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Copper Uptake by *Adesmia atacamensis* in a Mine Tailing in an Arid Environment

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ABSTRACT: This work evaluates the potential of *Adesmia atacamensis*, an endemic species from the North of Chile, in phytoremediation processes of copper mining tailings. The selection of this species was based on the fact that its presence was found in 4 copper mining companies that are close to each other, its endemic quality, and its great capacity to adapt to the adverse climatic conditions of the sector of the project, characterized by having a semi-arid climate. In the experiment, the concentrations of 5 metals of environmental connotation for the country's mining were measured: Cu, Fe, Cd, Pb, and Zn; however, given the small concentrations in Cd, these could not be measured by the team. The applied experimental design quantified the variation of bioconcentration and translocation factors (BCF and TF) for the following treatments: (1) tailing control without amendment (T0), (2) tailing plus 4% $CaCO_3$ + 3% vermicompost (VC), and (3) tailing + 8% $CaCO_3$ + 6% VC. In addition, for treatments T1 and T2 (T0 was not considered as it is the control treatment), the following levels of mycorrhiza were considered: 0, 10, 15, and 20 g m^{-2}. The Baker and Brooks criteria and the BCF were used to evaluate the species as hyperaccumulators. Regarding the first criterion, high concentrations of copper were found in the shoots (shoots) of the specimens, which generally exceeded 1000 mg kg^{-1}, with an average of 1513 mg kg^{-1}, which allowed classifying the species as a copper hyperaccumulator; however, when compared with the BCF criterion, given that all the values were less than 1, they indicated that the species was an excluder of all the evaluated metals. Given the high concentrations of metals in the tailings, in this work, the plant has been considered as a hyperaccumulator of copper. Another indicator was the TF, which, for all experiments, resulted in a value greater than 1 for Fe, Pb, and Zn, which shows that *A atacamensis* translocates effectively these metals from the roots toward the aerial part and, therefore, presents the potential to accumulate metals in the aerial part. Regarding the treatments carried out, no significant impact was detected.

KEYWORDS: Mine tailing, phytoremediation, phytostabilization, arid, hyperaccumulators

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Introduction

Mining is the main economic activity in Chile,[1–3] where copper exploitation has been predominant for several decades. In addition, new industrial needs have opened new possibilities for national mining, as in the case of lithium, where the country concentrates practically one-third of the world's mineral reserve.[4]

Because of the large mining reserves that Chile presents, different companies have returned their interest to invest in such projects in the national territory. There is a millionaire investment projected for the coming years, concentrated mainly in the Antofagasta region. Bringing with it both economic and social development, thanks to the generation of new jobs and the consequent increase in the socioeconomic level of workers in the sector.[5]

However, there is a less friendly face of mining because it causes significant impacts on the environment such as air pollution associated with the movement of land in the case of open-pit mining and emissions of toxic gases in smelters, depletion of the resource water pollution and pollution due to discharges of industrial liquid waste to surface channels, or infiltration from tailings to underground water.[6–8] In addition, there may be a potential archeological impact and an impact on the flora and fauna of the area.[9]

It must be considered that mining does not only cause an environmental impact while the projects are in operation,[10,11] as once the mining projects are finished or closed, the facilities and waste generated remain, those that constitute the mining environmental liabilities, among which the ones with the greatest environmental connotation are the tailings, disposed to the weather affected by wind action, precipitation, or even seismic activity.[12,13]

In Chile, as in many parts of the world, there are a great number of mining environmental liabilities, mainly composed of tailings, which are potential risk sources for people and the environment.[14] The great number of tailings distributed in the urban and peri-urban area of Chile (more than 700) have been considered as environmental liabilities by the Chilean mining agency (Survey by the National Geology and Mining Agency of Chile[15]). Most of them do not have a protective surface layer and have not been closed under environmentally sound plans.[16]

Phytoremediation can be carried out with vascular plants[17–19] or algae[20,21] that have the capacity to extract (by absorption or adsorption), store, precipitate, volatilize, or degrade organic and inorganic toxic substances in their surroundings. Phytostabilization and phytoextraction have been demonstrated to be more suitable for soils contaminated with heavy metals.[22–24] Application of phytostabilization in arid regions, such as the north of Chile, implies that the plants used must be tolerant to drought and salinity.

On the contrary, phytoextraction involves the capture of contaminants from the solid substrate by plant roots and their transport and accumulation in upper parts of the plant such as buds.[25,26] Transport of contaminants to buds is a critical biochemical process for an effective phytoextraction because collection and storage in roots cannot be viable.[27,28] Phytoextraction requires the use of plants able to accumulate and transport high concentrations of toxic metals.[29,30] The most easily absorbed inorganic materials are As, Cd, Cu, Ni, Se, and Zn; moderate bioavailable metals include Co, Fe, and Mn, whereas Cr and Pb present low bioavailability.[31]

There are 2 main phytoextraction strategies: first, the use of hyperaccumulators which are wild species of plants that can accumulate large amounts of specific metals in their shoots, but they often grow slowly and have a low biomass[32,33]; second, fast-growing plants with high biomass are usually not metal specific and can extract lower heavy metal concentrations.[34] *Adesmia atacamensis* would fall in the first category, being a low biomass plant. This factor has to be considered when defining phytoextraction projects.

Although phytoremediation has been extensively applied in soils with organic and inorganic contaminants,[35,36] it is a relatively new technique to remediate mine tailings.[37,38] Before applying phytoremediation to mine tailings, it is necessary to modify physic-chemical characteristics of the tailings so that the plants can be self-sustaining over time. This can be done specifically by increasing the concentrations of organic matter (OM) and nutrients as tailings have low levels of microbial activity.[39] In arid regions, the contributions that increase the potential for a successful coverage include the addition of organic and inorganic amendments and irrigations. It is well known that organic amendments immediately reduce the bioavailability of metals and irrigation increases growth of plant species.[30]

Baker and Brooks[40] define hyperaccumulator plants as species that reach metal concentrations in their leaves as high as 10 to 100 times the "normal" concentrations. For example, a plant is said to be a hyperaccumulator when it is capable of accumulating more than 10 000 $\mathrm{mg\,kg^{-1}}$ of Zn, more than 1000 $\mathrm{mg\,kg^{-1}}$ of Cu and Pb, and more than 100 $\mathrm{mg\,kg^{-1}}$ of Cd. These concentrations are based on the milligram of metal in a kilogram of dry matter in some tissue of their biomass.

Many species present the capability of tolerating high concentrations of certain metals in the substrate, preventing their translocation to the aerial part of the plant. This allows the plants to maintain relatively low and constant concentrations in the aerial biomass, independent of the concentration in the substrate that contains them. These plant species are called exclusors.[41]

The aim of this article is to evaluate the accumulation capacity of one endemic plant species, *A atacamensis*, in copper mine tailings in the Antofagasta Region in Chile.

Material and Methods

Site description and sampling techniques

This work was conducted in copper mine tailings located in the Antofagasta region, in the north of Chile, which we will call CMZ in this work. This area is characterized by high levels of evaporation and low levels of water infiltration resulting in high salt concentrations; these conditions make the area for scarce vegetation.[42]

In previous studies, the tailing was characterized as being extremely saline and sodic, with highly clayey soils, which are not suitable for vegetation.[43,44] Regarding chemical characterization, high concentrations of metals such as Cu, Fe, Mn, and Zn were measured. Table 1 summarizes the maximum and minimum values considering 30 samples (10 samples per depth: 0-10, 10-20, and 20-30 cm). The average values of pH, electrical conductivity, exchangeable sodium percentage, and OM for each depth are given in Table 2.

Selection of A atacamensis

The region where the project is located is one of the most arid areas of the world and is characterized by high solar radiation and high saline concentration in the soils, environmental conditions that determine a scarce presence of vegetation, concentrated mainly in the fluves and absent, in general, in the interfluves. From the biogeographical point of view, this region is inserted in the so-called xeromorphic ecosystems, conditioned by extreme aridity (high diurnal temperatures, wide thermal oscillations, minimal, and cyclical precipitation), specifically in the tropical peri-arid desert ecoregion, which extends from the rocky coastline to the Andean foothills above 2500 m altitude and includes an intermediate area called desert plain. In this region, altitude and relief are the factors that determine the presence of vegetation. This region is characterized by a short vegetative period, defining a very particular plant physiognomy.

The information of the Environmental Impact Studies of 4 mining sites was analyzed (CMZ, Minera Escondida Limitada, Minera Lomas Bayas, and Minera Gaby). All are close to each other, extract Cu, and share common characteristics in their processes. It was found that the plant species *A atacamensis* and *Cistanthe salsoloides* are common in the 4 mining areas.

Given the presence of the species *A atacamensis* and *C salsoloides* in sites disturbed by copper mining, it is expected that the metals present, in addition to the conditions of the soils such as

Table 1. Maximum and minimum values of chemical characteristics of the tailing used in the experiment.

PARAMETERS	UNITS	MINIMUM VALUE	MAXIMUM VALUE
Fe	mg kg^{-1} tailing	19236	41923
Cu		1008	16296
Zn		108.6	306.6
Pb		75.7	215.4
Cd		<0.1	3.9
SAR	—	31.5	90.1
pH	—	8.2	8.6
EC	dS m^{-1} (at 25°C)	15.3	40.1

Abbreviations: EC, electrical conductivity; SAR, sodium adsorption ratio.

Table 2. Characteristics of the different tailing profile samples.

SAMPLING DEPTH	UNITS	0-10 CM	10-20 CM	20-30 CM
Acidity pH (H_2O)	—	8.16 (±0.05)	8.39 (±0.08)	8.4 (±0.06)
EC	dS m^{-1}	40.8 (±2.3)	23.6 (±3.5)	42.1 (±2.8)
ESP	%	33.4	34.1	37.2
OM	%	0.08	0.06	0.05

Abbreviations: EC, electrical conductivity; ESP, exchangeable sodium percentage; OM, organic matter.
Data in parentheses are standard deviations.

pH and electrical conductivity, along with the climate, favor the growth of these endemically.

The average dimensions found for *A atacamensis* were 60-cm long, 57-cm wide, and 45-cm high, and the distance between the specimens was approximately 120 cm. The species *C salsoloides* has a length of approximately 5 cm, a width of 12 cm, and a height of 4 cm, and the distance between the specimens is close to 100 cm.

However, in the period in which the experiment was conducted, the availability of *C salsoloides* in conditions of being transplanted was very scarce (only 8 plants). So, in order not to affect the biodiversity of the place, it was decided not to extract specimens of this species. For this reason, only *A atacamensis* was evaluated as a potential species to remedy soils exposed to copper mining.

Amendments

The pot experiment was conducted using 3 amendments ($CaCO_3$, vermicompost [VC], and arbuscular mycorrhizal fungi [AMF]). For the inorganic amendment, the dose of $CaCO_3$ was determined using the method of Sobek et al[45] as applied in previous works in the zone.[43,44] Considering that the average apparent density of tailings is 1.3 g/cm^3, to evaluate the effect of this amendment, 3 levels were considered: $T0_{IA}$: Tailing tailing + 0% $CaCO_3$ (0 kg in 1 kg of tailing), $T1_{IA}$: Tailing + 4% $CaCO_3$ (0.04 kg in 1 kg of tailing), $T2_{IA}$: Tailing + 8% $CaCO_3$/(0.08 kg in 1 kg of tailing).

With respect to the organic amendments, the mining company is served by a wastewater treatment plant that employs the “Tohá System,” also known as “Dynamic Aerobic Biofilter” or “Earthworm Filter,” which consists of a trickling filter with different layers and earthworms.[44] The residual water percolates through various filter beds and the OM is retained and then consumed by earthworms. Subsequently, solid wastes are processed by earthworms to produce a bioproduct known as VC, which was used as organic amendment in this work. Its chemical and physical characteristics are presented in Tables 3 and 4, respectively.

Following the indications of Lam et al,[44] the method of Hirzel[50] was employed to calculate the dose of organic amendment. Considering that the quantity of OM ranges in the interval 2% to 4% and the physic-chemical characterization of the VC (see Tables 3 and 4), from the application of this method, it results that the required dose of organic amendment for 20 cm depth is between 0.03 and 0.06 kg VC/kg tailing. Based on this result, 3 levels were considered: $T0_{OA}$: Tailing + 0% VC (0 kg in 1 kg of tailing), $T1_{OA}$: Tailing + 3% VC (0.03 kg in 1 kg of tailing), $T2_{OA}$: Tailing + 6% VC (0.06 kg in 1 kg of tailing).

Table 3. Chemical characterization of vermicompost.

PROPERTIES	UNITS	VALUE	METHOD
pH	—	6.64	4.1[a]
EC	$dS\,m^{-1}$	0.49	5.1[a]
Organic matter	%	90.5	6.1[a]
Total nitrogen	%	0.75	8.1.1[a]
Total phosphorus	%	0.08	5.8.1[c]
Total potassium	%	0.04	9.1[a]
Total sodium	%	0.09	9.1[a]
Ca	%	0.30	13650[b]
Mg	%	0.10	13650[b]
Ammoniacal nitrogen	$mg\,kg^{-1}$	4.20	8.2.1[a]
Nitric nitrogen	$mg\,kg^{-1}$	62.7	8.3.1[a]
Zn	$mg\,kg^{-1}$	99.5	11.3.1[a]
Mn	$mg\,kg^{-1}$	23.3	10.1[a]
Fe	$mg\,kg^{-1}$	1173	10.1[a]
Cu	$mg\,kg^{-1}$	936	11.4.1[a]
B	$mg\,kg^{-1}$	56.6	11.1[c]
Ammonium/nitrate ratio	—	0.07	14.2[a]
C/N ratio	—	67.1	14.1[a]

[a]Abbreviation: EC, electrical conductivity.
[b]Method reference: AENOR.[46]
[c]Method reference: Sadzawka et al.[47]

Table 4. Vermicompost physical characterization.

PROPERTIES	UNITS	VALUE	METHOD
Moisture	%	35.0	2.1[a]
Real density	$g\,cm^{-3}$	1.52	13039[b]
Apparent density	$g\,cm^{-3}$	0.17	13041[b]
Total porous space	% Vol	89.1	13041[b]
Aeration capacity	% Vol	49.5	13041[b]
Water volume	% Vol	39.6	13041[b]
Water retention total capacity	$mL\,L^{-1}$	396	13041[b]

[a]Method reference: Sadzawka et al.[47]
[b]Method reference: AENOR.[48,49]

The AMF used was *Glomus intraradices*. Contact time for the plants was 8 weeks due to the time required for the fungal inoculation process to occur as in the work by Lam et al.[44] The amount of mycorrhiza, in accordance with the recommendation of the supplier, is a ratio of substrate: mycorrhiza 2% to 4% v/v, which is approximately equivalent to a dosage between 10 and 20 g m−2 (mycorrhiza/substrate). The levels considered in this work were 0, 10, 15, and 20 $g\,m^{-2}$ for *A atacamensis*. Considering the surface of the holes that contained the plants (0.0625 m^2, see next section), the dose of mycorrhiza applied was 0 g (0 cm^3), 0.625 g (2.1 cm^3), 0.94 g (3.1 cm^3), and 1.25 g (4.2 cm^3).

Experimental development

Preparation of the substrate. Tailings were extracted from the dam at 2 depths: 0 to 20 cm and 20 to 30 cm. The material was sieved through a 2-mm mesh screen. These substrates were placed in black plastic bags 20 cm in diameter by 60 cm in length. The inorganic amendment ($CaCO_3$) was applied all at once on the sifted tailings of the surface layer (0-20 cm), and the prepared substrate was thoroughly mixed using a plastic shovel. This was then placed on the tailings extracted at the depth of 20 to 30 cm. This material was irrigated with distilled water and incubated for 2 months.

The total substrate was placed into black plastic bags to avoid interference from sunlight on the process. First, the 20- to 30-cm-depth unamended tailing was added and then the homogenized tailing with $CaCO_3$ was incorporated. It was irrigated for 2 months to lime the system. After 2 months, the organic amendment (VC) was added and it was homogenized with the limed substrate with the help of a plastic shovel. Irrigation was performed and after 1 month the plants were transplanted to black plastic bags.

Transplant of A atacamensis and inoculation with AMF. The first step was to select the specimens for transplant; for this, the younger and smaller plants were selected, with similar characteristics in height and state of preservation—young individuals. Once the specimens were selected, the area was cleaned, and the dry branches were removed. Subsequently, a circumference was marked around the selected plants, considering as diameter the extension that the branches had before being pruned, thus estimating the root area. A deep ditch was dug around the circumference, releasing the roots that were stuck in the ground. It was then proceeded to dig under the root ball, removing woody roots with the help of a shovel when needed.

The entire area was moistened with water, and finally, after 24 hours, the specimens were extracted, keeping as much root ball as possible. Figure 1 shows the sequence of the selection and preparation process prior to the extraction of the plant.

Finally, the extracted specimens were placed in the black bags with the prepared substrate, and they were left in this state for 15 days, to slowly change the state of the plant, as they are more affected by abrupt changes. In Figure 2, the sequence of the extraction process of the specimens is presented.

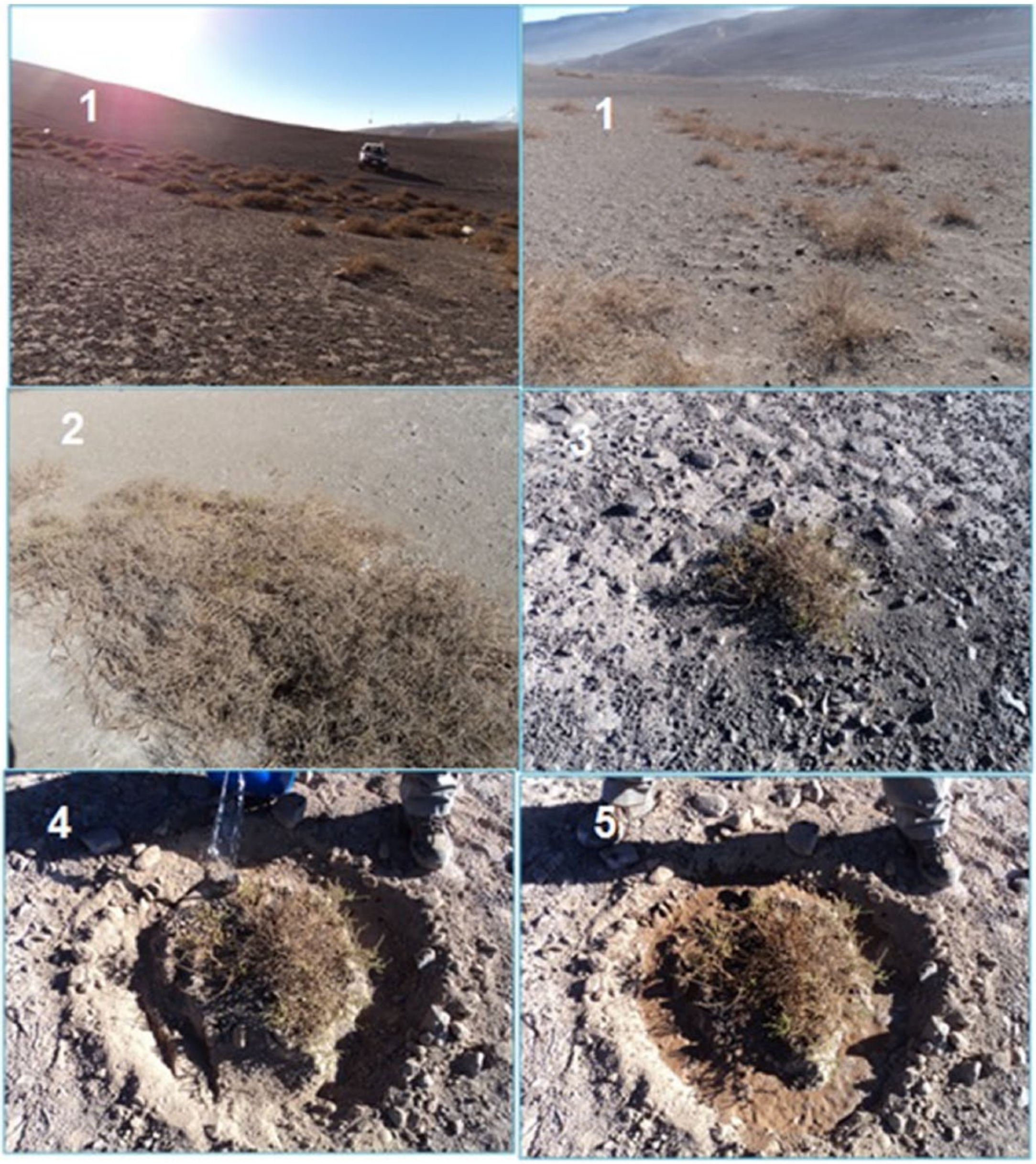

Figure 1. Photographic sequence of the field identification of the plant species and preparation prior to their extraction: (1) extraction site, (2) mound of dry branches, (3) cleared plant, (4) circular delimitation and irrigation, and (5) specimen ready to be extracted.

The plants in the black plastic bags were taken to the tailings dump for their definitive transplant.

The specimens of *A atacamensis* were transplanted to the tailings site. The root ball was included when transplanting the species that were in the bags. Holes of 25 cm depth and 25 cm diameter (0.0625 m^2 surface) were dug, and the plants were placed 50 cm apart. After the plants were transplanted, these were kept in direct contact with the mycorrhiza for a period of 8 weeks, to allow the activation of mycorrhiza in the roots. Prior to the experiments, the roots were analyzed to determine whether there was a presence of native AMF. This possibility was subsequently discarded.

After the initial conditioning period (8 weeks inorganic amendment + 4 weeks organic and inorganic amendment + 8 weeks of mycorrhizal activation), the process of experimentation for the measurement of variables was started; this lasted for 16 weeks.

Experimental design. The field experiment was conducted following the indications of Lam et al,[44] which are detailed in the following paragraphs for clarity and replicability. In particular, a randomized block design was used considering 2 factors: tailing treatment and mycorrhiza. To examine the accumulation potential and response of *A atacamensis* on copper mine tailings, the treatments consisted of (1) control tailing without amendment (T0), (2) tailing plus 4% $CaCO_3$ + 3% VC, ie, 0.04 kg $CaCO_3$ + 0.03 kg VC in 1 kg of tailing (T1), and (3) tailing + 8% $CaCO_3$ + 6% VC, ie, 0.08 kg $CaCO_3$ + 0.06 kg

Figure 2. Photographic sequence of the extraction process of the specimens: (1) excavation of the delimiting furrow, (2) pruning of the plant, (3) extraction of a specimen, and (4) transfer of the specimen to a black bag.

VC in 1 kg of tailing (T2). In addition, for treatments T1 and T2 (T0 was not considered because it is the control treatment), the previously mentioned levels of mycorrhiza were considered: 0, 10, 15, and 20 $g\,m^{-2}$.

The symbols used for the treatment are as follows: M(X)-T_j, where M represents mycorrhiza, X (0, 10, 15, 20) the corresponding level of mycorrhiza in grams per square meter, and T_j is the treatment j ($j = 0, 1, 2$). The following treatments were evaluated for *A atacamensis*: M(0)-T0, M(0)-T1, M(0)-T2, M(10)-T1, M(10)-T2, M(15)-T1, M(15)-T2, M(20)-T1, and M(20)-T2. The treatments applied to the plant are shown in Table 5.

Under this experimental design, the hypothesis is that the different treatments and mycorrhizal fungi levels will have a positive impact on the adaptation of the plant. In this sense, the null hypothesis is that the different amendments have no significant effect on the adaptation of the plants.

Effect of the treatments

To evaluate the mobility of the metals from the substrate into the roots of the plants and the ability of the plants to translocate metals from roots to shoots, the BCF and the TF of Cu, Fe, Pb, and Zn were estimated (Cd was omitted due to the lack of enough data). The evaluation and selection of the plants for phytoremediation purposes depend on these factors.[50] The BCF was calculated as follows[52]:

$$\mathrm{BCF} = \frac{[\mathrm{metal}]_{\mathrm{roots}}}{[\mathrm{metal}]_{\mathrm{tailing}}} \tag{1}$$

According to Azlan et al[53] and Lotfy et al,[54] BCF is a measure of the ability of a plant to accumulate metals from the soil (tailings in this case). According to Baker[41] and Rezvani and Zaefarian,[55] the following criteria must be considered: if BCF <1 the plant is an excluder; if $1 < \mathrm{BCF} < 10$, the plant is an accumulator; and if BCF >10, the plant is a hyperaccumulator. Plants with a BCF value greater than 1 are suitable for phytoextraction.[52,56]

It is worth pointing out that the greater than 1 criterion for the BCF should not be applied when doing studies in environments with as high a concentration of heavy metals as it can be found in mine tailings, as it would be difficult that the high concentration of bioavailable metals in the tailings could be matched by the concentration of metals in the plants.[43,44]

The TF indicates the efficiency of the plant in translocating accumulated metals from their roots to shoots[57,58] and is a measure of the ability of the plant to translocate metals from the roots to the shoots[59,60]:

$$\mathrm{TF} = \frac{[\mathrm{metal}]_{\mathrm{shoots}}}{[\mathrm{metal}]_{\mathrm{roots}}} \tag{2}$$

Table 5. Treatments applied to *Adesmia atacamensis*.

M(X)-TREATMENT	NO. OF PLANTS
M(0)-T0	5
M(0)-T1	5
M(0)-T2	5
M(10)-T1	5
M(10)-T2	5
M(15)-T1	5
M(15)-T2	5
M(20)-T1	5
M(20)-T2	5

M(X): X g mycorrhiza m^{-2}.

where $[\text{metal}]_{\text{shoots}}$ is the metal concentration in the shoots, $[\text{metal}]_{\text{roots}}$ is the metal concentration in the roots, and $[\text{metal}]_{\text{tailing}}$ is the metal concentration in the tailing. The following criteria must be considered for these factors: TF >1 means that the plant effectively translocates metals from the roots to its aerial parts,[40] and therefore, it presents potential to accumulate metals in the aerial part. If TF <1, it indicates that the plant does not effectively translocate metals to its aerial parts due to which it possesses a potential for metal phytostabilization in its roots.

A 3-way analysis of variance (ANOVA) was applied to compare the effects on BCF and TF produced by the different experimental factors, at a .05 level of significance on the data. The factors were as follows: mycorrhizal level (0, 10, 15, 20), treatment (T0, T1, T2), and metals (Cu, Fe, Pb, Zn). All statistical analyses were performed using the R language for statistical computing.[61] The data were analyzed using the general linear model of a 3-way ANOVA, followed by Tukey test at a significance level of $P = .05$ for the comparison of means.

Analyses of metals

The analysis was performed in accordance with the indications of Lam et al,[44] and the concentrations of metals were measured in tailings (mg/kg) and plant (mg/kg dry weight). Sampling and chemical analyses were run in triplicate to evaluate experimental reproducibility. The details of the analysis are presented in this article to facilitate replicability, with the original description being found in the work by Lam et al.[44]

Plants. Shoots and roots were divided mechanically and cleaned with deionized distilled water for approximately 5 minutes to remove soil particles adhering to the plants. They were then rinsed and dried at 70°C in a gravity oven for 48 hours. Subsequently, they were ground into powder with an electric grinder and passed through a 2-mm sieve.[62] Afterward, samples were ground again with a mortar and pestle. For analysis, 2.0 g of dry plant matter was placed in a Pyrex beaker and digested with a mixture of aqua regia and perchloric acid, according to standard methods.[63]

Plant extracts were diluted to 50 mL with double distilled water and then digested in hot air oven at 95°C during approximately 2 hours until digestion was completed.[64] The solution was then filtered and concentrations of Al, Cd, Cu, Fe, Mn, Pb, and Zn were analyzed by atomic absorption spectrophotometry (AAS).[64,65]

Tailing. Substrate samples of about 1 kg were collected, properly labeled, and packed in polyethylene bags. They were oven-dried at 40°C until reaching a constant weight.[66,67] Rocks, stones, and any other extraneous material were removed, and the remaining particles were reduced in size with a mortar and pestle. Particles were then screened with a 2-mm sieve (US No. 10 mesh), which is the standard particle size for most soil testing methods.[66,68]

Bioavailable Fe, Zn, Cu, Cd, and Pb contents were measured by an atomic absorption spectrophotometer after extraction using a diethylenetriaminepentaacetic acid solution.[69] These metals were extracted by shaking 0.01 kg of oven-dried soil for 2 hours in 20 mL of 0.005 Diethylenetriaminepentaacetic Acid (MDTPA). The filtrate was analyzed for these metals by AAS. All solutions were filtered with Whatman GF/C fiberglass filter paper.

Chloride and SO_4^{2-} anions[70,71] and K^+, Na^+, Ca^{2+}, and Mg^{2+} cations[71,72] were determined by ionic chromatography (Metrohm 861-Compact IC).

The pH was measured potentiometrically from the saturated paste extract (SPE) using a pH meter. This method involves saturating the material with water and subsequently extracting the liquid phase under partial vacuum. Electrical conductivity was measured in the SPE with a conductivity cell.[73] Water saturation percentage was calculated as the sum of the water added and that initially present in the field, expressed on an oven-dry basis.

Plant species

In field inspections of the mine, 2 sites of floristic relevance were observed; the first corresponds to the slopes of the hills, on the way to the tailings dams, the species *A atacamensis* and *C salsoloides* were found, observing the characteristics presented in Table 6. The average was calculated as the arithmetic mean of the values taken from 3 randomly chosen plants, taking 3 replications in each of them, excluding the maximum and minimum value for each species. However, as mentioned before, in order not to affect the biodiversity of the place, *C salsoloides* were not taken from the site.

Table 6. Characteristics of plant species on hill slope in CMZ.

SPECIES	RECORDED NUMBER	DISTANCE BETWEEN INDIVIDUALS, M	AVERAGE ROOT LENGTH, M	AVERAGE RELATIVE CHLOROPHYLL[a]	AVERAGE PLANT DIMENSIONS, CM × CM × CM
Adesmia atacamensis	21	0.6	>1	0.14	40 × 30 × 40
Cistanthe salsoloides	7	1	0.15	0.01	5 × 12 × 4

[a] Relative chlorophyll was measured based on relative absorbance using double optical wavelength (620 and 940 nm wavelength), the equipment used is Chlorophyll Content Meter model CL-01 of Hansatech Instruments. The relative chlorophyll content measures in the range of 0 to 2000 units.

Table 7. Metal concentrations in the substrate of *Adesmia atacamensis.*

SAMPLE	CD, MG KG^{-1}	CU, MG KG^{-1}	FE, MG KG^{-1}	PB, MG KG^{-1}	ZN, MG KG^{-1}
M(0)-T0	0.39	9602.8	22 175.8	87.2	219.5
M(0)-T1	<0.25	10 320.5	24 537.3	93.6	301.0
M(0)-T2	<0.25	10 517.2	19 745.3	127.4	124,9
M(10)-T1	<0.25	9467.9	19 437.0	117.8	134.6
M(10)-T2	<0.25	1238.2	34 654.2	211.0	145.2
M(15)-T1	<0.25	11 943.9	20 432.1	134.8	221.8
M(15)-T2	<0.25	7687.3	32 935.2	82.8	187.3
M(20)-T1	<0.25	3453.5	23 003.4	90.3	272.6
M(20)-T2	<0.25	2354.2	26 953.2	87.9	283.8

Table 8. Metal concentrations in shoots of *Adesmia atacamensis.*

SAMPLE	CD, MG KG^{-1}	CU, MG KG^{-1}	FE, MG KG^{-1}	PB, MG KG^{-1}	ZN, MG KG^{-1}
M(0)-T0	nd	92.3	1112.3	11.6	23.2
M(0)-T1	nd	101.2	1563.6	15.3	32.1
M(0)-T2	nd	87.2	1437.2	12.9	27.8
M(10)-T1	nd	90.3	1092.5	19.2	25.4
M(10)-T2	nd	80.2	2258.3	16.1	35.7
M(15)-T1	nd	153.8	1769.2	11.8	43.6
M(15)-T2	nd	112.3	2111.7	19.2	40.8
M(20)-T1	nd	97.8	1382.9	9.5	38.7
M(20)-T2	nd	76.5	3294.1	13.6	43.2

Abbreviation: nd, not detected.

Results and Discussion

Statistical analysis

The total metal contents (Cu, Fe, Pb, Zn, and Cd) were measured in tailings, plant shoots, and plant roots. Tables 7 to 9 present the results of the concentrations of Cd, Cu, Fe, Pb, and Zn in the tailing, in the shoots, and in the roots of *A atacamensis*. From these results, the BCF and TF were calculated. The results are shown in Tables 10 and 11, respectively.

As it can be observed in Table 10 (BCF), even though all the values of BCF are less than 1, it should be considered that given the high values of metal concentrations in the substrate, it is hard for the concentration in the roots to be greater or

Table 9. Metal concentrations in roots of *Adesmia atacamensis.*

SAMPLE	CD, MG KG^{-1}	CU, MG KG^{-1}	FE, MG KG^{-1}	PB, MG KG^{-1}	ZN, MG KG^{-1}
M(0)-T0	nd	4382.5	788.4	4.7	17.5
M(0)-T1	nd	5132.5	924.2	4.3	23.2
M(0)-T2	nd	4206.4	856.2	4.1	35.6
M(10)-T1	nd	4002.5	862.3	5.3	25.8
M(10)-T2	nd	1089.5	1123.1	3.5	45.2
M(15)-T1	nd	5035.7	900.2	5.2	36.2
M(15)-T2	nd	4965.8	956.1	6.1	56.3
M(20)-T1	nd	3004.5	764.7	5.7	48.7
M(20)-T2	nd	1956.8	836.9	4.6	55.2

Abbreviation: nd, not detected.

Table 10. Bioconcentration factor values in *Adesmia atacamensis*.

SAMPLE	CD, MG KG^{-1}	CU, MG KG^{-1}	FE, MG KG^{-1}	PB, MG KG^{-1}	ZN, MG KG^{-1}
M(0)-T0	nd	0.46	0.04	0.05	0.08
M(0)-T1	<0.25	0.50	0.04	0.05	0.08
M(0)-T2	<0.25	0.40	0.04	0.03	0.29
M(10)-T1	<0.25	0.42	0.04	0.04	0.19
M(10)-T2	<0.25	0.88	0.03	0.02	0.31
M(15)-T1	<0.25	0.42	0.04	0.04	0.16
M(15)-T2	<0.25	0.65	0.03	0.07	0.30
M(20)-T1	<0.25	0.87	0.03	0.06	0.18
M(20)-T2	<0.25	0.83	0.03	0.05	0.19

Abbreviation: nd, not detected.

equal than the value of the concentration of the substrate. Nevertheless, it can be observed that the plants are able to accumulate a great concentration of Cu, placing it in the hyperaccumulator class according to the criterion of Baker and Brooks. On the contrary, *A atacamensis* can be seen as an excluder of Fe, Pb, and Zn. However, the ability of these plants to translocate and accumulate metals may be useful for phytostabilization.[25]

The ANOVA tests show that there is a statistically significant difference on the BCF value depending on the metal ($P = 1.233\mathrm{e}{-11}$), in particular, the values for Cu were significantly higher. Although the treatment and the mycorrhiza had marginally effect on the results, the ANOVA test showed that they did not have a statistically significant contribution to the value of BCF ($P > .05$). Analyzing the results without taking the values for Cu yields similar results in terms of statistical significance. The application of Tukey post hoc tests indicates that the values for Cu are indeed higher by a statistically significant margin.

From Table 11 (TF) it can be seen that *A atacamensis* presents potential to accumulate Fe, Pb, and Zn in its aerial part, even without need of treating the tailing with any amendment, although the effect seems to increase when applying different amendments, with the highest value being reached for M(10)-T2 for Cu, M(20)-T2 for Fe, and M(10)-T2 for Pb and Zn. However, the ANOVA tests did not find any statistically significant effect for both the amendment factors, attributing all the effects to the different metals ($P = 1.036\mathrm{e}{-08}$).

Furthermore, TF values below 1 suggest that *A atacamensis* could prove useful for phytostabilization for Cu. Tukey post hoc analysis shows that there is a statistically significant difference between different pairs of metals, in particular, Pb and Zn

Table 11. Translocation factor values in *Adesmia atacamensis*.

SAMPLE	CD, MG KG^{-1}	CU, MG KG^{-1}	FE, MG KG^{-1}	PB, MG KG^{-1}	ZN, MG KG^{-1}
M(0)-T0	nd	0.57	1.41	2.47	3.85
M(0)-T1	<0.25	0.51	1.69	3.56	4.71
M(0)-T2	<0.25	0.44	1.68	3.15	2.88
M(10)-T1	<0.25	0.48	1.27	3.62	4.49
M(10)-T2	<0.25	0.72	2.01	4.60	2.72
M(15)-T1	<0.25	0.23	1.97	2.27	3.97
M(15)-T2	<0.25	0.23	2.21	3.15	2.39
M(20)-T1	<0.25	0.30	1.81	1.67	3.60
M(20)-T2	<0.25	0.39	3.94	2.96	3.03

Abbreviation: nd, not detected.

obtain similar values ($P > .05$) and significantly higher values than Fe ($P = .0301071$ and $P = .0011514$, respectively), and Fe obtains significantly higher values than Cu ($P = .0007825$). Both results suggest that the metals themselves have the greatest impact on the BCF and TF; this concurs with previous analysis done on other plants, such as in the work by Lam et al.[44]

In general, the untreated tailing presented a lower removal efficiency than the tailings treated by the different amendments. However, there were not statistically significant effects according to the ANOVA tests performed during the analysis of results. On one hand, the analysis of the BCF values suggests that *A atacamensis* is a clear excluder of Pb, Fe, and Zn, and thus they are not appropriate for phytoextraction of these metals; however, for Cu, the results could suggest that *A atacamensis* is a hyperaccumulator. On the other hand, the analysis of the TF values for Zn suggests that *A atacamensis* could be adequate for phytostabilization of Cu in the roots of the plants, but this would not be the case for the other metals because *A atacamensis* tends to move the metals from the roots to the shoots for Fe, Pb, and Zn accordingly based on their TF values.

It is worth pointing out that the greater than 1 criterion for the BCF should not be applied when doing studies in environments with as high a concentration of heavy metals as it can be found in mine tailings, as it would be difficult that the high concentration of bioavailable metals in the tailings could be matched by the concentration of metals in the plants.[44]

Phytoremediation potential

The soils of the region where the mine site under study is located are characterized by having developed in essentially abiotic conditions due to low rainfall and high average annual temperatures, both typical characteristics of the Atacama Desert. These soils are characterized by being very poorly developed and with absence of OM. The red soils of the Atacama Desert, called entisols, are characterized by a high degree of oxidation of the minerals and the formation of salt crusts on the surface. Originally, on one hand, the natural substrate (before the mining production process) presented high contents of salts, being classified as saline sodic. This natural characteristic led to high pH values. However, on the other hand, there is the presence of tailings which contain several metals whose main contents are Fe and Cu, in addition to presenting a potential acid generation capacity due to the excess of SO_4^{2-}. This is a complex and dynamic system, all the processes presented are interdependent and interactive; thus, any factor could have an influence on the diverse processes that occur.

In Tables 7 to 9, it can be observed that the highest concentrations in the tailing correspond to Fe (average of 76%) followed by Cu with approximately 23%. Regarding the concentrations in the tissues of the plant, it is observed that in the shoots, Fe is the most abundant with an average close to 52%, followed by copper with an average of 44%; in the roots, Fe was the most found with an approximate average of 22% and 77% of Cu, respectively.

Considering the criteria for hyperaccumulator plants given by Baker and Brooks, *A atacamensis* is capable of accumulating between 768 and 2603.7 mg kg^{-1} of Cu. Therefore, it could be considered a hyperaccumulator. Also, the ability of this plant to translocate and accumulate metals may be useful for phytostabilization.[25]

Regarding the TF for Fe, Zn, and Pb, in all the treatments (T0, T1, and T2), values above 1 were found, which indicates that there is translocation of these metals from the roots to the aerial part, and therefore, the plant presents a potential to accumulate metals in the aerial part. Regarding Cu, a value lower than unit was always obtained, however, the values were all above 1000 mg kg^{-1}, and therefore, the plant could be considered to have a potential for translocation of Cu and the TF values also indicate that the plant has a potential to phytostabilize Cu in the roots. In the case of Cd, given that its concentrations could not be detected by the measuring equipment, the potential of the plants as accumulators of this metal could not be determined.

With respect to mycorrhiza, the activation time for *A atacamensis* was between 20 and 35 days. When the plants that had been subjected to treatments with mycorrhiza were extracted, they were examined before starting analysis to determine whether there was presence of activation of mycorrhiza. Their activation occurs when the hyphae penetrate the roots and are established, this effect was observed in all the specimens of the species. Finally, concerning the effect of the different treatments and the amount of mycorrhiza added, it is established that there is no significant effect on any of the metals.

Conclusions

The results show that *A atacamensis* is an endemic species with characteristics of hyperaccumulator of Cu, without requiring any amendment, and therefore, it is suitable for phytoextraction. However, it is worth noting that the addition of mycorrhiza contributes to increasing this potential. Although the lowest BCF values were obtained for the tailings without any type of treatment, the effects of the treatments themselves were not found to be statistically significant.

The BCFs for all metals were found to be less than 1, which indicates that *A atacamensis* could be considered excluder of Fe, Pb, and Zn, but not so for Cu. With respect to Cu, even though the values of BCF are less than 1, given the high concentration of copper in the tailing and the criterion given by Baker and Brooks, the plant will be classified as hyperaccumulator of copper, and therefore, it is adequate for phytoextraction.

Regarding the translocation of the analyzed metals, it is observed that Fe, Pb, and Zn are translocated from the roots toward the aerial part, obtaining the highest TF value for the unamended tailing; therefore, the contribution of the amendments might be enhancing the phytostabilization of these metals in the roots.

In general terms, according to the statistical evidence, it can be concluded that for an endemic species, such as *A atacamensis*, its phytoremediation depends fundamentally on the metal content of the tailing. This is because the species possesses the capability of natural adaption to the characteristics of the study site.

The results of this study, alongside the previous work on other plant species,[42,44] make us think of copper phytomining as an emerging technology that must be further studied for the recovery of copper from tailings which are currently environmental liabilities of the mining industry.

The procedure to remove particles from the tissue is not efficient, and we also have to consider the low biomass of the plant. However, given the excellent capabilities of this plant to grow and develop in the environmental conditions of the tailing, there is a high potential for revegetation.

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Author Contributions

Conceived and designed the experiments: ELE, MGE. Analyzed the data: BKN, IMB. Wrote the first draft of the manuscript: ELE, BKN. Contributed to the writing of the manuscript: ELE, BKN, IMB, MGE. Agree with manuscript results and conclusions: ELE, BKN, IMB, MGE.

Jointly developed the structure and arguments for the paper: ELE, BKN.

Made critical revisions and approved final version: ELE, BKN, IMB, MGE. Reviewed and approved of the final manuscript: ELE, BKN, IMB, MGE.

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