Journal of Geochemical Exploration 182 (2017) 210–217

Contents lists available at ScienceDirect

Journal of Geochemical Exploration

journal homepage: www.elsevier.com/locate/gexplo

Evaluation of the phytoremediation potential of native plants growing on a copper mine tailing in northern Chile

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ARTICLE INFO

Keywords:
Copper mine tailings
Phytoextraction
Phytostabilization
Metal translocation
Bioconcentration factor

ABSTRACT

Mining operations in northern Chile are responsible for numerous mine tailings deposits, which may represent a risk to human health and the environment. This study evaluated the phytoremediation potential of three plant species (*Prosopis tamarugo*, *Schinus molle* and *Atriplex nummularia*) to remediate copper mine tailings in northern Chile. To improve the characteristics of the tailing, three treatments based on *arbuscular mycorrhizal fungi* and organic and inorganic amendments were applied. Plant roots and shoots and the associated tailings samples were collected and analyzed for total metal concentrations of Cu, Mn, Fe, Pb, Zn and Cd. Bioconcentration Factor (BCF) and Translocation Factor (TF) were estimated to evaluate the plant species potential for phytoremediation. The results show that *A. nummularia* presents high TF values on untreated tailings for Pb and Zn; on tailings treated with $CaCO_3$/compost for Mn, Fe, Pb and Zn, and on tailings treated with $CaCO_3$/compost/mycorrhizae only for Zn. *P. tamarugo* presents high TF values on the untreated tailings for Pb and Zn; and on tailings treated with $CaCO_3$/compost for Mn. *S. molle* presents high TF values on untreated tailings for Cu, Mn, Pb and Zn; on the tailing treated with $CaCO_3$/compost for Mn and Zn, and on the tailing treated with $CaCO_3$/compost/mycorrhizae for Mn and Pb. High TF values show that the plants studied under the specific characteristics of the treated or untreated tailings, present the potential of being used for phytoextraction. Regarding the BCF, the experiments showed values lower than 1 for all the metals, except for Cd, which for *A. nummularia* developed in the amended tailing, presented potential characteristics of a hyperaccumulator. This metal even had in general BCF values higher than 1 and TF lower than 1, which shows the potential of the three evaluated species as phytostabilizers for Cd. Regarding the effect of the amendments, the untreated tailing presented lower removal efficiency than the tailings treated with $CaCO_3$/compost (T1) and $CaCO_3$/compost/mycorrhizae (T2). On the other hand, no significant difference was found between treatments T1 and T2.

1. Introduction

Mining is the most important economic activity in Chile. However, it is also responsible for significant environmental damage, reflecting the large amount and diversity of residues generated from mining operations, which are often untreated, thus affecting the quality of water, soil and air (Bech et al., 1997; Valladares et al., 2013; Kossoff et al., 2014; Feyen et al., 2015).

Historically, because of the lack of adequate legislation, environmental liabilities increased in number and magnitude, leaving a legacy of major environmental problems. In particular, mining operations in northern central Chile have resulted in numerous mine tailings deposits that contain approximately 50% of the solid residues generated from such operations (Rubio, 2007). Solid tailings may also contain fines and slimes which may affect the stability of the tailings storage facilities (Edraki et al., 2014). Given the high content of metals, these tailings represent a risk to human health and the environment. Fine particles are exposed to physical agents like wind and rain that can disperse them into the surrounding area, contaminating waterways and neighboring soils (Mendez and Maier, 2008; Yadav and Jamal, 2015). Mine tailings in semiarid regions are highly susceptible to erosion and are sources of dust pollution and potential avenues of human exposure to toxic metals. Many factors can influence the mobility of metals in tailings, such as pH and organic composition and concentration (Bolan et al., 2014).

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http://dx.doi.org/10.1016/j.gexplo.2017.06.015
Received 6 July 2016; Received in revised form 21 June 2017; Accepted 21 June 2017
Available online 22 June 2017
0375-6742/

In 2012, the new Chilean law 20,551, which regulates mine closure and mining facilities, entered into force. Its main objectives are to protect the life, health and safety of people and the environment, to mitigate the negative effects of mining, to hold the mining industry accountable after cessation of operations and to ensure the physical and chemical stability of the ground where mining was carried out.

The last three decades have seen the emergence and development of ecologically-friendly, gentle soil remediation techniques using plant species. These techniques, known as phytoremediation, are generally considered to be less invasive, more cost-effective and restorative of soil compared to conventional methods (Kidd et al., 2009). Phytoremediation is based on the use of plants and their associated microorganisms to reduce concentrations of contaminants such as metals, radioactive isotopes, hydrocarbons or pesticides (Bennett et al., 2003; Greipsson, 2011; Ali et al., 2013). To apply phytoremediation to mine tailings, it is necessary to modify the physicochemical characteristics of the tailings so that plants can be self-sustaining over time, specifically by increasing the concentrations of organic matter and nutrients, given that tailings have low levels of microbial activity (Pepper et al., 2012; Young et al., 2015). In most cases, phytoremediation is supported with additives, termed amendments, which improve the characteristics of the substrate by reducing the mobility and bioavailability of metals (Alcantara et al., 2015; Puga et al., 2015; Zeng et al., 2015). Plant-based technologies in mine tailings are mostly limited to phytostabilization and phytoextraction. Phytostabilization involves using plants to minimize metal mobility from contaminated soils (Root et al., 2013; Barajas-Aceves et al., 2015; Li and Huang, 2015). The strategy of phytostabilizing mine tailings in arid or semiarid climates involves using plants tolerant to drought, salinity and metals (Mendez et al., 2007; Mendez and Maier, 2008). Phytoextraction involves the capture of contaminants from the solid substrate or water by plant roots and the transport and accumulation of these in the upper parts of the plant (Rafati et al., 2011; Dhankher et al., 2012).

Bioconcentration factor (BCF) and translocation factor (TF) were determined, both factors can be used to estimate a plant's potential for phytoremediation purpose (Yoon et al., 2006). From the perspective of phytoremediation, an efficient phytoextraction strategy should have high TF values because metals must be in the aerial parts of the plants so that they can be removed at harvest (Kamari et al., 2014). TF < 1 and TF > 1 represent, respectively, a low and high capacity to translocate metals from the roots to the shoots (Bazihizina et al., 2015).

This work was carried out on a copper mine tailing from the Antofagasta Region, one of the driest zones in the world. The tailing was classified as saline-sodic, with low Ca and high Na and SO_4^{2-} contents, high concentrations of metals such as Cu, Fe and Al, and pH 8.4. The tailing under study presents high concentrations of SO_4^{2-} and sulfides, which can generate sulfuric acid by oxidation and irrigation. *Arbuscular mycorrhizal fungi*, organic (compost) and inorganic amendments ($CaCO_3$) were added to improve the characteristics of the tailing. The aim of this study was to assess the potential of three native plant species (*Prosopis tamarugo*, *Schinus molle* and *Atriplex nummularia*) for phytoremediation of metals on amended copper mine tailings.

2. Material and methods

2.1. Site description and sampling techniques

The study area is located in the western margin of the Atacama Desert in northern Chile. Tailings samples were collected from a post-operative copper mine tailing site, which was active until 2006, located at latitude 24° 9′58.33″ S and longitude 69° 2′33.36″ W at an altitude of 3200 m.a.s.l. The tailing contains high concentrations of metals such as Cu, Fe, Zn, Mn and Pb. The approximate area of the tailing deposit is 10,000 m^2. This region is characterized by a desert climate with high levels of solar radiation. Temperatures range approximately from −15 °C in July to 30 °C in January. Precipitation typically falls in the summer months, with 2–6 mm/year.

Table 1
Maximum, minimum and average values and standard deviations of basic characteristics of tailing.

Parameter	Minimum value	Maximum value	Average value	Standard deviation
Sand (%)	–	–	75	–
Silt (%)	–	–	24	–
Clay (%)	–	–	1	–
TOC (%)	–	–	0.03	–
SAR	31.5	90.1	34.1	15.3
pH	8.13	8.63	8.42	0.06
Saturated water (%)	63.1	74.0	71.6	5.8
EC (dS m^{-1} at 25 °C)	15.3	40.1	35.5	3.9
Fe (mg kg^{-1})	19,236	41,923	35,015	3552
Cu (mg kg^{-1})	1008	16,296	11,151	2262
Pb (mg kg^{-1})	75.65	215.4	183.2	27.2
Mn (mg kg^{-1})	279.3	467.8	352.5	78.9
Zn (mg kg^{-1})	108.6	306.6	282.2	89.5
Na^+	1639	6086	3265	2134
Ca^{2+}	163.7	293.3	292.5	115.2
S-SO_4	2459	4441	2198.2	1941.6

TOC: Total organic carbon; SAR: Sodium adsorption ratio.

In order to characterize the tailings, 80 equidistant sampling sites were established in a grid of 15 m × 7 m, covering a total area of 6615 m^2. Tailing samples of about 1 kg were collected at three depths: 0–10 cm, 10–20 cm and 20–30 cm.

2.2. Physicochemical characterization of mine tailing

Physical and chemical characteristics of the tailings used in this study at the three different depths are presented in Table 1.

pH was measured potentiometrically in a 1: 2.5 tailing–water suspension. EC was measured in the saturated tailing-paste extract using the Hanna HI4321 benchtop conductivity meter. Total metal concentrations were measured by atomic absorption spectrophotometry (AAS). All the samples were run in triplicate. The exchangeable sodium percentage (ESP) was used to assess sodicity (Fan and Kong, 2013; Emami et al., 2014). ESP was estimated from SAR (sodium adsorption ratio) based on the linear equation (USDA. U.S. Salinity Laboratory Staff, 1954):

$$ESP = \frac{100 \cdot K_g \cdot SAR}{1 + K_g \cdot SAR} \quad (1)$$

This relationship is based on the Gapon equation by assuming Gapon selectivity coefficient, K_g, of Na − (Ca + Mg) exchange as 0.0147 (Wang et al., 2014). Constant K_g was evaluated by the USDA. U.S. Salinity Laboratory Staff (1954). There are various classification systems of salt-affected soils. The most widely used system is the classifications of the US Salinity Laboratory, which are based on EC and ESP. Based on the results obtained, the tailing was classified as saline sodic.

2.3. Amendments and plant species

Soil salinity and sodicity are escalating problems worldwide, especially in arid and semi-arid regions (Mahmoodabadi et al., 2013). In this case, the tailing is sandy and saline-sodic, with a very low content of Ca^{2+} and a very high concentration of S-SO_4. High levels of metal sulfides, such as pyrite (FeS_2) and sulfide wastes, H_2SO_4 could be produced by pyrite oxidation. The amelioration of saline-sodic soils needs sodium to be removed from soil and this can be managed by adding soluble calcium salts such as gypsum, and afterward leaching the exchanged Na^+ out of the root zones (Hafez et al., 2015). Recently, (Lam

Table 2
Characteristics of the organic amendment used.

Parameter	Unit	Value
pH		7.3
C/N		27
Moisture content	%	40
Organic content	%	40
EC	$dS\ m^{-1}$	0.2

et al., 2016) stated that sulfuric acid reacts with calcium carbonate present in tailings to produce calcium sulfate, which crystallizes as gypsum. It is expected that the generated gypsum has the same effect as the direct application of gypsum and the potential advantage of inhibiting the toxic effects of sulfuric acid. Based on the above hypothesis, $CaCO_3$ was chosen as the inorganic amendment. $CaCO_3$ was obtained from eggshells since calcium carbonate makes up about 94% of the shell (Murakami et al., 2007). The dose of $CaCO_3$ was determined based on the method described by Sobek et al. (1978), which theoretically estimates maximum acidity and neutralization capacity or potential, as a result of providing the amount of $CaCO_3$ required to neutralize concentrations of total sulfur and sulfates that can produce H_2SO_4 on contact with water. The estimated amount of eggshells was $40.5\ g\ kg^{-1}$.

The addition of organic matter to tailings has been shown to improve physical and chemical properties of tailings by increasing metal immobilization, promoting aggregated structure development, providing nutrients for vegetation and increasing water-holding capacity (Lee et al., 2014) and it also improves textural properties and fertility (Milczarek et al., 2004). Table 2 shows the characteristics of the commercial compost. The dose of compost was calculated by the method of Hirzel (2010), which is based on the apparent density of the soil, soil organic matter content and the moisture level of the amendment. The result provided by this method was a dose of $52.5\ kg\ m^{-2}$.

Arbuscular mycorrhizal fungi (AMF) were applied with the purpose of alleviating metal stress on plants (Kohler et al., 2015). The AMF used was *Glomus intraradices*. The fungi were distributed in the tailing and placed in contact with the plant species for eight weeks. The quantity of mycorrhizae, in accordance with the supplier's recommendations, was $15\ g$ mycorrhizal m^{-2}, which was applied directly to the roots of the plants.

Establishing plant species on mine tailings in arid and semiarid regions is impeded by physicochemical factors including extreme temperatures, low precipitations, high winds and high salt concentrations (Munshower, 1994). Three native plant species were used in this study: *P. tamarugo*, *S. molle* and *A. nummularia*. These plants were selected on the basis of their natural presence in northern Chile and their capacity to grow in sites with similar characteristics to those of the tailing under study (Mooney et al., 1980; Mur, 2015; Fernández et al., 2016). The species were planted in March 2014 and collected in February 2015. First, they were transferred to a vivarium in the mine for a period of one month and later transplanted to the tailing site. The average height of each species was 57, 40 and 65 cm for *A. nummularia*, *P. tamarugo* and *S. molle*, respectively.

2.4. Plant analyses

Shoots and roots were divided and mechanically cleaned with deionized distilled water for approximately 5 min to remove soil particles adhering to the plants, rinsed and dried at 70 °C in a gravity oven for 48 h. They were ground into powder with an electric grinder and passed through a 2-mm sieve (Máthé-Gáspár and Anton, 2005), after which 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 (Ryan et al., 2001). Plant extracts were diluted to 50 ml with doubled distilled water and then digested in a hot air oven at 95 °C for two hours until digestion was completed (Mkumbo et al., 2012). The solution was then filtered, followed by atomic absorption spectrophotometry (AAS) analysis of Cu, Mn, Fe, Pb, Zn and Cu. Total metal content was measured in shoots and roots. All the analyses were run in triplicate.

2.5. Experimental design and statistical analysis

To evaluate the phytoremediation potential of the species, the following treatments were applied: T0, control tailing without amendment; T1, $CaCO_3$ and compost and T2, $CaCO_3$, compost and *arbuscular mycorrhizal fungi*. When adding the amendments to the tailing, $CaCO_3$ was the first to be incorporated, being mixed and homogenized with the tailing. The conditioned substrate was allowed to stand for 6 weeks, subsequently compost was added, homogenizing the new substrate. Plantation was done 3 weeks after conditioning the tailing with both amendments.

Each of the three treatments was applied to 10 specimens of each species, therefore, a total of 90 specimens were examined in the course of this study (3 species × 3 treatments × 10 specimens). The spatial distribution was: $20\ m^2$ for *A. nummularia*, where the distance between plants was 60 cm; in the case of *P. tamarugo*, the block area was $72\ m^2$ and the separation between units was 1.5 m, and for *S. molle*, the experimental block area was $53\ m^2$, with a separation of 1.2 m between plants.

In order 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 Bioconcentration Factor (BCF) and the Translocation Factor (TF) of Cu, Mn, Fe, Pb, Zn and Cd were estimated. The evaluation and selection of plants for phytoremediation purposes entirely depends on these factors (Ali et al., 2013). BCF was calculated as follows (Kamari et al., 2014):

$$BCF = \frac{[\text{metal}]_{\text{roots}}}{[\text{metal}]_{\text{tailing}}} \tag{2}$$

As discussed by Azlan et al. (2014) and Kamari et al. (2014), BCF is a measure of the ability of a plant to accumulate metals from soil (tailing in this case). According to Baker (1981) and Rezvani and Zaefarian (2011) the following criteria must be considered: if $BCF < 1$ the plant is an excluder, if $1 < BCF < 10$ the plant is an accumulator and if $BCF > 10$ the plant is a hyperaccumulator. Plants with a BCF value > 1 are suitable for phytoextraction (Kamari et al., 2012; Kamari et al., 2014).

The translocation factor indicates the efficiency of the plant in translocating accumulated metals from their roots to shoots (Ahmadpour and Soleimani, 2015; Stefanowicz et al., 2015), and is a measure of the ability of a plant to translocate metals from the roots to the shoots (Mahdavian et al., 2015; Swarnalatha and Radhakrishnan, 2015).

$$TF = \frac{[\text{metal}]_{\text{shoots}}}{[\text{metal}]_{\text{roots}}} \tag{3}$$

where $[\text{metal}]_{\text{roots}}$ is the metal concentration in the roots, $[\text{metal}]_{\text{shoots}}$ is the metal concentration in the shoots 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 (Baker and Brooks, 1989) 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 three-way ANOVA was applied to compare the effects of bioconcentration and translocation factors, at a 0.05 level of significance on the data (Gomez and Gomez, 1984). The factors were: species, treatment and metals. The first two factors had three levels each (*P. tamarugo*, *S. molle* and *A. nummularia* for the species and T0, T1 and T2 for the treatments) and the last factor had six levels (Cu, Mn, Fe, Pb, Zn and Cd). Similarly, for the analysis of the removal efficiency, two separate three-way ANOVA procedures were applied. The first one had treatment, species and nutrients (SO_4, Na, Mg and Ca) as factors, while the second one had treatment, species and metals as factors. Finally, for the analysis of the effects on pH, density, water saturation and electrical conductivity, four separate two-way ANOVA procedures were applied, using the species and treatments as factors. All statistical analyses were performed using the R language for statistical computing (R Development Core Team, 2008). The data were analyzed using the general linear model of a three-way analysis of variance (ANOVA), followed by Tukey's test at a significance level of $p = 0.05$ for the comparison of means.

3. Results and discussion

3.1. Effects on tailings

Table 3 shows the results for pH, apparent density, saturated water and electrical conductivity of the tailings under the initial conditions and the properties of the substrate after transplanting the species and applying treatments T0, T1 and T2 for 11 months. To evaluate the effect of the species on the substrate, the results for the untreated tailings and treatments T1 and T2 were compared. All the evaluated properties decreased due to the presence of the plants.

With most species identified as highly adaptive, pH tended to decrease, which may be due to irrigation and the addition of organic matter during plant development. This trend was observed in the substrates of *A. nummularia* and *P. tamarugo* for the three treatments. Tailing samples of *S. molle* presented the highest pH values. Apparent density is an important parameter to measure compaction. The tailing for *S. molle* presented the highest apparent density values, indicating problems in the structure. Tailing samples of *A. nummularia* and *P. tamarugo* with treatments T1 and T2 presented the lower values, representing tailings with better properties for plant adaptation. In general terms, tailing density decreases due to the effects of amendments and the presence of vegetal species.

Tailings presented a high level of compaction owing to small particle size, with 71.6% of saturated water. Given the large volumes of water eliminated by surface evaporation, tailings can be considered water-retaining substrates. Treatments based on amendments increased aeration capacity and, therefore, the capacity to filter water. The average values of saturated water for *P. tamarugo*, *A. nummularia* and *S. molle* were 60%, 49% and 37%, respectively. Electrical conductivity decreased significantly for all the treatments. Among the possible causal factors are tailing conditioning, plant development and irrigation, which is responsible of salt wash. The average decreases of electrical conductivity for tailings containing *P. tamarugo*, *A. nummularia* and *S. molle* were 74%, 69% and 66%, respectively.

From these results, four analyses of variance at a significance level of 5% were carried out for the four parameters under study. No statistical evidences were found for the values in tailings containing the three species and the applied treatments.

According to these results, *A. nummularia and P. tamarugo* presented high adaptation capacity and positive impacts on the substrate – plant interaction. The best results were obtained for *P. tamarugo* in tailings treated with organic amendment and mycorrhizae.

In order to evaluate the effects of the amendments, the initial total concentrations of the tailings and the total concentrations of the same tailings after the treatments were compared. This was done through the calculation of the removal efficiency (RE), which is based on the following equation:

$$RE = \frac{100\bullet(C_i - C_f)}{C_i} \quad (4)$$

where C_f and C_i are the final and the initial total concentration, respectively. Table 4 and Table 5 present the chemical characterization of the tailings after the treatments with respect to nutrients and metals, respectively.

Concentrations of SO_4 decreased significantly from the initial levels. This inorganic anion is responsible for acidification and is a good indicator of the tendency to decrease potential acid drainage risk produced by the reaction between SO_4 and water. On average, SO_4 concentrations decreased in the tailing samples of the species *A. nummularia*, *P. tamarugo* and *S. molle* by 68%, 62% and 68%, respectively. Significant decreases in Na concentrations were detected. This was attributed to irrigation, which dissolves and transports salts due to water infiltration. Salinity causes stress and affects the adaption of plants. The average value of the decrease in sodium content of the treated tailing was 86%. Magnesium concentrations decreased significantly in all the tailings. The data show a decrease of 86, 80 and 91% for the tailings of *A. nummularia*, *P. tamarugo* and *S. molle*, respectively. Calcium is a vital nutrient for plant development, since it allows for cell division and internal permeability (Russell, 2002). The decrease in calcium is caused by plant–tailing interaction. The average decreases in the tailing samples for *A. nummularia*, *P. tamarugo* and *S. molle* were 41%, 31% and 63%, respectively.

The concentrations of essential elements for plant growth decreased for all the tailings, demonstrating in the case of untreated tailings, that they were absorbed by the plants. In the case of the tailings under treatment T1 and T2, the same tendency is observed, which could be attributed to the dilution of the nutrients in the added amendments and/or the absorption of these nutrients by the plants. In general, from the results of the three-way ANOVA and the subsequent Tukey's tests, it appears that the removal efficiency of *A. nummularia* is reduced when the additional amendments are applied, though no statistically significant difference was found at a 0.05 level of significance. For the other two species, *P. tamarugo* and *S. molle*, applying amendments showed an increase in the removal efficiency, though again no statistically significant difference was found between treatments. However, a statistically significant difference was found between the effects of the *P. tamarugo* and *S. molle* species. In particular, *S. molle* provided greater removal efficiency, especially when combined with the additional amendments.

Cu concentrations decreased in all the tailing samples. In average,

Table 3
pH, ρ_A, Sat. water and EC of the tailings before and after the treatments.

Species	–	*Atriplex nummularia*			*Prosopis tamarugo*			*Schinus molle*		
Treatment	Original tailing	T0	T1	T2	T0	T1	T2	T0	T1	T2
pH	8.42	7.95	7.89	7.90	7.91	7.92	7.94	8.02	7.94	7.97
ρ_A (ton m^{-3})	1.47	1.19	0.98	0.91	1.14	0.87	0.89	1.15	1.26	1.21
Sat. water (%)	71.6	45.2	51.1	51.6	62.9	58.9	57.3	41.4	33.0	36.7
EC (dS m^{-1})	35.5	7.25	12.7	15.6	13.0	8.03	8.86	20.9	11.6	6.61

EC: Electrical conductivity at 25 °C; ρ_A apparent density.

Table 4
Chemical characterization of the tailings after treatments for nutrients.

Species	Treatment	SO_4 (2198.2)[a]	Na (3264.8)[a]	Mg (402.3)[a]	Ca (292.5)[a]
		C_f mg kg^{-1} (%RE)			
Atriplex nummularia	T0	558.5 (74.59)	238.7 (92.69)	25.3 (93.71)	161.8 (44.68)
	T1	733.4 (66.64)	488.5 (85.04)	64.3 (84.02)	176 (39.83)
	T2	843.4 (61.63)	608.6 (81.36)	75.4 (81.26)	176.5 (39.66)
Prosopis tamarugo	T0	965.5 (56.03)	652.9 (80.00)	88.6 (77.98)	217.8 (25.54)
	T1	688.3 (68.69)	327.8 (89.96)	65 (83.84)	174.2 (40.44)
	T2	822.9 (62,56)	371.4 (88.62)	89.3 (77.80)	211.7 (27.62)
Schinus molle	T0	1327 (39.63)	823.9 (74.76)	41.9 (89.58)	127.2 (56.51)
	T1	463 (78.94)	283.7 (91.93)	43.2 (89.26)	120.6 (58.77)
	T2	343.2 (84.39)	163.7 (94.99)	24.9 (93.81)	78.1 (73.3)

[a] The values in parentheses are the average values of the tailing initial concentrations (mg kg^{-1}).

Table 5
Chemical characterization of tailings after treatments for metals.

Species	Treatment	Cu (11151.4)[a]	Mn (352.5)[a]	Zn (282.2)[a]	Fe (35015.3)[a]	Cd (1.7)[a]	Pb (183.2)[a]
		C_f mg kg^{-1} (%RE)					
A. nummularia	T0	6055 (45.70)	324.1 (8.06)	170.9 (39.44)	22,451 (35.88)	3.093 (−81.94)	93.7 (48.85)
	T1	3120 (72.02)	428.7 (−21.62)	180.8 (35.93)	23,525 (32.82)	0.1 (94.12)	97.6 (46.72)
	T2	4526 (59.41)	406.4 (−15.29)	163 (42.24)	22,996 (34.33)	0.449 (73.59)	82.0 (55.24)
P. tamarugo	T0	7232 (35.15)	449.1 (−27.40)	210.7 (24.34)	29,408 (16.01)	3.387 (−99.24)	117.7 (35.75)
	T1	3450 (69.06)	428.7 (−21.62)	147.6 (47.70)	23,525 (32.82)	1.6 (5.88)	77.0 (57.97)
	T2	1758 (84.24)	350.7 (0.51)	147.4 (47.77)	19,570 (44.11)	1.596 (6.12)	76.7 (58.13)
S. molle	T0	4492 (59.72)	211.3 (40.06)	112.1 (60.28)	19,911 (43.14)	1.596 (6.12)	76.4 (58.30)
	T1	1014 (90.91)	185.5 (47.38)	177.31 (37.17)	29,855 (14.74)	0.199 (88.29)	83.0 (54.69)
	T2	1147 (89.71)	252.4 (28.40)	108 (61.73)	28,426 (18.82)	0.1 (94.12)	18.8 (89.74)

[a] The values in parentheses are the average values of the tailing initial concentrations (mg kg^{-1}).

Fig. 1. Distribution of decreases in Cu, Zn, Fe, Pb concentrations in the substrate for the three treatments T0, T1 and T2 (An: *Atriplex nummularia*, Pt: *Prosopis tamarugo* and Sm: *Schinus molle*).

the tailing of *S. molle* provided the best results, with a decrease of Cu concentration of approximately 91% for treatment T1 and 90% for treatment T2. Given the pH range, bioavailability could increase if pH decreased. Mn concentrations tended to increase, except in the tailing of *S. molle*. The increment is attributed to the heterogeneity of the tailing, taking into account that the original characterization was the average of the points defined in the sampling design.

Zn plays an important role in many plant functions. Zn stimulates plant growth; however, above certain concentrations it can be toxic for plants. Therefore, it is necessary to evaluate Zn availability and the plant's capacity to absorb this element (González and Rivero, 2011). A decrease of Zn was observed in all the tailings, which can be attributed to the tailing's heterogeneity or to the absorption of Zn by the plant, and for the specific case of the tailing under T1 and T2 treatments, it could also be due to an effect of dilution caused by the addition of amendments. The average concentrations decreased by 39, 40 and 53% for the tailings of *A. nummularia*, *P tamarugo* and *S. molle*, respectively. As with Cu, due to the pH range, bioavailability could increase if pH decreased.

Fe plays an important role in the process of photosynthesis. Lack of Fe causes serious effects on the plant, from the roots to the shoots (Sequi and Piaggesi, 2004). Results suggest that Fe is assimilated by the three plants. The average concentrations decreased by 34, 31 and 36% for the tailings of *A. nummularia*, *P. tamarugo* and *S. molle*, respectively.

Cd is not essential for plants, in fact, it is considered toxic in low concentrations for most living organisms. Cd interferes with the absorption of essential elements for plants such as Ca, Mg, K and P (Navarro-Aviñó et al., 2007). There are no clear tendencies, but it appears that phytoremediation can remove Cd. The high values of the final sample can be explained by the heterogeneity of the tailings.

Pb concentrations decreased significantly in all the amended substrates. The data show an average decrease of 50, 50 and 68% for the tailings of *A. nummularia*, *P. tamarugo* and *S. molle*, respectively. The effect of treatment T2 on the substrate containing the species *S. molle* should be noted, it had a removal capacity for Pb of almost 90%, which can be explained by the heterogeneity of the tailing, a dilution effect due to the amendments added and/or the plant's removal capacity.

Fig. 1 shows the average removal efficiency for Cu, Zn, Fe and Pb for each applied treatment. The average decreases in total metal concentrations in the substrate were 43%, 50% and 54% for treatments T0, T1 and T2, respectively. Mn and Cd were not considered because the concentrations increased over the initial values (for same cases).

In general, the results from the three-way ANOVA and the subsequent Tukey test showed a statistically significant difference between the effects of treatment T0 and the other two treatments. Treatment T0 presented lower removal efficiency than treatments T1 and T2. Furthermore, no significant difference was found between treatments T1 and T2. Therefore, the use of amendments seems to improve the removal efficiency of the different species compared to the use of plants alone. *S. molle* showed higher removal efficiency than *P. tamarugo* at the 0.05 significance level (this holds even if Mn is not considered in the analysis), however no other significant differences were found among species.

3.2. Accumulation and translocation of metals in plants

The total metals contents (Cu, Mn, Fe, Pb, Zn and Cd) were measured in tailings, plant shoots and plant roots. From these results the bioconcentration and translocation factors were calculated. The results are shown in Tables 6 and 7, respectively. The data showed that total metal content in plant tissue differed among the species at the tailing, indicating different capacities for metal uptake. *A. nummularia* accumulated average concentrations of Mn (82.7 and 68.8 mg kg^{-1}-dry) and Pb (11.2 and 15.4 mg kg^{-1}-dry) in its roots and shoots respectively, which were higher than the accumulations in *P. tamarugo and S. molle*.

Considering the criteria for hyperaccumulator plants, none of the plant species showed metal concentrations as high as those values, so none of them can be considered as hyperaccumulators. However, the ability of these plants to translocate and accumulate metals may be useful for phytostabilization (Yoon et al., 2006).

As it can be observed in Table 6, the three species can be considered as excluders of Cu, Mn, Fe, Pb and Zn. In the case of Cd, *A. nummularia* presented characteristics of an accumulator for the tailing treated with $CaCO_3$/compost/mycorrhizae and hyperaccumulator for the tailing treated with $CaCO_3$/compost. *P. tamarugo* showed characteristics of an accumulator for the tailing treated with $CaCO_3$/compost/mycorrhizae and *S. molle* presented characteristics of an accumulator for the tailing treated with $CaCO_3$/compost.

Table 6
Bioconcentration factor of Cu, Mn, Fe, Pb, Zn and Cd in selected plants for different treatments.

Scientific name	Treatment	Bioconcentration factor (BCF)					
		Cu	Mn	Fe	Pb	Zn	Cd
A. nummularia	T0	0.12	0.33	0.06	0.11	0.14	0.34
	T1	0.17	0.11	0.03	0.06	0.11	17.89
	T2	0.17	0.23	0.10	0.21	0.27	4.58
P. tamarugo	T0	0.08	0.10	0.06	0.06	0.09	0.33
	T1	0.18	0.11	0.06	0.01	0.29	0.72
	T2	0.31	0.21	0.08	0.07	0.34	1.33
S. molle	T0	0.02	0.10	0.13	0.05	0.17	0.42
	T1	0.15	0.07	0.03	0.08	0.09	3.36
	T2	0.28	0.02	0.03	0.06	0.37	nd

nd stands for not detected.

Table 7
Translocation factor of Cu, Mn, Fe, Pb, Zn and Cd in selected plants for different treatments.

Scientific name	Treatment	Translocation factor (TF)					
		Cu	Mn	Fe	Pb	Zn	Cd
A. nummularia	T0	0.17	0.71	0.39	1.33	1.05	0.75
	T1	0.18	1.15	1.69	2.74	2.35	0.67
	T2	0.13	0.80	0.40	0.96	1.57	0.33
P. tamarugo	T0	0.24	0.59	0.35	0.86	1.44	1.13
	T1	0.14	1.15	0.65	0.004	0.65	0.58
	T2	0.34	0.34	0.35	0.42	0.56	0.97
S. molle	T0	2.78	1.61	0.16	1.36	1.22	nd
	T1	0.96	1.25	0.40	0.70	1.97	nd
	T2	0.83	3.83	0.46	1.95	0.68	nd

nd stands for not detected.

The average BCF values of metals in the tailing were: Cd (1.46) > Zn (0.21) > Cu (0.16) > Mn (0.14) > Pb (0.08) > Fe (0.06). A BCF > 1 indicates high root accumulation. The BCFs were > 1 only for Cd for the three plants, *A. nummularia* presented the highest BCF. Among the three treatments applied with the three species, T2 showed the highest BCF for Cu and Zn. For Mn, *A. nummularia* had the highest BCF with all the treatments.

A three-way ANOVA was applied to compare the means of the different treatments for BCF at a 0.05 level of significance. Initially, the analysis included Cd and significant differences were found between the BCF for Cd and all the other metals at a 0.05 level of significance. However, because of this anomalous behavior of Cd compared to the other metals, it was omitted to better analyze the other results. From the results obtained for the BCF, it can be noted that the type of treatment has significant effects with a *p*-value of 0.006. Moreover, a *p*-value of 0.001 indicates an effect on the metals, that is, the type of metal significantly influences bioconcentration. No significant differences were found between the different species. The subsequent Tukey's test revealed that treatment T2 has a greater BCF than treatments T0 and T1 with *p*-values of 0.0149 and 0.0229, respectively.

As it can be seen in Table 7, *A. nummularia* presents potential to accumulate Pb and Zn in the aerial part, without needing to treat the tailing with any amendment. It was observed that when the tailing was treated with $CaCO_3$/compost, there was an increase in the accumulation potential of Mn, Fe, Pb and Zn. The results show that the addition of mycorrhizae decreased the translocation of metals from the roots to the aerial parts.

Regarding *P. tamarugo*, it presents potential to accumulate Zn and Cd in the aerial part, without needing to treat the tailing with any amendment. It was also observed that the addition of amendments decreases the effectiveness of the transport of metals in the aerial part of the plant, favoring the phytostabilization potential of the metals in its roots. It only showed potential accumulation of Mn for the tailing treated with $CaCO_3$/compost.

For its part, *S. molle* presents potential to accumulate Cu, Mn, Pb and Zn in the aerial part, without need of amending the tailing. It was also observed that the addition of $CaCO_3$/compost decreased the transport potential of Cu, Mn and Pb from the root to the aerial part, favoring phytostabilization in the roots. On the contrary, an increase in the transport of the metals Fe and Zn to the aerial part was observed. Regarding the addition of Mycorrhizae, in contrast to the other two species, it showed a significant increase in the translocation of Mn and Pb from the roots to the aerial part of the plant.

A three-way ANOVA was applied to compare the means of the different treatments for TF at a 0.05 level of significance. The ANOVA indicated that the species significantly affects metal distribution, with a *p*-value of 0.0166. A Tukey test was applied to analyze the differences in mean values. Statistically significant differences between the species

P. tamarugo and *S. molle* were found for the translocation factor at a significance level of 5%. Specifically, *S. molle* has a higher TF than *P. tamarugo*, as can be seen in Table 7.

As shown in Tables 6 and 7, none of the sampled plants were suitable for phytoextraction of Cu, Mn, Fe, Pb and Zn, but *A. nummularia* was the most suitable for phytoextraction of Cd for treatments T1 and T2 (BCF = 17.89 and BCF = 4.58 and TF = 0.67 and TF = 0.33 respectively) and *P. tamarugo* for treatment T2 (BCF = 1.3 and TF = 0.97).

4. Conclusions

Regarding the effect of the amendments, in general, the untreated tailing presented lower removal efficiency than the tailings treated with $CaCO_3$/compost (T1) and $CaCO_3$/compost/mycorrhizae (T2). Fig. 1 shows the potential effect of the amendments and plant species in the decrease of the metal content in the tailing under study, indicating that the addition of amendments favors the removal (or dilution) of Cu. In the case of Fe, it is observed that the interaction amendment/plant species has an effect. There is no significant effect of the addition of amendments on the removal (or dilution) of Zn from the substrate. The decrease of Pb concentration from the tailing was significantly higher when the substrate was under treatment T2 with the species *S. molle*.

The BCF and TF values allowed comparing the ability of species *A. nummularia*, *P. tamarugo* and *S. molle*, in taking up metals from tailings and translocating them to the shoots. The three species can be considered as excluders of Cu, Mn, Fe, Pb and Zn, since they presented BCF values lower than 1 (see Table 6). Considering TF, *A. nummularia* was found to be an accumulator mainly of Mn, Pb and Zn, while *S. molle* showed characteristics of an accumulator of Cu, Mn, Pb, and Zn. On the contrary, *P. tamarugo* in average presented a TF lower than 1, and therefore, it is not considered in this work as a metal accumulator, except in the case of Mn (under T1 treatment), Zn (untreated tailing) and Cd (under T1 treatment). Our results also showed that in the tailing treated with amendments (T1 and T2), BCF values were higher than 1 and TF values were lower than 1 for the species *A. nummularia*, and therefore, it can be considered as the most promising species for phytostabilization of Cd in tailings.

Acknowledgments

This study was conducted in the framework of a CORFO-INNOVA project (08CM01-05), titled "Integrated development of magneto-chemical technologies and phytotechnologies applied to the remediation of heavy metals in the development of mining environmental liabilities".

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