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Assessment of the adaptive capacity of plant species in copper mine tailings in arid and semiarid environments

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Abstract
Purpose The objective of this work was to identify hyperaccumulator plants and evaluate their capacity on copper mine tailings in the Antofagasta Region (Chile), considered one of the most arid in the world.
Materials and methods Two native plant species, *Gazania rigens* and *Pelargonium hortorum*, were grown during 11 weeks on mine tailings. The physico-chemical characterization of the mine tailings under study indicated that the substrate required conditioning to support a phytoremediation system. In this respect, organic and inorganic amendments and mycorrizhal fungi were added to the substrate. Three treatments were designed to assess the effects of the amendments through an analysis of variance.
Results and discussion Indicators of plant growth and development were measured weekly, and concentrations of Cd, Cu, Fe, Mn, Pb, Al, and Zn in roots of tailing-grown plants and substrate were measured at the end of the experiment.
Conclusions The results were used to determine the bioconcentration factor (BCF), which demonstrated that both species act as excluders of Fe, Mn, Pb, Al, and Zn. In addition, it was found that both species present characteristics of potential accumulators of Cu.

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1 Introduction

The abundance of mineral resources in Chile is well-known; it is, in fact, the largest copper producer in the world. The Atacama Desert has important deposits of copper, gold, silver, molybdenum, and mineral products from saltpans like nitrates and others (Ghorbani and Kuan 2016). However, mining extraction is also responsible for significant environmental impacts such as the generation of mine tailings which affect the quality of air, soil, and water (Salomons 1995; Bell and Donnelly 2006; Sims et al. 2013; Sağlam and Akçay 2016). Mine tailings consist of a mixture of ground rock and process effluents generated in the mine's processing plant. Their composition and size depend on the mining method applied (Kossoff et al. 2014).

During the last years, after the closure of a mine, tailings were abandoned without an adequate management plan. Due to the high content of heavy metals, tailings represent an important risk to human health and environment (Lim et al. 2008). Currently, the new Chilean legislation on mining (Law 20,551) establishes that tailings must be physically and chemically stabilized. The mine closure plan must include a description of the methodology and effectiveness of the remediation and rehabilitation works. Among all the techniques employed in this regard, phytoremediation is an emerging technique that can be used to stabilize mine tailings. This method has been extensively used to reduce and control pollutant concentrations in contaminated environments. This technique is based on the use of plants and associated soil microorganisms which are able to degrade or eliminate contaminants reducing concentrations

and their toxic effects on the environment (Salt et al. 1995; McIntyre 2003).

Phytoremediation can be carried out with vascular plants (Rahimi and Manavi 2010; Martínez-Fernández and Walker 2012; Alizadeh et al. 2012) or algae (Pinto et al. 2002; Gomes and Asaeda 2013) that have the capacity to extract (by absorption or adsorption), store, precipitate, volatilize, or degrade organic and inorganic toxic substances in their surroundings. Phytoremediation can occur through several processes such as phytoextraction (also known as phytoaccumulation or phytoadsorption), phytofiltration, phytoestabilization, phytovolatization, phytodegradation, rhizodegradation, and phytodesalination (Ali et al. 2013). Among all the processes, phytoestabilization and phytoextraction have demonstrated to be more suitable for soils contaminated with heavy metals (Robinson et al. 2006; Meeinkuirt et al. 2012; Yang et al. 2014). The basis of phytostabilization is the coverage of the surface of the contaminated soil with plants which reduce mobility of contaminants through accumulation by roots or immobilization in the rhizosphere. Phytostabilization immobilizes the contaminants in the soil, preventing its migration and reducing availability of heavy metals. It has been demonstrated to be more effective in fine-textured soils with high concentrations of organic matter (Padmavathiamma and Li 2007). 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 other hand, 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 (Yoon et al. 2006; Rafati et al. 2011). Transport of contaminants to buds is a critical biochemical process for an effective phytoextraction because collection and storage in roots cannot be viable (Zacchini et al. 2009; Tangahu et al. 2011). Phytoextraction requires the use of plants able to accumulate and transport high concentrations of toxic metals (Padmavathiamma and Li 2007; Mendez and Maier 2008). The most easily absorbed inorganic materials are As, Cd, Cu, Ni, Se, and Zn; moderate bioavailable metals include Co, Fe, and Mn, while Cr and Pb present low bioavailability (Wuana and Okieimen 2011).

While phytoremediation has been extensively applied in soils with organic and inorganic contaminants (Weyens et al. 2013; Afzal et al. 2014; Doni et al. 2015), it is a relatively new technique to remediate mine tailings (Novo et al. 2013; Sánchez-López et al. 2015). Before applying phytoremediation to mine tailings, it is necessary to modify physico-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 and nutrients since tailings have low levels of microbial activity (De la Iglesia et al. 2006). 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 bioavailability of metals and irrigation increases growth of plant species (Mendez and Maier 2008).

This paper is aimed to evaluate the accumulation capacity of two native plant species, *Gazania rigens* and *Pelargonium hortorum*, in abandoned copper mine tailings in the Antofagasta Region in Chile.

2 Material and methods

2.1 Mine tailing samples

This work was carried out in copper mine tailings located in the Antofagasta Region, in the North of Chile. The economic development of this region is mainly based on copper mining. According to CODELCO, the National Copper Corporation of Chile, the region has a production capacity of 150,000 t per year of high-purity copper cathodes.

This area is characterized by high levels of evaporation and low levels of water infiltration resulting in high salt concentrations; these conditions make for scarce vegetation (Cavieres et al. 2002). Tailing samples were collected from a copper mine tailings site in a mining area approximately 175 km southeast of the city of Antofagasta in the Andean foothills at an altitude of 3200 m above mean sea level. In this site, the tailings were arranged in terraces until the year 2006. These terraces have high concentrations of heavy metals, thus representing an important environmental problem.

In previous studies, the tailing was characterized as being extremely saline and sodic, with highly clayey soils which are not suitable for vegetation (Lam et al. 2016). Regarding chemical characterization, high concentrations of metals such as Al, 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, (EC), exchangeable sodium percentage (ESP), and organic matter (OM) for each depth are given in Table 2.

2.2 Plant species

The species considered in this work were *G. rigens* and *P. hortorum* (see Fig. 1), which were initially selected due to their natural presence in Northern Chile and their ability to grow in sites with similar physico-chemical characteristics than the tailings under study.

2.3 Amendments

The pot experiment was conducted using three amendments (eggshells, vermicompost, and arbuscular mycorrhizal fungi).

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	19,236	41,923
Mn		279.3	467.8
Cu		1008	16,296
Zn		108.6	306.6
Pb		75.7	215.4
Cd		< 0.1	3.9
Cr		2.9	10.4
Al		18,052	23,841
Mo		59.9	133.7
As		2.8	4.2
B		57.3	94.1
K^+		18.0	68.5
Na^+		1639	6086
Mg^{+2}		30.1	126.5
Ca^{+2}		163.7	293.3
Cl^-		2161	10,001
SO_4^{-2}		2459	4441
SAR	–	31.5	90.1
pH	–	8.2	8.6
Saturated water	%	63.1	74.0
EC	dS m^{-1} (at 25 °C)	15.3	40.1

SAR sodium adsorption ratio, *EC* electrical conductivity

2.3.1 Inorganic amendment

Inorganic amendment was obtained from eggshells because they offer a rich source of calcium carbonate (approximately 94%) with small amounts of magnesium carbonate, calcium phosphate, and other organic matter including proteins (Tangboriboon et al. 2012). The dose of inorganic amendment was determined by the method of Sobek et al. (1978), which theoretically estimates the maximum acidity and the neutralization potential as a result of providing the amount of $CaCO_3$ required to neutralize concentrations of total sulfur and sulfates that could produce sulfuric acid in contact with water. Considering that the average apparent density of tailings is 1.3 g/cm^3, to evaluate the effect of this amendment, three levels were considered: $T0_{IA}$: 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), and $T2_{IA}$: tailing + 8% $CaCO_3$/(0.08 kg in 1 kg of tailing).

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

Data in parentheses are standard deviations

2.3.2 Organic amendment

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. The residual water percolates through various filter beds, and the organic matter is retained and then consumed by earthworms. Subsequently, solid wastes are processed by earthworms to produce a bioproduct known as vermicompost (VC), which was used as organic amendment in this work. Its chemical and physical characteristics are presented in Tables 3 and 4, respectively.

The method of Hirzel (2010) was employed to calculate the dose of organic amendment using Eqs. (1) and (2).

$$OM_d = \frac{OM_a \times AD \times SD}{0.33} \quad (1)$$

Where OM_d is the dose of organic matter (t ha^{-1}), OM_a is the percentage of organic matter added, AD is the apparent density (g cm^{-3}), SD is the sample depth (cm), and 0.33 is the estimated efficiency of net organic matter discharge in the soil.

$$OAD = \frac{OM_d \times 10,000}{OAD_{OM} \times (100 - H_{OAD})} \quad (2)$$

where OAD is the dose of organic amendment (t ha^{-1}), 10,000 is a conversion factor, OAD_{OM} is the percentage of organic matter in the amendment, and H_{OAD} is the percentage of moisture in the amendment.

Considering that the quantity of organic matter ranges the interval 2–4% and the physico-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 the 20-cm depth is between 0.03 and 0.06 kg VC/kg tailing. Based on this result, three 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), and $T2_{OA}$: tailing + 6% VC (0.06 kg in 1 kg of tailing).

2.3.3 Arbuscular mycorrhizal fungi

The arbuscular mycorrhizal fungi (AMF) used was *Glomus intraradices*. *Glomus intraradices* are particles with diameters between 40 and 140 μm, due to which they barely migrate in the soil. Therefore, it is necessary to apply the product in the vicinity of the roots. Contact time for both plants species was 8 weeks; this is due to the time required for the fungal inoculation process to occur.

Fig. 1 Plant species used in this work. *Gazania rigens* (left) and *Pelargonium hortorum* (right)

The amount of mycorrhizal, in accordance with the recommendation of the supplier, is a ratio of substrate/mycorrhizal 2–4% *v*/*v*, which is approximately equivalent to a dosage between 10 and 20 g m^{-2} (mycorrhizal/substrate). The levels considered in this work were 0, 10, 15, and 20 g m^{-2} for *P. hortorum* and 0, 10, and 15 g m^{-2} for *G. rigens*. Considering the surface of the holes that contained the plants (0.0625 m^2, see section 2.3.3), the dose of mycorrhizal applied for *P. hortorum* 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) and for *G. rigens* it was 0 g (0 cm^3), 0.625 g (2.1 cm^3), and 0.94 g (3.1 cm^3). In the case of the latter species, since the volume of the roots was smaller than that of *P. hortorum*, the level of 20 g m^{-2} of mycorrhizal (1.25 g per plant) was not considered.

Table 3 Chemical characterization of VC

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] AENOR (2001a)

[b] Method reference: AENOR (2002)

[c] Method reference: Sadzawka et al. (2005)

2.4 Experimental development

2.4.1 Preparation of the experimental site

The experiments were carried out on the tailing, which was found as consolidated sediments. In order to proceed to experimentation in the field, the first step was to demarcate the site where the plantations would be carried out. The site was plowed with a backhoe loader, which allowed improving terrain structure to facilitate the development of plants.

The second step was the adaptation of the plant species to the specific conditions of the site, such as temperature, winds, and height. To accomplish this, the following three phases were carried out:

Phase 1: 50 specimens of *P. hortorum* and 40 specimens of *G. rigens* were transferred from a municipal vivarium located in the city of Antofagasta. The seedlings were put in polyethylene bags with a capacity of 2.2 kg of substrate per bag. The 90 specimens were kept in a vivarium installed inside the mining company during 45 days. In this period of time, the only change that the seedlings had to undergo was the site change; variables such as substrate and irrigation system were the same as before starting the

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. (2005)

[b] Method reference: AENOR (2001b)

experiment. The vivarium was built in such a way that it allowed protecting the species from the winds and the extreme temperatures.

Phase 2: once the first conditioning was carried out, the species were taken outside the vivarium, keeping the original conditions of substrate and irrigation. The color of the polyethylene bags was black to prevent solar radiation from damaging plants' roots. In this phase, plants were not protected from winds or temperatures. This second conditioning phase lasted for 15 days.

Phase 3: the species were transplanted to the tailing, which was previously conditioned, undergoing the treatments during 9 weeks (the first 6 weeks were used for liming, and the three following weeks for liming and vermicompost). 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 seedlings were placed 50 cm apart. After the seedling transplant, these were kept in direct contact with the mycorrhizal for a period of 8 weeks, to allow the activation of mycorrhizal in the roots.

After the initial conditioning period (6 weeks inorganic amendment + 3 weeks organic and inorganic amendment + 8 weeks of mycorrhizal activation), the process of experimentation for the measurement of variables was started; this lasted for 11 weeks. Three replicates for each treatment and species were grown.

2.4.2 Experimental design

The field experiment was carried out in a randomized block design taking into account three factors: plant species, tailing treatment, and mycorrhizal. To examine the accumulation potential and response of *P. hortorum* and *G. rigens* on copper mine tailings, the treatments consisted of (1) control tailing without amendment (T0), (2) tailing plus 4% $CaCO_3$ + 3% VC, i.e., 0.04 kg $CaCO_3$ + 0.03 kg VC in 1 kg of tailing (T1), and (3) tailing + 8% $CaCO_3$ + 6% VC, i.e., 0.08 kg $CaCO_3$ + 0.06 kg VC in 1 kg of tailing (T2).

Additionally, for treatments T1 and T2 (T0 was not considered since it is the control treatment), the previously mentioned levels of mycorrhizal were considered, specifically:

- *P. hortorum*: the levels considered in this work were 0, 10, 15, and 20 g m^{-2}.
- *G. rigens*: the levels considered in this work were 0, 10, and 15 g m^{-2}.

The symbols used for the treatment are as follows: M(X)-T_j, where M represents mycorrhizal, X (0, 10, 15, 20) is the corresponding level of mycorrhizal in grams per square meter, and T_j is the treatment j (j = 0, 1, 2). The following treatments were evaluated: for *P. hortorum*: M(0)-T0, M(0)-T1, M(0)-T2, M(10)-T1, M(10)-T2, M(15)-T1, M(15)-T2, M(20)-T1 y M(20)-T2; and for *G. rigens*: M(0)-T0, M(0)-T1, M(0)-T2, M(10)-T1, M(10)-T2, M(15)-T1, M(15)-T2. The treatments applied to the plants are shown in Tables 5 and 6.

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 two plant species. In this sense, the null hypothesis is that the different amendments have no significant effect on the adaptation of the plants.

2.5 Effect of the treatments

2.5.1 Effect of the amendments on the adaptation phase of the plants

The effect of the amendments on the adaptation of the plant species was determined by monitoring physical parameters. A linear mixed-effects analysis was carried out using the following tools: (R Core Team 2016), lme4 (Bates et al. 2015), and lmertest (Kuznetsova et al. 2015). Four response variables were considered: height and number of leaves for *G. rigens*, and height and stem thickness (diameter) for *P. hortorum*. The relationship between these response variables and the treatments was studied, in addition to their interaction over time. Thus, four mixed models were evaluated, one for each of the response variables.

The baseline model for each response variable over time for each plant is given by a random intercept model which considers time as having a fixed effect on the response variable. The model is as follows:

$$y_{ij} = \beta_0 + \beta_1 T_{ij} + \eta_i + \varepsilon_{ij}$$

Table 5 Treatments applied to *P. hortorum*

M(X)-treatment	Number 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 mycorrhizal m^{-2}

Table 6 Treatments applied to *G. rigens*

M(X)-treatment	Number 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(X): X g mycorrhizal m^{-2}

where

- y_{ij} is the value of the response variable for plant ($j = 1, 2, 3$) in sample j ($j = 1, \ldots, 11$)
- T_{ij} is the time (in weeks) of the corresponding sample
- β_0 is the fixed intercept parameter
- β_1 is the fixed slope parameter of the model
- η_i is the random intercept element of the model, $\eta_i \sim N\left(0, \sigma_\eta^2\right)$
- ε_{ij} is the error term of the model, $\varepsilon_{ij} \sim N(0, \sigma^2)$

The mixed model used for the contrast for each response variable is given by

$$y_{ij} = \beta_0 + \beta_1 T_{ij} + \beta_2 S_i + \beta_3 M_i + \beta_4 T_{ij} S_i + \beta_5 T_{ij} M_i + \beta_6 S_i M_i + \beta_7 T_{ij} S_i M_i + \eta_i + \varepsilon_{ij}$$

where

- y_{ij} is the value of the response variable for plant ($j = 1, 2, 3$) in sample j ($j = 1, \ldots, 11$)
- T_{ij} is the time (in weeks) of the corresponding sample for the plant
- S_i is the soil treatment of the corresponding plant (note that it does not depend on time)
- M_i is the quantity of mycorrhizal fungi of the corresponding plant (note that it does not depend on time)
- β_0 is the fixed intercept parameter
- $\beta_1, \ldots, \beta_7$ are the fixed slope parameters of the model
- η_i is the random intercept element of the model, $\eta_i \sim N\left(0, \sigma_\eta^2\right)$
- ε_{ij} is the error term of the model, $\varepsilon_{ij} \sim N(0, \sigma^2)$

The individual effect of time and the interaction between time and the two types of treatment were considered as fixed effects in the model. The different values of the intercept for each of the plants were considered as random factors. The p values of the models were obtained by performing a likelihood ratio test of the complete model with all the effects compared against the model that only considered the effects of time and omitted the differences resulting from treatments and their interaction. The p values to test the significance of each one of the parameters of the model were obtained through the use of Satterthwaite approximations as implemented in the *lmerTest* package (Kuznetsova et al. 2015).

Following the results obtained by the initial model, further analyses were carried out to study more thoroughly the effects of the independent variables on the response. Repeated-measurements ANOVA has not been used due to the low flexibility of this approach and to its sensitivity to missing data points (Krueger and Tian 2004).

2.5.2 Phytoremediation potential of the plant species

The phytoremediation potential of the plant species was determined by measuring concentrations of metals both in the substrate and in each plant species, and for all the treatments. Concentrations of Al, Cd, Cu, Fe, Mn, Pb, and Zn were measured, and the bioconcentration factor was calculated using the following formula (Kulkarni et al. 2014):

$$\mathrm{BCF} = \frac{[metal]_{roots}}{[metal]_{tailing}} \quad (3)$$

where $[\mathrm{metal}]_{\mathrm{roots}}$ is the concentration of metal in the roots and $[\mathrm{metal}]_{\mathrm{tailing}}$ is the concentration of metal in the tailing. Azlan et al. (2014) and Kamari et al. (2014) discuss the BCF as a measure of the capability of a plant to accumulate metals from soil, in this case from the tailings. According to the works of Baker (1981) and Rezvani and Zaefarian (2011), the following criteria must be considered for different values of the BCF: if BCF < 1, then the plant is an excluder, if 1 < BCF < 10, the plant is an accumulator, and if BCF > 10, the plant is a hyperaccumulator. According to Kamari et al. (2012, 2014), plants with a BCF value greater than 1 are suitable for phytoextraction.

2.6 Analyses of metals

2.6.1 Plants

Shoots and roots were divided mechanically and cleaned with deionized distilled water for approximately 5 min to remove soil particles adhering to the plants. They were then rinsed and dried at 70 °C in a gravity oven for 48 h. Subsequently, they were ground into powder with an electric grinder and passed through a 2-mm sieve (Máthé-Gáspár and Anton 2005). Afterwards, 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 double distilled water and then digested in hot air oven at 95 °C during approximately 2 h until digestion was completed (Mkumbo et al. 2012). The solution was then filtered, and concentrations of Al, Cd, Cu, Fe, Mn, Pb, and Zn were analyzed by atomic absorption spectrophotometry (AAS) (Jones 2001; Mkumbo et al. 2012).

2.6.2 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 (Fellet et al. 2007; Marchiol et al. 2007). 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 N° 10 mesh), which is the standard particle size for most soil testing methods (Fellet et al. 2007; Clemente et al. 2008).

Bioavailable Fe, Mn, Zn, Cr, Cu, Cd, and Pb contents were measured by an atomic absorption spectrophotometer after extraction using a diethylenetriaminepentaacetic acid (DTPA) solution (Lindsay and Norvell 1978). These metals were extracted by shaking 0.01 kg of oven-dried soil for 2 h in 20 mL of 0.005 MDTPA. The filtrate was analyzed for these metals by AAS. The analysis for As was carried out separately by hydride generation atomic absorption spectrometry (HG-AAS). Hydride was generated using a Perkin-Elmer 100 FIAS system (Hartley et al. 2004). All solutions were filtered with Whatman GF/C fiberglass filter paper.

Boron was extracted (Bingham 1982; Watson 1998) by boiling 0.025 kg of oven-dried soil under reflux with 50 mL of 0.01 M $CaCl_2$ for 5 min in fiber digestion equipment. The samples were cooled and then filtered through a 0.45-μm PTFE, and the filtrate was acidified and analyzed by inductively coupled plasma–atomic emission spectroscopy (ICP-AES, Fassel and Kniseley 1974; Dahlquist and Knoll 1978). Aluminum was extracted from the tailing using potassium chloride (1 M, 1:10 *w/v* tailing: extractant ratio, 30 min shaking). Aluminum in the filtrate was measured by inductively coupled plasma–atomic emission spectroscopy (ICP-AES, Blakemore et al. 1972; Westerman 1990). Molybdenum was analyzed by inductively coupled plasma–atomic emission spectrometry after partial digestion with HCl-H_2O_2 leach and DIBK extract (Briggs 1996).

Chloride and SO_4^{2-} anions (Nieto and Frankenberger 1985a; Nieto and Frankenberger 1985b) and K^+, Na^+, Ca^{2+}, and Mg^{2+} cations (Nieto and Frankenberger 1985b; Basta and Tabatabai 1985) 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 saturated paste extract with a conductivity cell (Rhoades et al. 1989). 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.

Concentrations of metals were measured in tailings (mg/kg) and plant (mg/kg dry weight). Sampling and chemical analyses were run in triplicate in order to evaluate experimental reproducibility.

3 Results

3.1 Adaptation of the plant species

Three seedlings underwent each of the treatments under assessment. For the purposes of the experiment, they were considered as those of better physical evolution when at least two of the three specimens that were subjected to a treatment survived the adverse conditions of the system. The seedlings of *G. rigens* that showed better evolution regarding their physical development were those subjected to the treatments M(0)-T0, M(0)-T2, M(15)-T1, and M(15)-T2. While the seedlings of *P. hortorum* that showed better evolution were those subjected to the treatments M(0)-T0, M(10)-T1, M(20)-T2. Given these results, only the seedlings that presented better evolution were considered.

The behavior of the four dependent variables (height and number of leaves for *G. rigens*, and height and stem thickness for *P. hortorum*) over time under the different treatments has been studied.

3.1.1 Number of leaves (G. rigens)

Figure 2 shows the influence of the treatments on the behavior of this variable over time. The mixed model for the number of leaves fits the data with a high degree of statistical significance ($\chi^2_{(12)} = 33.405, p = 0.0008368$). Although not all the model's coefficients have been found significant with a 95% of confidence, a highly significant effect has been detected in the M(15)-T1 treatment over time (second-order interaction) with $p = 0.000353$; the effect of the interaction corresponds to an increase of 1.9091 [leaves] ± 0.5256 per week.

These results imply that, over time, the M(15)-T1 treatment brings about an increase in the number of leaves of the plant, and thus an improvement of the plant's health. It is concluded that there is a significant difference due to the use of the treatment over time and, in particular, that M(15)-T1 treatment has a positive impact on the plant species during the adaptation phase.

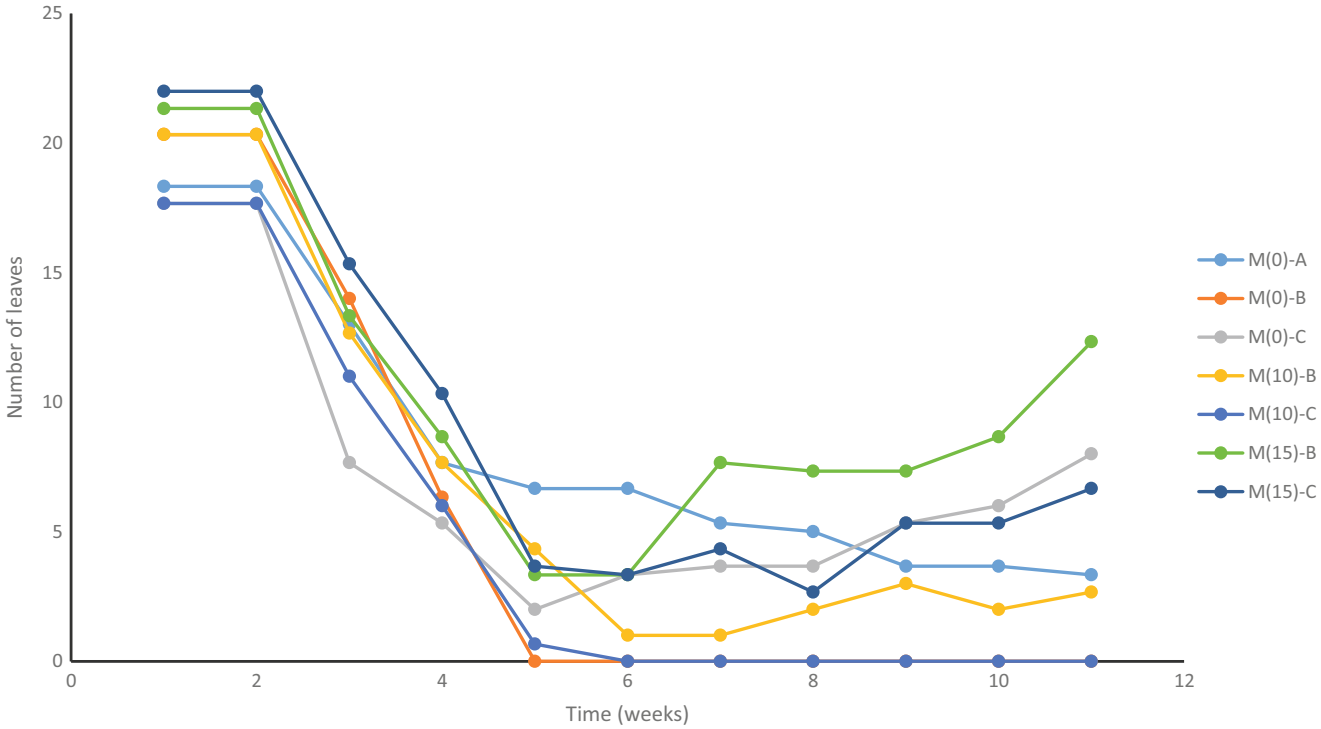

Fig. 2 Number of leaves vs time for each treatment (*G. rigens*)

3.1.2 Height (G. rigens)

The mixed model for height fits the data with a high level of statistical significance ($\chi^2_{(12)} = 117.375, p < 2.2{\cdot}10^{-16}$), but upon observing the parameters and their p values, it could be noted that first-order interactions have a higher influence on the dependent variables than third-order interactions. For this reason, the model was simplified to consider only those variables.

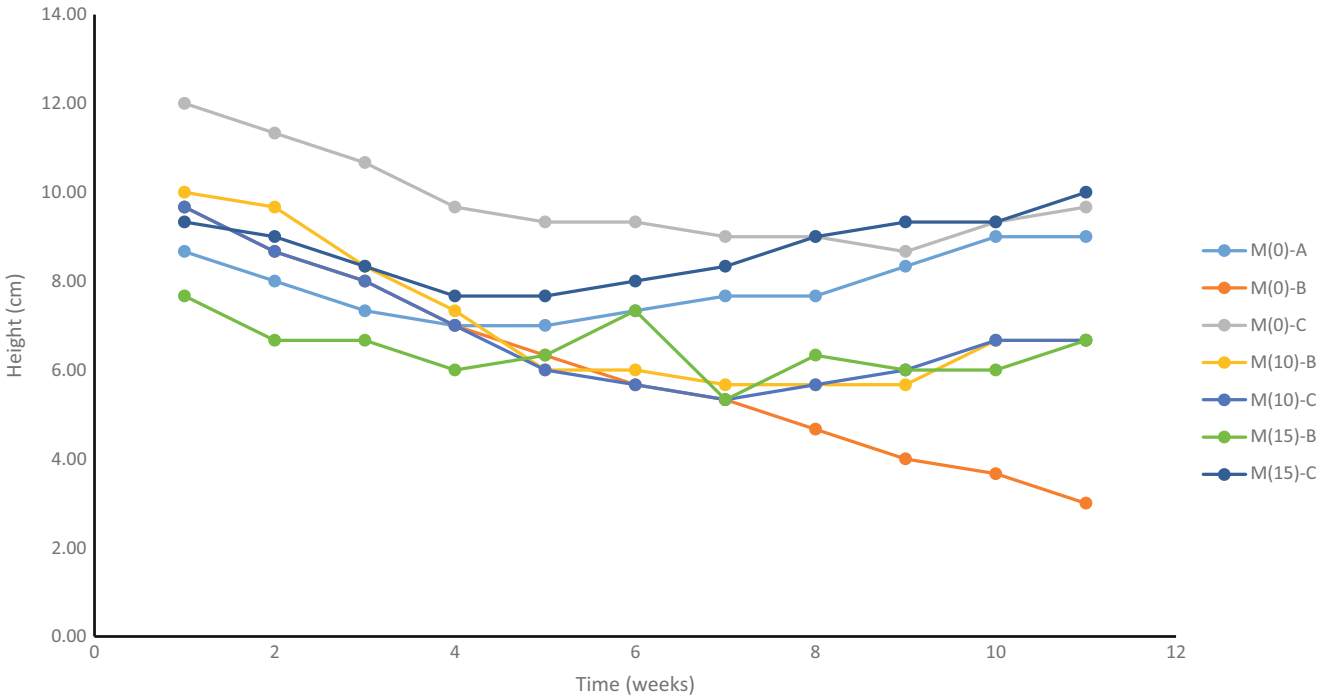

Fig. 3 Plant height vs time for each treatment (*G. rigens*)

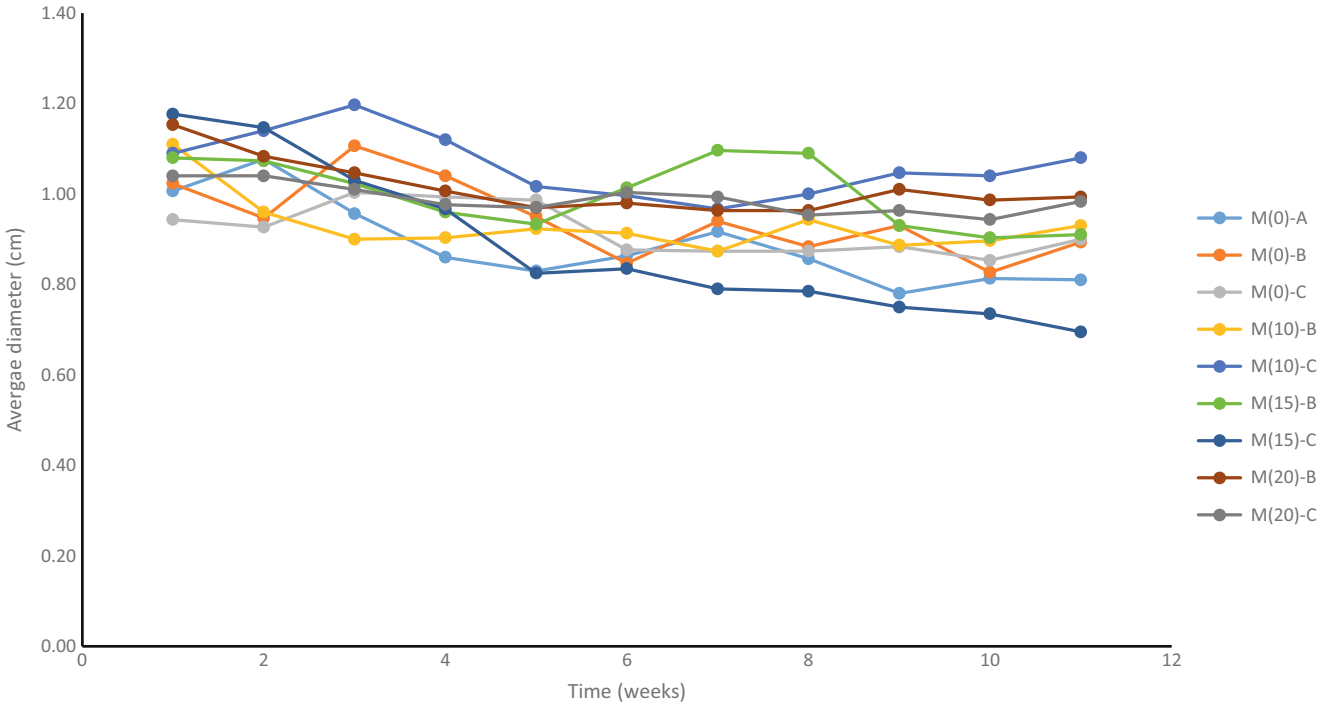

Fig. 4 Average diameter vs time for each treatment (*P. hortorum*)

The new model considers as fixed effects time, the effect of mycorrhizal treatment over time and of the organic and inorganic amendments over time (the interaction between both types of treatment has not been considered in this model). The p value of the new model is less than 2.2×10^{-16}, just like the first model. However, the initial model has been simplified and the redundant interactions that did not make a significant contribution to the model have been removed.

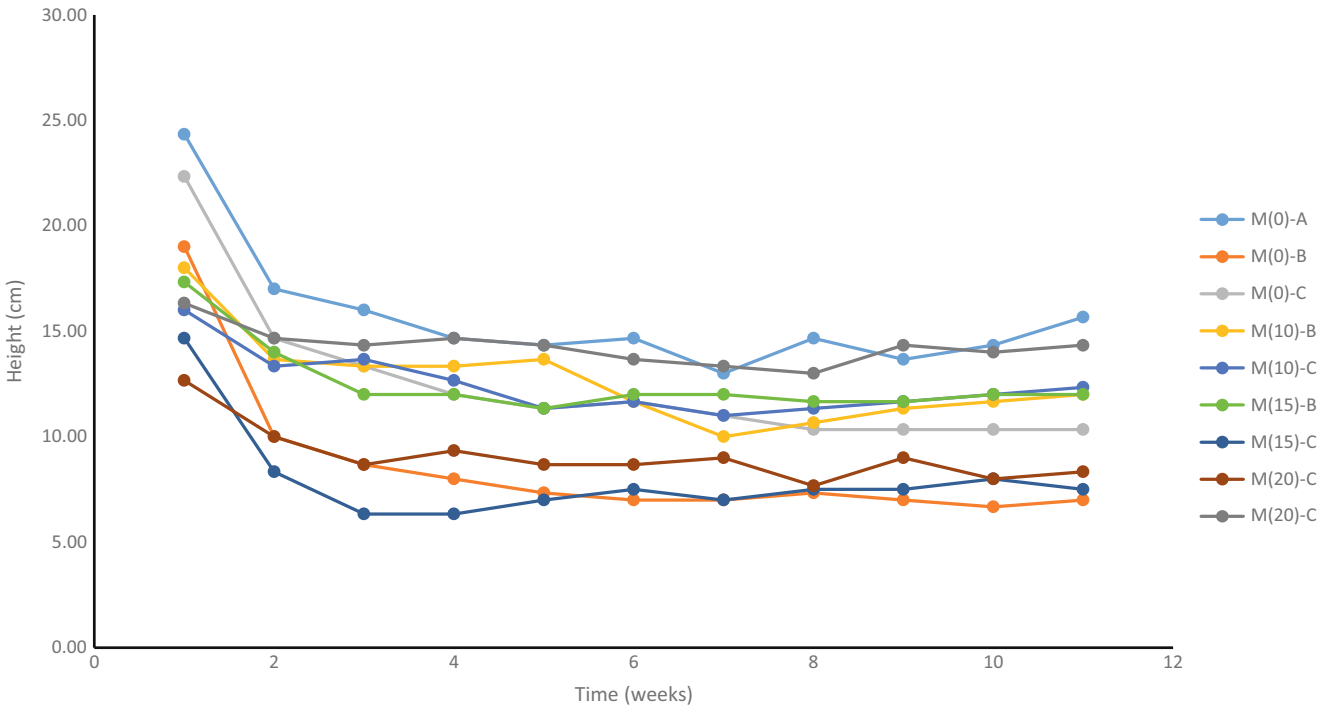

Fig. 5 Plant height vs time for each treatment (*P. hortorum*)

Table 7 Metal concentrations in the substrate of *G. rigens*

Sample	Cd	Cu	Fe mg kg^{-1}	Mn	Pb	Al	Zn
M(0)-T0	< 0.25	10.7	47,949	649	233	15,621	356
M(15)-T1	< 0.25	17.1	67,559	912	369	26,523	619
M(0)-T2	< 0.25	13.2	48,814	743	303	28,775	529
M(15)-T2	< 0.25	15.7	47,401	694	246	27,895	505

The main effects noted in the new model indicate that the effect of the type of amendment over time has a significant influence on the plants' height. Furthermore, there is a significant positive effect of the levels of mycorrhizal on the plant's height. Treatment with 10 g of mycorrhizal had an effect of 0.11667 [cm] ± 0.05645 per week with a p value = 0.04, and treatment with 15 g showed an effect of 0.45152 [cm] ± 0.05645 per week with a p value = 8.39×10^{-14} (Fig. 3).

Based on the results obtained, it can be concluded that the application of the treatments has a significant influence on height over time. An increase in mycorrhizal levels implies a higher growth rate of the plant over the adaptation period, while an increase in organic and inorganic amendments brings about a lower growth rate of the plant over the adaptation period.

3.1.3 Diameter (P. hortorum)

Based on the statistical analysis, it has been determined that there is no significant influence of the applied treatments on the plants' diameter ($\chi^2_{(16)} = 21.74, p = 0.1515$). Following these results, other models were tried to be adjusted, for instance, a model that would consider first-order interactions among the treatments or another that would not consider interactions; however, none of these models yielded significant results. It is concluded that the treatment used does not influence the diameter of the plants over time (Fig. 4).

3.1.4 Height (P. hortorum)

Initial calculations determined that the model fitted the data well ($\chi^2_{(16)} = 36.233, p = 0.002685$). Nevertheless, upon observing the coefficients and their p values, it was found that second-order interactions and the effects of organic and inorganic amendments were not significant. Only the effect of the different levels of mycorrhizal over time brought about differences in plants' height (Fig. 5).

Based on these results, it was proposed to simplify the model considering only different levels of mycorrhizal treatments. A new mixed-effects model was designed which considers the effects of time and those of the treatment over time. This new model fits the data even better ($\chi^2_{(16)} = 22.068, p = 0.001177$) and shows that all the levels of mycorrhizal present a significant positive effect on plant height over time. The most significant result was obtained for the effect of the treatments with 20 g of mycorrhizal ($p = 1.84 \times 10^{-5}$) consisting of an increase of 0.48283 [cm] ± 0.11065 per week. The rest of the treatments present similar effects, an increase of 0.31465 [cm] ± 0.11065 with a p value = 0.00481 for the treatment with 10 g, and an increase of 0.33845 [cm] ± 0.11659 with a p value = 0.00401 for the treatment with 15 g.

It is concluded that the application of mycorrhizal has a positive effect on this species height during the adaptation phase. On the other hand, organic and inorganic amendments do not present significant effects on the development of this plant species.

Table 8 Metal concentrations in roots of *G. rigens*

Sample	Cd	Cu	Fe mg kg^{-1} dry weight	Mn	Pb	Al	Zn
M(0)-T0	nd	227.76	8979.1	553.43	13.43	6984.8	61.49
M(15)-T1	nd	278.52	8112.6	471.41	16.88	6164.2	66.07
M(0)-T2	nd	262.95	7396.7	420.98	18.36	5656.4	84.92
M(15)-T2	nd	780.59	7540.6	330.3	21.78	5181.8	69.31

nd not detected

Table 9 Metal concentrations in the substrate of *P. hortorum*

Sample	Cd	Cu	Fe mg kg^{-1}	Mn	Pb	Al	Zn
M(0)-T0	< 0.25	10.4	38,567	610	171	17,122	300
M(10)-T1	< 0.25	14.5	43,683	620	215	16,673	429
M(20)-T2	< 0.25	12.6	43,736	620	276	22,384	410

3.2 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 organic matter. The red soils

Table 10 Metal concentrations in roots of *P. hortorum*

Sample	Cd	Cu	Fe mg kg^{-1} dry weight	Mn	Pb	Al	Zn
M(0)-T0	nd	109.15	6061	338.98	7.46	400.1	58.31
M(10)-T1	nd	117.74	1006.5	87.74	nd	693.6	34.19
M(20)-T2	nd	105.23	8440.3	538.26	11.54	7157.3	68.99

nd not detected

Table 11 BCF values in *G. rigens*

Sample	Cd	Cu	Fe	Mn	Pb	Al	Zn
M(0)-T0	0.00	*21.29*	0.19	0.85	0.06	0.45	0.17
M(15)-T1	0.00	*16.29*	0.12	0.52	0.05	0.23	0.11
M(0)-T2	0.00	*19.92*	0.15	0.57	0.06	0.20	0.16
M(15)-T2	0.00	*49.72*	0.16	0.48	0.09	0.19	0.14

Italicized values are greater than unity

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, 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, Al, 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, and thus any factor could have an influence on the diverse processes that occur. Tables 7, 8, 9, and 10 present the results of the concentrations of Cd, Cu, Fe, Mn, Pb, Al, and Zn in the tailing and the roots of both plant species, considering only the treatments that showed better evolution.

It can be observed that for all the treatments, the plants accumulated in their roots a high concentration of Al. Given the original pH of the substrate, pH about 8, this behavior is completely anomalous, since this metal is bioavailable at a much lower pH (pH ~ 5–5.5). According to the characterization of the substrate presented in Table 1, the high content of Al in comparison with Ca and Mg and the destruction of the components of the clays as a consequence of the preparation of the terrain lead to the liberation of great amounts of Al (Casierra and Aguilar 2007). On the other hand, in horizons poor in organic matter with a high concentration of sulfates under potential conditions of acidic reaction of the soil, a part of the OH^- ions can be replaced by SO_4^{-2}; Al can associate with the sulfates forming hydroxysulfates of Al, which can be adsorbed in clays. Chemical reactions of Al in the soil are complex and diverse and mainly include hydrolysis, polymerization, and element replacement. The presence of Al in the plant tissue could be attributed to two factors:

1. The majority of mechanisms of metal tolerance are in the root. Exclusion of Al can be achieved immobilizing Al in the cell wall, decreasing thus the permeability of the plasma membrane to Al, and attaching it to mucigel associated to the root apex (Castillo-Rodríguez 2005). Al has the capacity of attaching to the cell wall, altering its structure and increasing its rigidity (Klimashevskii and Dedov 1980; Gunsé et al. 1997; Horst 1995). On the other hand, the mechanisms of absorption of Al are not well known; entry paths could be simple permeability through the cell membrane in the form of neutral compounds (Haug and Foy 1984), through micellar lipid structures (Cullis and Kruijff 1979) or through some kind of transporter linked to phospholipids of the membrane or some other chelating agent (Green et al. 1980).
2. The washing of the roots using only distilled water was not enough for the desorption of Al. It is recommended for a further study to focus on the high concentration of Al present in the substrate and utilize adequate solvents to optimize the cleaning process of the samples of plant tissue (Sadzawka et al. 2007).

Based on the concentration data in the tailing and roots of plants (Tables 7, 8, 9, and 10), BCF has been calculated using Eq. (3). As discussed by Yoon et al. (2006), BCF is a measure of the ability of a plant to accumulate metals from contaminated soils (tailings in this case). Tables 11 and 12 show the BCF values in the two plant species studied. As it can be seen in these tables, both species show a high accumulation potential for Cu, since their values are greater than unity in all cases; values above 16 were found for the species *G. rigens* and above 8 for the species *P. hortorum.* Hence, in light of these data, it is concluded that both species are hyperaccumulators of this metal. On the contrary, for Fe, Mn, Pb, Al, and Zn, BCF values lower than 1 were found, due to which both species would be classified as excluders of these metals. 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.

Table 12 BCF values in *P. hortorum*

Sample	Cd	Cu	Fe	Mn	Pb	Al	Zn
M(0)-T0	0.00	*10.50*	0.16	0.56	0.04	0.23	0.19
M(10)-T1	0.00	*8.12*	0.02	0.14	0.00	0.04	0.08
M(20)-T2	0.00	*8.35*	0.19	0.87	0.04	0.32	0.17

Fig. 6 Mycorrhizal fungi activated in the roots of *G. rigens*

Regarding mycorrhizal, the activation time for both species was between 15 and 25 days. When the plants that had been subjected to treatments with mycorrhizal were extracted, they were examined before starting analysis to determine if there was presence of activation of mycorrhizal. Their activation occurs when the hyphae penetrate into the roots and are established; this effect was observed in all the specimens of both species. Figure 6 shows the activated mycorrhizal in the roots of *G. rigens*, and Fig. 7 shows the insertion of mycorrhizal in the roots of *P. hortorum*. On observing Figs. 6 and 7, it can be seen that mycorrhizal fungi are able of being activated in the roots inserted in the tailing substrate; however, it was also observed that they require more contact time between plant/substrate to bring about effects in phytoremediation. It can be observed that there was a percentage of fungi that were not activated (50–70% for both species), which could be due to the fact that the root ball was too fixed to its structure, preventing the hyphae of the fungi from penetrating in the root. Another factor could be the time required by the fungi. Furthermore, excess of water may have played a role, since during experimentation time there was rainfall in the tailing site. This saturated the substrate for a long period, which is not suitable for mycorrhizal fungi.

4 Conclusions

The results show that neither the organic nor the inorganic amendment significantly affected the adaptation of the species *G. rigens* and *P. hortorum*, indicating that both species can adapt without amendments. However, the amendment with mycorrhizal had a positive effect on the adaption of *P. hortorum*, and this effect increased with the use of organic or inorganic amendments.

The bioconcentration factors in both species for all metals, with the exception of copper, were found to be less than 1, which indicates that *G. rigens* and *P. hortorum* could be considered excluders of these metals. In contrast, the elevated Cu bioconcentration factors (> 8 for *P. hortorum* and > 16 for *G. rigens*) could be a good measure of the high capacity of these two native plants to accumulate Cu.

It is worth pointing out that the greater than 1 criterion for the bioconcentration factor 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, since 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. The results of this study 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.

Fig. 7 Image of mycorrhizal fungi inserted in the roots of *P. hortorum*

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