MTS-AMF = mine tailings with 20 % pine sawdust and arbuscular mycorrhizal fungi (AMF). Bars represent the mean of three replicates ± the standard deviation.



Figure 5. Bacteria and fungi in the different treatments at the end of the experiment. MT = mine tailings; MTS = mine tailings with 20 % pine sawdust; MT-AMF = mine tailings with arbuscular mycorrhizal fungi (AMF); MTS-AMF = mine tailings with 20 % pine sawdust and arbuscular mycorrhizal fungi (AMF). Bars represent the mean of three replicates ± the standard deviation.



Figure 6. AMF structures observed in Prosopis sp. roots in the treatments inoculated with mycorrhizal fungi. V = vesicles, H = hyphae.

Previous studies have demonstrated the ability of Prosopis sp. to adapt to mine tailings contaminated with potentially toxic elements. This plant species can alkalize the rhizosphere when the mine tailings are acidic (Solís-Domínguez, 2012). In this work, where the mine tailings were alkaline, a significant decrease in pH was observed. Moreover, pine sawdust contributed to plant survival by reducing the bioavailability of cyanide (Carrillo et al., 2022), thereby reducing toxicity in the plants. Additionally, pine sawdust increased moisture retention, improving the number of bacteria and filamentous fungi. Inoculated arbuscular mycorrhizal fungi (AMF) showed benefits in adapting Prosopis sp. in mine tailings by significantly increasing the number of bacteria and filamentous fungi. AMF mediates nutrient uptake, alleviates stress caused by drought, salinity, and heavy metals, and protects against various root pathogens. The control of mycorrhizae in root exudates increases the mycorrhizosphere's influence zone (Deepika et al., 2019). AMF also alleviated cyanide stress, which is an underexplored area of knowledge.

CONCLUSIONS

There is limited information on the recovery of cyanide-contaminated soils and mine tailings. The strategy of using Prosopis sp. in desert or semi-desert areas to remediate alkaline mine tailings with phytotoxic levels of electrical conductivity and

cyanide can be promising when pine sawdust and previously selected mycorrhizal fungi, such as the AMF2 inoculant used in this research, are added. The benefits of this strategy include increased moisture in the root growth zone, an increase in bacteria and filamentous fungi, and primarily, the reduction of cyanide phytotoxicity to allow plant adaptation in the mine tailings.

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Data availability. Data are available with Fernando Amílcar Solís Domínguez, solisf@uabc.edu.mx, upon reasonable request.

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