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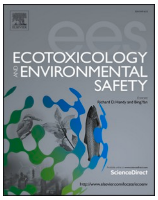

Research Paper

Interactive effect of compost application and inoculation with the fungus *Claroideoglomus claroideum* in *Oenothera picensis* plants growing in mine tailings

Rodrigo Pérez [a,b], Yasna Tapia [c], Monica Antilén [d], Manuel Casanova [c], Catalina Vidal [a,b], Christian Santander [a], Humberto Aponte [a,e], Pablo Cornejo [a,*]

[a] *Centro de Investigación en Micorrizas y Sustentabilidad Agroambiental, CIMYSA, Universidad de La Frontera, Avenida Francisco Salazar 01145, Temuco, Chile*
[b] *Programa de Doctorado en Ciencias de Recursos Naturales, Universidad de La Frontera, Temuco, Chile*
[c] *Departamento de Ingeniería y Suelos, Universidad de Chile, 8820808 Santiago, Chile*
[d] *Departamento de Química Inorgánica, Pontificia Universidad Católica de Chile, 7820436 Santiago, Chile*
[e] *Laboratory of Soil Microbiology and Biogeochemistry, Institute of Agri-Food, Animal and Environmental Sciences (ICA3), Universidad de O'Higgins, San Fernando, Chile*

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ABSTRACT

Different techniques have been developed for the remediation of Cu contaminated soils, being the phytoremediation a sustainable and environmentally friendly strategy, but its use in mine tailings is scarce. Arbuscular mycorrhizal fungi (AMF) can decrease the Cu concentration in plants by favouring the stabilization of this metal through different mechanisms such as the production of glomalin, immobilization in the fungal wall of hyphae and spores, and the storage of Cu in vacuoles. Additionally, the use of organic amendments promotes the beneficial effects produced by AMF and improves plant growth. Based on the above, the aim of this study was to determine the effect of AMF inoculation and compost application at different doses on the growth of *Oenothera picensis* in a Cu mine tailing. One group of plants were inoculated with *Claroideoglomus claroideum* (CC) and other was non-inoculated (NM). Both CC and NM were grown for two month under greenhouse conditions in pots with the Cu mine tailing, which also had increasing compost doses (0%, 2.5%, 5%, and 10%). Results showed greater biomass production of *O. picensis* by CC up to 2-fold compared with NM. This effect was improved by the compost addition, especially at doses of 5% and 10%. Therefore, the increase of mycorrhizal and nutritional parameters in *O. picensis*, and the decreasing of Cu availability in the mine tailing, promoted the production of photosynthetic pigments together with the plant growth, which is of importance to accomplish phytoremediation programs in Cu mine tailings.

1. Introduction

The soil contamination by high metal(loid) concentration has increased in recent years, becoming an environmental threat worldwide (Aponte et al., 2020b). Mining activities have produced an estimate of 5–14 million tons of mine wastes or tailings in recent decades (Schoenberger, 2016), which generate a great negative environmental impact that covers large areas of lands and reduce the plant cover and diversity (Mendez and Maier, 2008). Tailings often show low organic matter (OM) and nutrients amounts, with a high metal(loid) concentration and acidic pH, which promotes the erosion and negative effects on the vegetation cover and soil microbial activity (Kossoff et al., 2014; Lima et al., 2016; Aponte et al., 2020a).

Phytostabilization is recommended for the remediation of mine tailings since it limits the toxicity of the metal(loid)s, maintaining the plant cover that stabilizes the surface of the tailings, which prevents wind and water erosion (Miller and Zégre, 2014; Lima et al., 2016). To improve Cu phytostabilization, metallophytes tolerant to high Cu concentration are required. Also, the presence of other benefic traits is desirable such as the tolerance to other adverse conditions as extreme pH values, severe drought and salinity, and low content of OM and mineral nutrients, which are conditions commonly present in mine tailings (Baker et al., 2010; Huang et al., 2012). In this context, *Oenothera picensis* Phil. subsp. *picensis* (described as *Oenothera affinis* for

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[*] Corresponding author.
E-mail address: pablo.cornejo@ufrontera.cl (P. Cornejo).

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González et al., 2008; and later referenced as *Oenothera picensis*) is a biennial plant commonly present in degraded soils, with a natural distribution in central Chile and border areas with Argentina (Rodriguez et al., 2018). Additionally, this plant can grow in highly Cu contaminated soils by mine wastes (González and Neaman, 2015) with total and available Cu concentration up to 830 and 330 mg kg^{-1}, respectively (Cornejo et al., 2008). Furthermore, *O. picensis* has shown high Cu accumulation in root organs (Meier et al., 2012), high biomass production, and drought resistance, by which it is a potential candidate in phytoremediation assays of Cu contaminated soils (González-Guerrero et al., 2007; Meier et al., 2011, 2015, 2017; Cornejo et al., 2017).

In phytostabilization, some of the major limitations for the in situ plant establishment are the physicochemical conditions of the mine tailings, by which it is needed the use of organic amendments and other strategies to improve soil conditions for plants (Doronila et al., 2014; Medina et al., 2015; Tapia et al., 2016). In this sense, the main function of the organic amendments in phytostabilization processes is to improves the soil pH (reach an optimal value) and water holding capacity, as well as, provides nutrients for plants and OM to promote the complex formation with potentially toxic elements. Several sources of organic amendments and nutrients have been evaluated for stabilizing mine tailings. Recently, Tapia et al. (2017) showed that the addition of potassium humates on Cu mine tailings decreased the transport of Cu to the shoot of the halophyte *Carpobrotus aequilaterus*, promoting the Cu stabilization in the root. The mitigation of metal(loid) toxicity can facilitate the plant establishment and also the root colonization by arbuscular mycorrhizal fungi (AMF) (Bolan et al., 2014; Cicatelli et al., 2012; Medina et al., 2015, 2017; Wang et al., 2017). AMF belong to the phylum Glomeromycota and are commonly associated with the roots of most terrestrial plants, forming the arbuscular mycorrhizal (AM) symbiosis (Kleinert et al., 2018). In the AM association, the fungus colonizes the root cortex and develops an extensive network of extraradical mycelium around the root (Meier et al., 2012; Cornejo et al., 2017). AMF are widely used in phytoremediation processes due to the benefits they have for the plant, especially in conditions of water limitation, low fertility, and high levels of toxic elements (Barea et al., 2013; Cicatelli et al., 2012; Meier et al., 2011, 2012; Seguel et al., 2012). It has been described that AMF colonization can decrease the Cu concentration in the whole plant but also can increase the Cu-stabilization through different mechanisms such as the production of glomalin, immobilization by part of the fungal wall of hyphae and spores, and the storage of Cu in vacuoles into resistance spores (Cornejo et al., 2013; Nayuki et al., 2014; Ferrol et al., 2016). The inclusion of compost can facilitate the development of the AM symbiosis and improve the plant growth even in extreme conditions as shown by mine tailings (Bolan et al., 2014; Medina et al., 2015, 2017; Wang et al., 2017). Although these studies suggest the potential usefulness of these tools in phytoremediation strategies, a deep understanding of the effects considering the application of AMF in mining tailings is necessary. We hypothesize that the combined effect of both tools (AMF inoculation and compost application) will improve phytoremediation processes in mine tailings by increasing plant growth and metal(loid) and reducing Cu bioavailability. Based on the above, here we aimed to determine the effect of 1) AMF inoculation, 2) the incorporation of increasing compost amounts, and 3) their interaction on the growth of *O. picensis* plants growing in Cu mine tailings. This will allow approaching the optimal conditions for further in field compost application and establishment of AMF colonized plants to be used in the stabilization of several abandoned Cu mine tailings.

2. Materials and methods

2.1. Mine tailings, compost, and biological material

Copper mine tailings here used were collected from the non-operative reservoir Piuquenes, located in the Aconcagua Valley, Los Andes, Valparaíso Region (32°59′47.96″ S; 70°15′14.16″ W). Additionally, we acquired compost from La Pintana municipality, through the Direction of Environmental Management (DIGA), which was produced using the organic wastes from the commune. The physicochemical characterization was determined for Cu mine tailing and compost (Table 1). The pH was measured in an aqueous suspension (1:2.5, w:v). Electrical conductivity (EC) was determined in a saturation extract. OM content was determined by loss weight after ignition at 360 °C for 16 h and available concentration of N, P, K were determined according to Tapia et al. (2020). Available N concentration was measured by extraction with KCl, distillation, and titration. Available P was measured according to the Olsen method (Olsen and Sommers, 1982). For the determination of the total concentration of metalloids (Cu, Fe, Mn, and Zn), the samples of mine tailings were digested with HF-$HClO_4$-HCl-HNO_3 (Dold and Fontbote, 2001) and analyzed by Atomic Absorption Spectrophotometer (AAS, Unicam SOLAAR, mod. 969). For the determination of available Cu, DTPA-$CaCl_2$-TEA at pH 7.3 was used as extractant (Baker and Amacher, 1982) and quantified by AAS as above. For the calibration of AAS equipment and accuracy of analytical procedures, we used the Montana Soil SRM 2711 as reference. Seeds of mature plants of *O. picensis* were obtained from the ecosystem surrounding the Ventanas smelter installation in the Puchuncaví Valley (Valparaíso Region). The seeds were surface-sterilized with 2% chloramine-T solution for 5 min and rinsed thoroughly with distilled water. Then, seeds were germinated in plastic trays using 10 g of the AMF inoculum as substrate. To produce non-AMF-colonized plants, we used the same inoculum after autoclave sterilization by 30 min at 121 °C. The plantlets were maintained in the trays for three weeks after their emergence to ensure the AM fungal colonization before transplanting. We used an inoculum of *Claroideoglomus claroideum* that was provided by *Centro de Investigación en Micorrizas y Sustentabilidad Agroambiental* (CIMYSA), Universidad de La Frontera, Temuco, Chile. For the inoculum production, an open pot culture was performed using a mixed substrate composed by sepiolite, quartz sand, and vermiculite (1:1:1, v:v:v), with *Sorghum bicolor* and *Trifolium repens* as host plants. After six months, the rhizosphere substrate and roots were sieved through a mesh of 0.5 mm and collected in a mesh of 53 μm (200 mature spores per g),

Table 1
Main physicochemical characteristics of mine tailing and compost.

	Compost	Mine tailings	Range of reference mine tailings[a]	Reference of total metal levels in non-contaminated soils
pH	8.5 ± 0.01	4.05 ± 0.01	1.8–9.4	–
Electrical conductivity (dS m^{-1})	4.5 ± 0.1	0.03 ± 0.001	0.1–22.4	–
Organic matter (%)	27.8 ± 0.29	0.3 ± 0.002	0.02–25	–
Available N (mg kg^{-1})	97 ± 2.3	9.6 ± 0.18	–	–
Available P (mg kg^{-1})	361.5 ± 18.9	28 ± 0.12	1.0–400	–
Available K (mg kg^{-1})	10,243 ± 106	58 ± 0.03	1.0–564	–
Total Cu (mg kg^{-1})	36.13 ± 2.89	396 ± 22.42	1.0–750	2.0–250[c]
Total Fe (mg kg^{-1})	12,948 ± 112	61,184 ± 356	4,000–57,0000	2,000–55,000[c]
Total Mn (mg kg^{-1})	206 ± 5.2	278 ± 4.7	1.0–4,000	100–4,000[b]
Total Zn (mg kg^{-1})	34 ± 0.38	41 ± 0.35	1.0–5,000	1.0–900[b]

Values are expressed as means ± standard error of three independent data.

[a] Hossner and Shahandeh (2005).

[b] Adriano (1986).

[c] Sparks (2003).

which was used as inoculum. Before the greenhouse test, the Cu mine tailing was mixed manually with the corresponding amount of compost and stored for two weeks. The pots (1 L) were filled with the substrate, and the plantlets were placed in the middle of the pot individually.

2.2. Experimental design

An in vivo pot experiment with mine tailings was performed using a full factorial randomized experimental design that included two levels of AMF inoculation: i) with *Claroideoglomus claroideum* (CC) and ii) non-inoculated (non-mycorrhizal, NM). Also, the compost addition was made at increasing doses (%) regarding the total weight of the pots that resulted in the following levels: i) without compost (C0%), 2.5% (C2.5%), 5% (C5%), and 10% (C10%) w/w. A Cu mine tailing was here used as the main substrate, which was mixed with the compost at the above rates (see Supplementary Material 1). The combination of the factors produced eight treatments with five replicates ($n = 5$) considering one plant in each plot. The plants were harvested after 60 days of growth after transplanting.

2.3. Growth conditions

The greenhouse experiment was carried out using a 16/8 light/dark photocycle, 18/26 °C night/day and 50% of relative humidity. The germinated seeds of *O. picensis* and the experiment were maintained under the same conditions. The experiment was preserved in the same conditions for 60 days, being the pots randomized weekly to avoid block effects. All the pots were water adjusted every two days with 100 mL of distilled water.

2.4. Plant mineral and photosynthetic pigments measurement

At harvest, shoot and root of *O. picensis* were separated and dried at 65 °C for 48 h and weighed. Subsequently, the plants were ground, converted to ashes in an oven at 550 °C and digested using an $H_2O/HCl/HNO_3$ mixture (8:1:1, v:v:v) (Meier et al., 2015). The plant extracts were used for total Cu determination by Flame-Atomic Absorption Spectrophotometer. Previous to the drying process, photosynthetic pigments were extracted from three leaf discs of 4 mm radius with a solution of 80% acetone at 4 °C for 24 h. The absorbance of extracts was measured using a spectrophotometer at wavelengths of 664, 647 and 430 nm for chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids, respectively (Wellburn, 1994). The concentrations of pigments were calculated according to equations reported by Lichtenthaler (1987).

2.5. Arbuscular mycorrhizal fungal measurements

Arbuscular mycorrhizal fungal colonization percentage was determined by visual observation of fresh root pieces (1 cm) obtained before the drying process using a stereomicroscope (40–60X magnification). Root fragments were cleared using KOH 10% w/v and stained using trypan blue solution 0.05% w/v in lactic acid according to Phillips and Hayman (1970). Quantification of AMF colonization (%) was calculated according to the gridline intersect method (Giovanetti and Mosse, 1980). AMF spores were isolated from 20 g of soil through wet sieving and decanting method, followed by sucrose centrifugation at 2,500 rpm for 10 min. After centrifugation, the supernatant was poured through 50 μm-pore size mesh and quickly rinsed with tap water. Spores were counted in a Petri dish under the dissecting microscope (Sieverding, 1991).

2.6. Statistical analysis

The main effect of CC inoculation, compost application, and their interaction was statistically analyzed by two-way analysis of variance (ANOVA). Data not meeting statistical assumptions for normality and homoscedasticity were transformed using Ln+ 1 function, but the results are presented in their original scale of measurement. Treatments with significant differences were analyzed using the Tukey's HSD as a post hoc test to compare the means between factor levels. In addition, data were also subjected to principal component analysis (PCA) to evaluate the multivariate effect of established treatments and the relationship between variables. For all the procedures, we considered a $P < 0.05$ as statistically significant. The software SPSS 22.0 (IBM) and R statistics 3.5.1 were here used for analyses.

3. Results

3.1. Characterization of Cu mine tailing and compost

The Cu mine tailing has very acid pH, high total Cu concentration, low OM and N content, and high EC (Table 1). On the other hand, the compost pH was alkaline (pH = 8.5) with high nutrient concentration (Table 1). Besides the low pH of Cu mine tailing, compost addition increased the pH on all treatments (Fig. 1; Supplementary Material 2), ranging from 4 in the non-amended treatments to ~7 in the C10% treatment. The total Cu concentration in the mine tailing was slightly higher in the treatments inoculated with CC at all the compost doses (Fig. 1b), with a significant increase in CC-C0% (192 mg Cu kg^{-1}), compared with NM-C0% (165.5 mg Cu kg^{-1}) and CC-C10% (160.2 mg Cu kg^{-1}). The increase of the compost doses did not generate changes in the total Cu concentration of the mine tailing, maintaining values of about 165 mg Cu kg^{-1} in NM, and 185 mg kg^{-1} in CC. On the other hand, extractable Cu DTPA decreased in CC-2.5%, CC-5%, and CC-10% respect to the NM treatments and NM-C0% (Fig. 1c). No differences were observed between CC-C5% and CC-C10%. Despite this, the lowest Cu extractable DTPA concentration was found in CC-C10% (20.8 mg kg^{-1}) with a decrease of approximately 3-fold compared with NM-C0% (61.3 mg kg^{-1}). In NM, the C10% treatment decrease the Cu extractable DTPA concentration at least 2-fold (27.5 mg Cu kg^{-1}), compared to C0% (61.3 mg kg^{-1}).

3.2. AMF characteristics

Increasing compost application did not affect the AMF colonization by CC in *O. picensis* roots. However, all the CC inoculated plants showed about 30% of AMF colonization (Fig. 2a; Supplementary Material 2), being C10% the treatment with the highest AMF colonization (33.2%). Compost application had a significant effect on spore density, especially in C5% and C10% (Fig. 2b), increasing by 1.5 and 2 fold, respectively, compared with C0%. AMF hyphae density presented a significant increase only in C10% (Fig. 2C), reaching 88 cm g^{-1}.

3.3. Plant biomass production and photosynthetic pigments

Plants of *O. picensis* presented classic features of Cu toxicity as chlorosis and necrosis, especially in NM-C0% treatment. Nevertheless, some plants were able to flower even under these stress conditions. Plants inoculated with CC had a significant increase on shoot dry weight in all the cases (Fig. 3a; Supplementary Material 2), with an increase approximately of 2-fold in C0%, C2.5%, and C10% compared to NM, reaching the highest shoot biomass production (1,7 g per plant) in C10%. Roots of AMF inoculated plants showed an increase in dry weight compared with NM plants (Fig. 3b), with significant differences in C0% respect to C10%. Detailing, the highest root biomass production was in CC-C10% with 0.9 g of dry root per plant. In non-inoculated treatments, compost had no significant effect on shoot and root biomass production. However, AMF enhanced the biomass increase in shoot at all compost doses and had a significant increase in root biomass at C10%.

Photosynthetic pigments (Chl a, Chl b, and carotenoids) showed a tendency to increase as the compost dose increases (Fig. 4; Supplementary Material 2). The combined application of AMF inoculation and

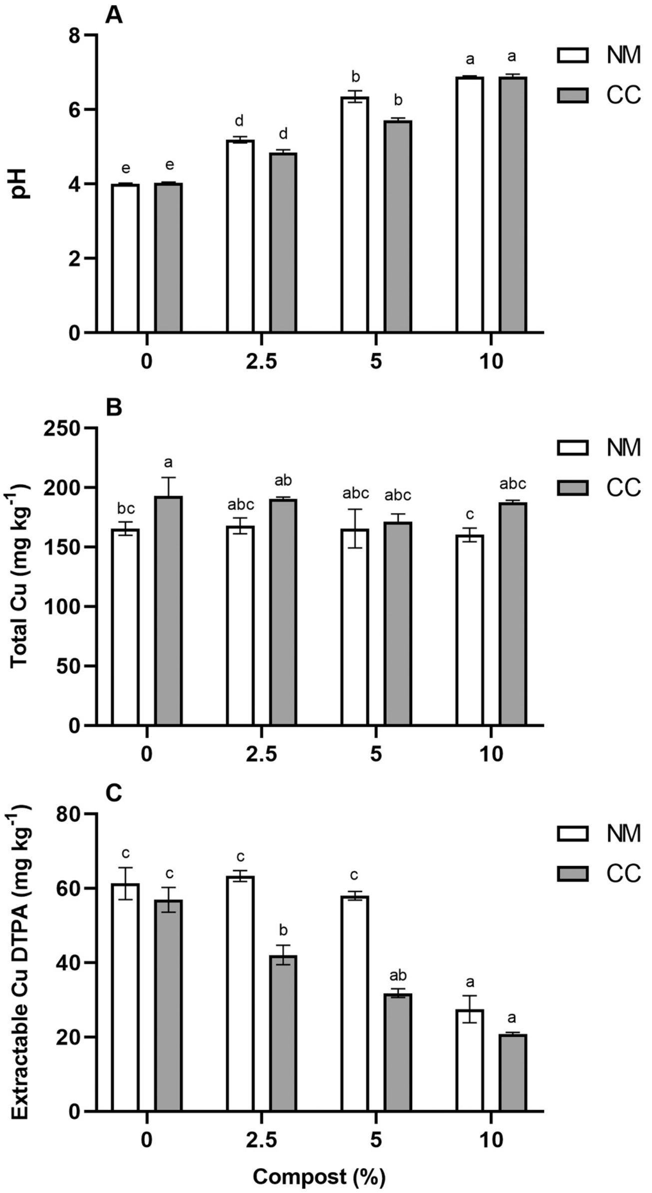

Fig. 1. Substrate pH (A), total Cu concentration on mine tailing (B) and extractable Cu-DTPA (C) at the end of the assay in non-inoculated *Oenothera picensis* plants (NM) and inoculated with the fungus *Claroideoglomus claroideum* (CC) amended with different doses of compost. Values are expressed as means ± S.E. Different letters over the bars indicate significant differences between means at $p < 0.05$ (two-way ANOVA, Tukey's HSD post-hoc test, n = 5).

compost supply at C5% and C10% had a significant increase for all pigments compared to C0%. The most noticeable increase of photosynthetic pigments was found in CC-C10%, which promoted higher values for all the pigments compared to NM, with a final production of 0.3, 0.09 and 0.19 mg cm^{-2}, respectively (Fig. 4).

3.4. Cu concentration in plant tissues

The Cu concentration in shoot was higher in plants inoculated with CC compared to NM plants, especially in C0% and C2.5% (Fig. 5a; Supplementary Material 2). The highest Cu concentration was observed in CC-C2.5%. Noticeably, this effect produced by the CC inoculation decreases at highest compost doses (C5%, C10%), with no differences in NM plants. In roots, the highest Cu concentration was registered in NM-C0%, reaching a Cu concentration of 1131.5 mg kg^{-1} (Fig. 5b). Inoculation with CC had a significant reduction on root Cu concentration at C5% and C10% (about 3.7-fold for both) compared with NM-C0%.

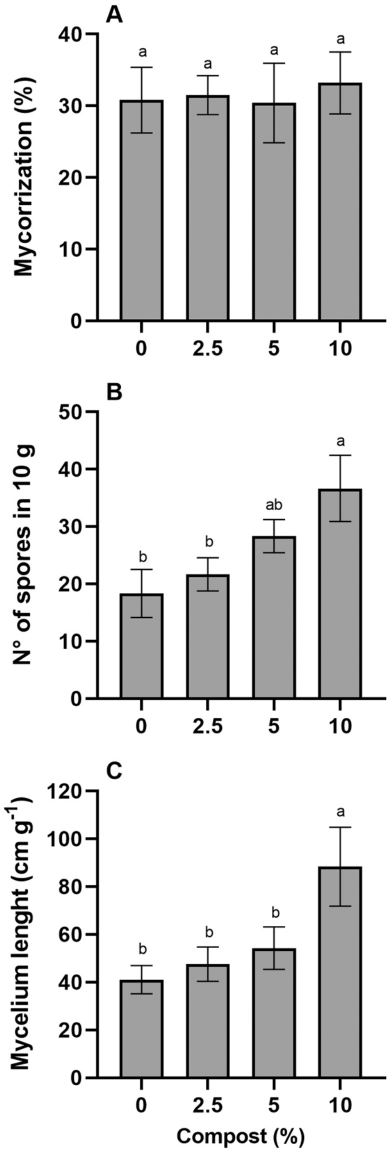

Fig. 2. Arbuscular mycorrhizal fungal parameters: Percentage of mycorrhization (**A**), N° of spores in 10 g of substrate (**B**) and mycelium length (**C**) present at different compost doses. Values are expressed as means ± S.E. Different letters over the bars indicate significant differences between means at $p < 0.05$ (two-way ANOVA, Tukey's HSD post-hoc test, n = 5).

3.5. Multivariate associations

A principal component analysis reflected the formation of highly homogeneous groups of experimental variables (Fig. 6a). The PC1 and PC2 explained 57.6% and 19.4% of the total variance, respectively (Supplementary Material 3). The confidence ellipsoids showed the separation of four well-defined groups of compost doses, which was more evident by the CC interaction where overlapping between groups was absent (Fig. 6). In this sense, PCA showed a clear separation between NM and CC, with higher plant attributes and less total Cu concentration in mine tailing in CC treatments (Fig. 6). The variables with more contribution were photosynthetic pigments, root and shoot biomass, and mycorrhizal attributes in PC1, as well as total Cu Concentration in shoot and mine tailing in PC2 (Fig. 6). Additionally, PCA displayed negative correlations between the mine tailing pH and

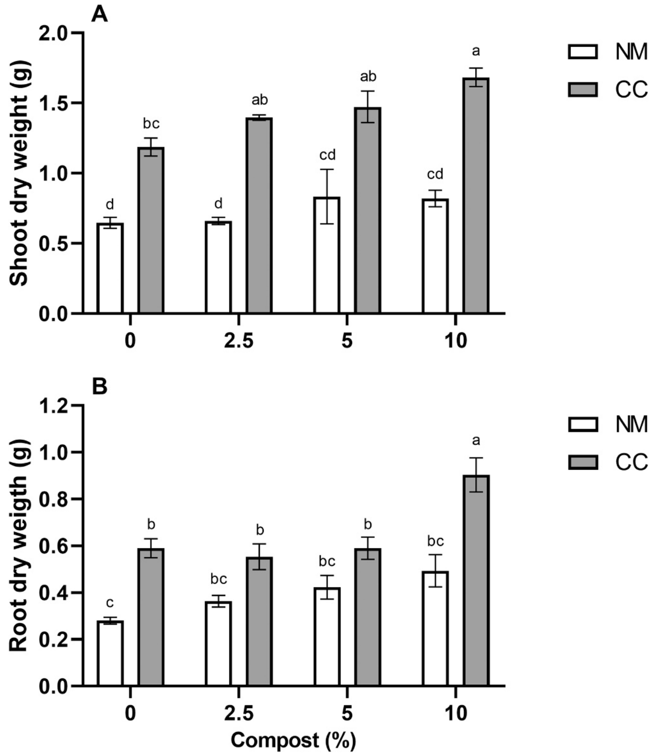

Fig. 3. *Oenothera picensis* dry weight values for root (**A**) and shoot (**B**) of non-inoculated plants (NM) and plants inoculated with the fungus *Claroideoglomus claroideum* (CC) and amended with different compost doses. Values are expressed as means ± S.E. Different letters in the bars indicate significant differences between means at $p < 0.05$ (two-way ANOVA, Tukey's HSD post-hoc test; n = 5).

photosynthetic pigments with total Cu concentration in roots and extractable Cu concentration in the substrate at the end of the experiment. Similarly, the total Cu concentration in the mine tailing was negatively correlated with all mycorrhizal variables and root and shoot biomass.

4. Discussion

The total Cu concentration in the mine tailing is similar to other present in areas from northern Chile (400 mg kg^{-1}; Santibáñez et al., 2008), but is lower than other Chilean soils where the Cu concentration was found between 2000 and up to 4393 mg kg^{-1} (Dold and Fontbote, 2001; Santibáñez et al., 2012). In Chile, plant communities present in Cu contaminated soils are dominated by few metallophyte species, which can grow under these restrictive conditions (Ginocchio and Baker, 2004). Metallophytes have developed biological mechanisms to resist or tolerate metal(loid) toxic conditions in soils and are typically endemic from the native plant communities present in the area (Meier et al., 2012). *Oenothera picensis* is a plant species that has been described naturally growing in Cu-contaminated soils (Cornejo et al., 2008; González et al., 2008), which has been suggested as an alternative for phytoremediation in these soils (Meier et al., 2011, 2012, 2017; Cornejo et al., 2017). Moreover, here we confirm that *O. picensis* is able to grow successfully in Cu mine tailings; however, the use of other technologies must be a key point to take into account. Our results demonstrate that the colonization with *C. claroideum* and the use of compost as an organic amendment synergistically improve the global performance of *O. picensis* plants.

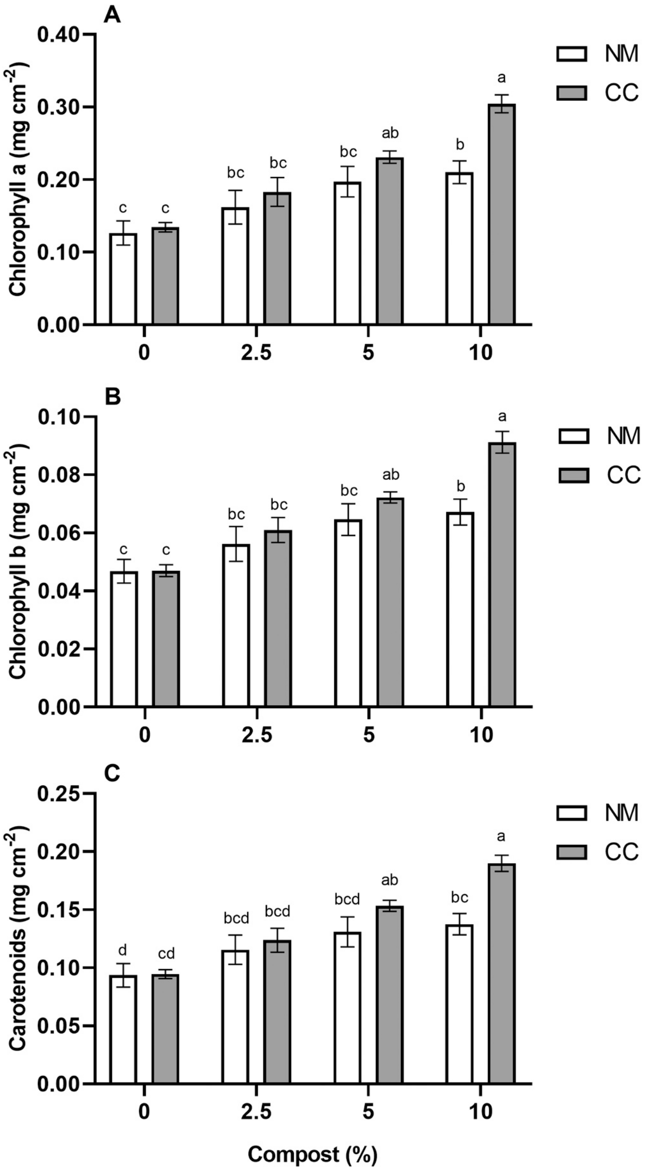

Fig. 4. Chlorophyll a (**A**), Chlorophyll b (**B**) and carotenoid (**C**) concentration in leaves of *Oenothera picensis* of non-inoculated plants (NM) and plants inoculated with the fungus *Claroideoglomus claroideum* (CC) amended with different compost doses. Values are expressed as means ± S.E. Different letters over the bars indicate significant differences between means at $p < 0.05$ (two-way ANOVA, Tukey's HSD post-hoc test, n = 5).

The joint use of *C. claroideum* with compost addition significantly reduced the bioavailability of Cu in the mine tailings, especially at the highest doses (5% and 10%). The AMF are recognized for producing multiple beneficial effects that improve plant tolerance to toxic elements, mainly because their interaction with the soil root interface, acting as a biological barrier against the transport of metal(loid)s towards the plant. This is mainly due to the presence of proteins with high affinity for metal(loid)s, such as chitin, melanin, and glomalin in the fungal cell wall, which allow the accumulation of these metal(loid)s in hyphae and spores (González-Guerrero et al., 2007; Ferrol et al., 2016; Cornejo et al., 2017). Additionally, it has been described that organic amendments can decrease Cu bioavailability in soil through interaction with humic substances, which also promote the plant cover formation in mine tailings (Gil-Loaiza et al., 2016; Medina et al., 2017; Pardo et al., 2017). On the other hand, it is well known that acidic conditions increase the bioavailability of several meta(loid)s in soil solution. Thus, the decrease of available Cu can be partially attributed to the significant increase of the pH in the C10%, which is supported by the negative correlation between both variables (Fig. 6). In this context, higher OM

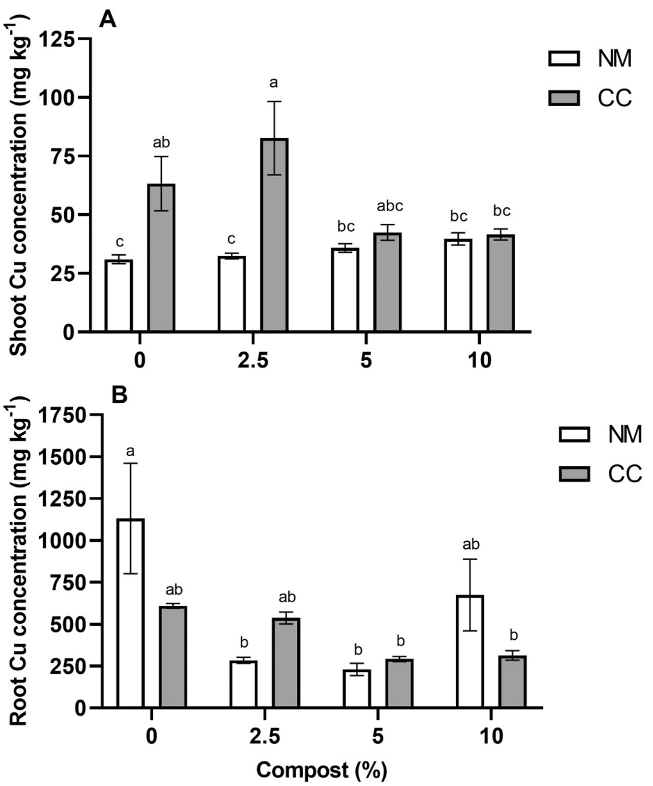

Fig. 5. Copper concentration in shoot (A) and root (B) of *Oenothera picensis* plants non-inoculated (NM) or inoculated with the fungus *Claroideoglomus claroideum* (CC) and amended with different compost doses. Values are expressed as means ± S.E. Different letters over the bars indicate significant differences between means at $p < 0.05$ (two-way ANOVA, Tukey's HSD, post-hoc test; n = 5).

content and pH by compost addition could reduce Cu bioavailability for plants on the substrate by increasing negative charge, Cu precipitation as hydroxides, and formation of hydroxyl species that are more strongly retained compared to free metal ion species (Jones et al., 2016; Medina et al., 2017). Similar results were observed by Solís-Dominguez et al. (2012), where the use of compost concentrations of 10% and 15%, reduced the availability of meta(loid)s such as As, Pb, Zn and Cu in acid mine tailing, suggesting a reduction on the mobility of these contaminants by compost. On the other hand, Mendez et al. (2007) observed that concentrations of compost from 10% to 25% significantly increased the growth of *Atriplex lentiformis* in mine tailing, with an increase in the accumulation of meta(loid)s as Na, K, Mn and Zn in shoot. These studies emphasize that the key factor in the establishment of plants in acidic mine tailing is the improvement of soil characteristics, mainly modifying the pH and providing a source of OM (Mendez et al., 2007; Solís-Dominguez et al., 2012; Gil-Loaiza et al., 2016).

About the root colonization by *C. claroideum*, this fungus was able to successfully colonize *O. picensis* plants growing in Cu mine tailings even without the compost addition. Furthermore, the increase of compost doses did not affect the mycorrhization capacity at least as total percentage of root colonization. However, compost addition positively affected other mycorrhizal parameters such as spore formation and hyphal density, especially at C5 and C10%. This result is consistent with previous studies where compost increased the development of the AMF and the spore production due to the increase in some components of OM such as humic acids and the contribution of nutrients such as N or P (Cavagnaro, 2015; Santana et al., 2015). Additionally, *C. claroideum* has previously demonstrated the particular capability to compartmentalize the Cu in spores (Cornejo et al., 2013), which in this case can be a relevant fungal trait to increase the tolerance of plant against the Cu present in high levels in the substrate.

The Cu toxicity affects various biochemical and physiological processes in the plant, due to several interactions expressed at cellular and molecular level (Kabata-Pendias and Pendias, 2001). Detailing, the decrease of chlorophyll concentration and membrane damage by oxidative stress are the main responses inducing the toxicity and the loss of plant growth (Xu et al., 2017). In this study, the interaction of *C. claroideum* and compost decreased the Cu bioavailability in the mine tailing and total Cu content in roots, which produced an increase of measured photosynthetic pigments at C5% and C10% in *O. picensis*. This beneficial effect was strongly related to the production of plant biomass and the increase of mycorrhizal propagule densities, such as the production of hyphae and spores. Also, the negative correlation between pH and the concentration of photosynthetic pigments with Cu extractable in mine tailings (Fig. 6) support the above mentioned effects. One effect of chlorophyll recovery is an increase in the amount of light captured by leaves, which in turn reduces the possibility of damage by reactive oxygen species produced by Cu excess (Kranner et al., 2002; Bagheri et al., 2011). In general, the combined use of AMF and high compost concentrations improved the growth of *O. picensis* in both shoots and roots (Fig. 3). In shoot, we observed an additive effect in growth produced by AMF and compost, in a similar way to the observed for photosynthetic

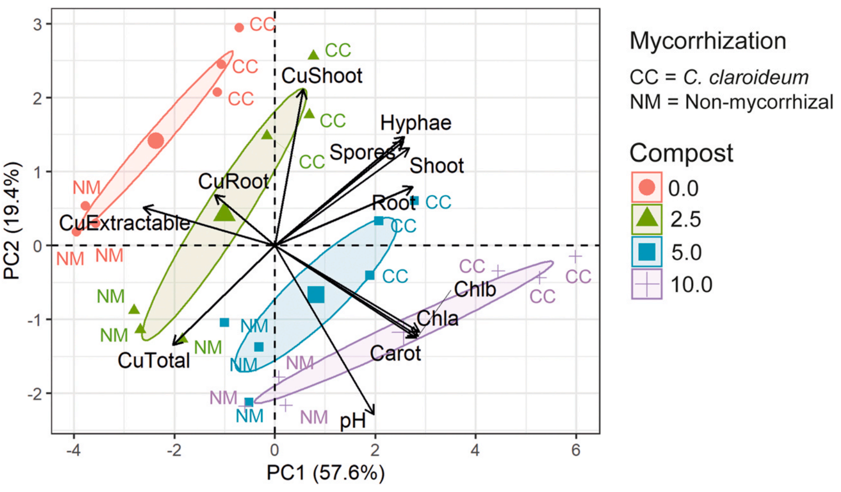

Fig. 6. Principal component (PC) analysis for arbuscular mycorrhizal fungal inoculation and compost addition for plants growing in a Cu mine tailing. The PCs are used for the ordination of both the experimental variables and all the individuals according the treatments established. Compost doses represent contents in %.

pigments. However, no significant difference was observed between CC-C5% and CC-C10% in Cu shoot concentration, suggesting that the difference in growth can be due to the nutrient increase of the mine tailing by compost, which is consistent with the increase in AM propagules density observed in CC-C10%. Additionally, the above results suggest that a diminish in shoot Cu concentration is mainly related to the reduction of Cu availability in the mining tailings with high compost doses. Therefore, our results suggest that the combined use of compost at a medium-high level (5% and 10%) can be successfully used to improve rhizosphere conditions, which reduced Cu availability and allow a wide AMF colonization both in the substrate and in the root of *O. picensis*. This reduced Cu concentration in root and shoot, finally generates an increase in photosynthetic rates and plant growth. Undoubtedly, the next step in the validation of this conceptual framework is the use of *O. picensis* under in field conditions, considering the production of "conditioned" plants colonized with effective AMF strains and with the most cost-effective level of organic amendments. These aspects are crucial since they could optimize the implementation of mitigation plans in mine tailings using effective metallophytes and AMF strains in an advantageous cost-effective way, using biotechnologies easy to be implemented.

5. Conclusion

Our results demonstrated that *Oenothera picensis* can efficiently growth in Cu mine tailing as a first noticeable step for further in-situ phytoremediation processes. Although *O. picensis* was able to establish without the colonization by the AM fungus *Claroideoglomus claroideum* or compost, the inoculation with *C. Claroideum* and the use of increasing doses of compost had a significant positive effect on the *O. picensis*, producing a noticeable increase in biomass up to 2-fold. Under this conditions, *C. Claroideum* was able to produce symbiosis with *O. picensis* regardless of compost concentration, showing great potential as a tool for plant growth in Cu mine tailing. Our results suggest that this better performance is mainly due to an increase in nutrient availability in *O. picensis* plants due to the supply through the compost addition and the uptake by the mycorrhizal way, and also by the reduction of Cu availability in the mine tailings. In this sense, a key aspect to be considered in future research for sustainable management of mine tailings should incorporate the use of effective AM fungal strains, including other microbial groups with plant-growth potential. Interestingly, one of the most evident variables representing the performance of *O. picensis* plants were the photosynthetic pigments, which could be a good indicator to analyze the behavior of plants in phytoremediation programs easily. Despite the best performance of *O. picensis* with *C. claroideum* inoculation and a compost dose of 10%, a dose of 5% can significantly improve pH, shoot biomass, photosynthetic pigments, Cu accumulation in shoot, and Cu available in the tailings. This factor regarding the cost-effectiveness of the program must be relevant to consider because the volume and transport can be the most important cost to decide its incorporation to improve phytoremediation plans in mine tailings.

CRediT authorship contribution statement

Rodrigo Perez: Conceptualization, Methodology, Formal analysis, Investigation, Writing - original draft, Visualization. **Yasna Tapia:** Resources, Supervision, Project administration, Funding acquisition. **Monica Antilen:** Resources, Supervision, Project administration, Funding acquisition. **Manuel Casanova:** Resources, Supervision, Project administration, Funding acquisition. **Christian Santander:** Investigation, Writing - review & editing. **Catalina Vidal:** Investigation, Writing - review & editing. **Humberto Aponte:** Visualization, Writing - review & editing. **Pablo Cornejo:** Conceptualization, Methodology, Writing - review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2020.111495.

References

Adriano, D. C.., 1986. Trace Elements in the Terrestrial Environment. Springer, New York.

Aponte, H., Herrera, W., Cameron, C., Black, H., Meier, S., Paolini, J., Tapia, Y., Cornejo, P., 2020a. Alteration of enzyme activities and functional diversity of a soil contaminated with copper and arsenic. Ecotoxicol. Environ. Saf. 192, 110264 https://doi.org/10.1016/j.ecoenv.2020.110264.

Aponte, H., Medina, J., Butler, B., Meier, S., Cornejo, P., Kuzyakov, Y., 2020b. Soil quality indices for metal(loid) contamination: an enzymatic perspective. Land Degrad. Dev. https://doi.org/10.1002/ldr.3630 (In press).

Bagheri, V., Shamshiri, M.H., Shirani, H., Roosta, H.R., 2011. Effect of mycorrhizal inoculation on ecophysiological responses of pistachio plants grown under different water regimes. Photosynthetica 49, 531–538. https://doi.org/10.1007/s11099-011-0064-5.

Baker, D.E., Amacher, M.C., 1982. Nickel, copper, zinc and cadmium. In: Page, A.L., Millers, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis Part 2: Chimical and Microbiological Properties, 2nd Ed. Agronomy Monograph No. 9, American Society of Agronomy, Madison, pp. 323–334.

Baker, A., Ernst, W.H., Van Der Ent, A., Malaisse, F., Ginocchio, R., 2010. Metallophytes: the unique biological resource, its ecology and conservational status in Europe, central Africa and Latin America. In: Batty, L., Hallberg, K. (Eds.), Ecology of Industrial Pollution. Cambridge University Press, New York, USA, p. 349.

Barea, J., Pozo, M., López-Ráez, J., Aroca, R., Ruiz-Lozano, J., Ferrol, N., Azcón, R., Azcon-Aguilar, C., 2013. Arbuscular mycorrhizas and their significance in promoting soil-plant system sustainability against environmental stresses. In: Rodelas, M.B., Gonzalez-Lopez, J. (Eds.), Beneficial Plant-Microbial Interactions: Ecology andApplications. CRC Press, USA, pp. 353–387 (Chapter 15). 〈https://doi.org/10.1201/b15251-16〉.

Bolan, N., Kunhikrishnan, A., Thangarajan, R., Kumpiene, J., Park, J., Makino, T., Beth, M., Scheckel, K., 2014. Remediation of heavy metal(loid)s contaminated soils - to mobilize or to immobilize? J. Hazard. Mater. 266, 141–166. https://doi.org/10.1016/j.jhazmat.2013.12.018.

Cavagnaro, T.R., 2015. Biologically regulated nutrient supply systems: compost and arbuscular mycorrhizas- a review. Adv. Agron. 129, 293–321. https://doi.org/10.1016/bs.agron.2014.09.005.

Cicatelli, A., Lingua, G., Todeschini, V., Biondi, S., Torrigiani, P., Castiglione, S., 2012. Arbuscular mycorrhizal fungi modulate the leaf transcriptome of a *Populus alba* L. clone grown on a zinc and copper-contaminated soil. Environ. Exp. Bot. 15, 25–35. https://doi.org/10.1016/j.envexpbot.2011.08.012.

Cornejo, P., Meier, S., Borie, G., Rillig, M.C., Borie, F., 2008. Glomalin-related soil protein in a Mediterranean ecosystem affected by a copper smelter and its contribution to Cu and Zn sequestration. Sci. Total Environ. 406, 154–160. https://doi.org/10.1016/j.scitotenv.2008.07.045.

Cornejo, P., Pérez-Tienda, J., Meier, S., Valderas, A., Borie, F., Azcón-Aguilar, C., Ferrol, N., 2013. Copper compartmentalization in spores as a survival strategy of arbuscular mycorrhizal fungi in Cu-polluted environments. Soil Biol. Biochem. 57, 925–928. https://doi.org/10.1016/j.soilbio.2012.10.031.

Cornejo, P., Meier, S., García, S., Ferrol, N., Durán, P., Borie, F., Seguel, A., 2017. Contribution of inoculation with arbuscular mycorrhizal fungi to the bioremediation of a copper contaminated soil using *Oenothera picensis*. J. Soil Sci. Plant Nutr. 17, 14–21. https://doi.org/10.4067/S0718-95162016005000070.

Dold, B., Fontbote, L., 2001. Element cycling and secondary mineralogy in porphyry copper tailings as a function of climate, primary mineralogy, and mineral processing. J. Geochem. Explor. 74, 3–55. https://doi.org/10.1016/S0375-6742(01)00174-1.

Doronila, A., Maddox, L., Reichman, S., King, D., Kolev, S., Woodrow, I., 2014. Vegetation response of Australian native grass species redgrass (*Bothriochloa macra*

(Steudel) S.T. Blake) and spider grass (*Enteropogon acicularis* (Lindl.) Lazarides) in saline and arsenic contaminated gold mine tailings: a glasshouse study. Miner. Eng. 56, 61–69. https://doi.org/10.1016/j.mineng.2013.10.025.

Ferrol, N., Tamayo, E., Vargas, P., 2016. The heavy metal paradox in arbuscular mycorrhizas: from mechanisms to biotechnological applications. J. Exp. Bot. 67, 6253–6265. https://doi.org/10.1093/jxb/erw403.

Gil-Loaiza, J., White, S., Root, R., Solís-Dominguez, F., Hammond, C., Chorover, J., Maier, R., 2016. Phytostabilization of mine tailings using compost-assisted direct planting: translating greenhouse results to the field. Sci. Total Environ. 565, 451–461. https://doi.org/10.1016/j.scitotenv.2016.04.168.

Ginocchio, R., Baker, A., 2004. Metalofitas en América Latina: un recurso biológico y genético único poco conocido y estudiado en la región. Rev. Chil. Hist. Nat. 77, 185–194. https://doi.org/10.4067/S0716-078X2004000100014.

Giovanetti, M., Mosse, B., 1980. An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. N. Phytol. 84, 489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x.

González-Guerrero, M., Cano, C., Azcón-Aguilar, C., Ferrol, N., 2007. GintMT1 encodes a functional metallothionein in *Glomus intraradices* that responds to oxidative stress. Mycorrhiza 17, 327–335. https://doi.org/10.1007/s00572-007-0108-7.

González, I., Muena, V., Cisternas, M., Neaman, A., 2008. Acumulación de cobre en una comunidad vegetal afectada por contaminación minera en el valle de Puchuncaví, Chile central. Rev. Chil. Hist. Nat. 81, 279–291. https://doi.org/10.4067/S0716-078X2008000200010.

González, I., Neaman, A., 2015. Evaluación de la tolerancia al cobre de dos poblaciones de *Oenothera picensis* Phil. subsp. *picensis* (Onagraceae). Gayana Bot. 72, 240–249. https://doi.org/10.4067/S0717-66432015000200008.

Hossner, L., Shahandeh, H., 2005. Rehabilitation of minerals processing residue (tailings). In: Lal, R. (Ed.), Encyclopedia of Soil Science. Marcel Dekker, New York, pp. 1450–1455.

Huang, L., Baumgartl, T., Mulligan, D., 2012. Is rhizosphere remediation sufficient for sustainable revegetation of mine tailings? Ann. Bot. 110, 223–238. https://doi.org/10.1093/aob/mcs115.

Jones, S., Bardos, R.P., Kidd, P.S., Mench, M., Leij, F., De, Hutchings, T., Cundy, A., Joyce, C., Soja, G., Friesl-hanl, W., Herzig, R., Menger, P., 2016. Biochar and compost amendments enhance copper immobilization and support plant growth in contaminated soils. J. Environ. Manag. 1–12. https://doi.org/10.1016/j.jenvman.2016.01.024.

Kabata-Pendias, A., Pendias, H. (2001). Trace Elements in Soils and Plants, New York. https://doi.org/10.1201/b10158-25.

Kleinert, A., Benedito, V.A., Morcillo, R.J.L., Dames, J., Cornejo, P., Zuniga-Feest, A., Delgado, M., Muñoz, G., 2018. Morphological and symbiotic root modifications for mineral acquisition from nutrient-poor soils. In: Giri, B., Prasad, R., Varma, A. (Eds.), Root Biology. Springer International Publishing, Switzerland, pp. 85–142.

Kranner, I., Beckett, R.P., Wornik, S., Zorn, M., Pfeifhofer, H.W., 2002. Revival of a resurrection plant correlates with its antioxidant status. Plant J. 31, 13–24. https://doi.org/10.1046/j.1365-313X.2002.01329.x.

Kossoff, D., Dubbin, W.E., Alfredsson, M., Edwards, S.J., Macklin, M.G., Hudson-Edwards, K.A., 2014. Mine tailings dams: characteristics, failure, environmental impacts, and remediation. Appl. Geochem. 51, 229–245. https://doi.org/10.1016/j.apgeochem.2014.09.010.

Lima, A.T., Mitchell, K., O'Connell, D.W., Verhoeven, J., Van Cappellen, P., 2016. The legacy of surface mining: remediation, restoration, reclamation and rehabilitation. Environ. Sci. Policy 66, 227–233. https://doi.org/10.1016/j.envsci.2016.07.011.

Lichtenthaler, H.K., 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. Methods Enzymol. 148, 350–382. https://doi.org/10.1016/0076-6879(87)48036-1.

Miller, A., Zégre, N., 2014. Mountaintop removal mining and catchment hydrology. Water 6, 472–499. https://doi.org/10.3390/w6030472.

Medina, J., Monreal, C., Barea, J.M., Arriagada, C., Borie, F., Cornejo, P., 2015. Crop residue stabilization and application to agricultural and degraded soils: a review. Waste Manag. 42, 41–54. https://doi.org/10.1016/j.wasman.2015.04.002.

Medina, J., Monreal, C., Chabot, D., Meier, S., González, M.E., Morales, E., Parillo, R., Borie, F., Cornejo, P., 2017. Microscopic and spectroscopic characterization of humic substances from a compost amended copper contaminated soil: main features and their potential effects on Cu immobilization. Environ. Sci. Pollut. Res. 24, 14104–14116. https://doi.org/10.1007/s11356-017-8981-x.

Meier, S., Azcón, R., Cartes, P., Borie, F., Cornejo, P., 2011. Alleviation of Cu toxicity in *Oenothera picensis* by copper-adapted arbuscular mycorrhizal fungi and treated agrowaste residue. Appl. Soil Ecol. 48, 117–124. https://doi.org/10.1016/j.apsoil.2011.04.005.

Meier, S., Borie, F., Bolan, N., Cornejo, P., 2012. Phytoremediation of metal-polluted soils by arbuscular mycorrhizal fungi. Crit. Rev. Environ. Sci. Technol. 42, 741–775. https://doi.org/10.1080/10643389.2010.528518.

Meier, S., Cornejo, P., Cartes, P., Borie, F., Medina, J., Azcón, R., 2015. Interactive effect between Cu-adapted arbuscular mycorrhizal fungi and biotreated agrowaste residue to improve the nutritional status of *Oenothera picensis* growing in Cu-polluted soils. J. Plant Nutr. Soil Sci. 178, 126–135. https://doi.org/10.1002/jpln.201400092.

Meier, S., Curaqueo, G., Khan, N., Bolan, N., Rilling, J., Vidal, C., Fernández, N., Acuña, J., González, M.E., Cornejo, P., Borie, F., 2017. Effects of biochar on copper immobilization and soil microbial communities in a metal-contaminated soil. J. Soils Sediment. 17, 1237–1250. https://doi.org/10.1007/s11368-015-1224-1.

Mendez, M.O., Glenn, E.P., Maier, R.M., 2007. Phytostabilization potential of quailbush for mine tailings. J. Environ. Qual. 36, 245–253. https://doi.org/10.2134/jeq2006.0197.

Mendez, M.O., Maier, R.M., 2008. Phytoremediation of mine tailings in temperate and arid environments. Rev. Environ. Sci. Biotechnol. 7, 47–59. https://doi.org/10.1007/s11157-007-9125-4.

Nayuki, K., Chen, B., Ohtomo, R., Kuga, Y., 2014. Cellular imaging of cadmium in resin sections of arbuscular mycorrhizas using synchrotron micro X-ray fluorescence. Microbes Environ. 29, 60–66. https://doi.org/10.1264/jsme2.me13093.

Olsen, S.R., Sommers, L.E., 1982. Phosphorus. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.), Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties. Agronomy Monograph No. 9. American Society of Agronomy and Soil Science of America, Madison, WI, pp. 403–430.

Pardo, T., Bernal, P., Clemente, R., 2017. The Use of Olive Mill Waste to Promote Phytoremediation, Olive Mill Waste: Recent Advances for Sustainable Management. Elsevier Inc.,. ⟨https://doi.org/10.1016/B978-0-12-805314-0.00009-1⟩.

Phillips, J.M., Hayman, D.S., 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Br. Mycol. Soc. 55, 158–161. https://doi.org/10.1016/S0007-1536(70)80110-3.

Rodriguez, R., Marticorena, C., Alarcón, D., Baeza, C., Cavieres, L., Finot, V.L., Fuentes, N., Kiessling, A., Mihoc, M., Pauchard, A., Ruiz, E., Sanchez, P., Marticorena, A., 2018. Catálogo de las plantas vasculares de Chile. Gayana Bot. 75, 1–430. https://doi.org/10.4067/s0717-66432018000100001.

Santana, N.A., Ferreira, P.A.A., Soriani, H.H., Brunetto, G., Nicoloso, F.T., Antoniolli, Z. I., Jacques, R.J.S., 2015. Interaction between arbuscular mycorrhizal fungi and vermi-compost on copper phytoremediation in a sandy soil. Appl. Soil Ecol. 96, 172–182. https://doi.org/10.1016/j.apsoil.2015.08.001.

Santibáñez, C., Verdugo, C., Ginocchio, R., 2008. Phytostabilization of copper mine tailings with biosolids: Implications for metal uptake and productivity of *Lolium perenne*. Sci. Total Environ. 395, 1–10. https://doi.org/10.1016/j.scitotenv.2007.12.033.

Santibáñez, C., Mar, L., Fuente, D., Bustamante, E., Silva, S., Ginocchio, R., 2012. Potential use of organic- and hard-rock mine wastes on aided phytostabilization of large-scale mine tailings under semiarid mediterranean climatic conditions: short-term field study. Appl. Environ. Soil Sci. 2012, 1–15. https://doi.org/10.1155/2012/895817.

Seguel, A., Medina, J., Rubio, R., Cornejo, P., Borie, F., 2012. Effects of soil aluminum on early arbuscular mycorrhizal colonization of wheat and barley cultivars growing in an andisol. Chil. J. Agric. Res. 72, 449–455. https://doi.org/10.4067/S0718-58392012000300022.

Schoenberger, E., 2016. Environmentally sustainable mining: the case of tailings storage facilities. Resour. Policy 49, 119–128. https://doi.org/10.1016/j.resourpol.2016.04.009.

Sieverding, E., 1991. Vesicular-Arbuscular Mycorrhiza Management in Tropical Agrosystems. GTZ,, Eschborn, Germany, p. 371.

Solís-Dominguez, F.A., White, S.A., Hutter, T.B., Amistadi, M.K., Root, R.A., Chorover, J., Maier, R.M., 2012. Response of key soil parameters during compost-assisted phytostabilization in extremely acidic tailings: effect of plant species. Environ. Sci. Technol. 46, 1019–1027. https://doi.org/10.1021/es202846n.

Tapia, Y., Diaz, O., Acuña, E., Casanova, M., Salazar, O., Masaguer, A., 2016. Phytostabilization of arsenic in soils with plants of the genus *Atriplex* established in situ in the Atacama Desert. Environ. Monit. Assess. 188. https://doi.org/10.1007/s10661-016-5247-x.

Sparks, D., 2003. Environmental Soil Chemistry, Second Edition. Academic Press, USA.

Tapia, Y., Bustos, P., Salazar, O., Casanova, M., Castillo, B., Acuña, E., Masaguer, A., 2017. Phytostabilization of Cu in mine tailings using native plant *Carpobrotus aequilaterus* and the addition of potassium humates. J. Geochem. Explor. 183, 102–113. https://doi.org/10.1016/j.gexplo.2017.10.008.

Tapia, Y., Loch, B., Castillo, B., Acuña, E., Casanova, M., Salazar, O., Cornejo, P., Antilén, M., 2020. Accumulation of sulphur in *Atriplex nummularia* cultivated in mine tailings and effect of organic amendments addition. Water Air Soil Pollut. 231, 1–14. https://doi.org/10.1007/s11270-019-4356-x.

Wang, L., Ji, B., Hu, Y., Liu, R., Sun, W., 2017. A review on in situ phytoremediation of mine tailings. Chemosphere 184, 594–600. https://doi.org/10.1016/j.chemosphere.2017.06.025.

Wellburn, A.R., 1994. The spectral determination of chlorophylls a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J. Plant Physiol. 144, 307–313. https://doi.org/10.1016/S0176-1617(11)81192-2.

Xu, Y., Yu, W., Ma, Q., Zhou, H., Jiang, C., 2017. Toxicity of sulfadiazine and copper and their interaction to wheat (*Triticum aestivum* L.) seedlings. Ecotoxicol. Environ. Saf. 142, 250–256. https://doi.org/10.1016/j.ecoenv.2017.04.007.