Phytostabilization of Cu in mine tailings using native plant *Carpobrotus aequilaterus* and the addition of potassium humates

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ABSTRACT

Mine tailings are a serious problem in many countries because their deposits cover large soil areas and are an important source of metal trace elements and sulphur. The objective of this study was to evaluate the phytostabilization capacity of metals and sulphur of the Chilean native plant *Carpobrotus aequilaterus* with the addition of potassium humates solution. Mine tailings presented a slightly acid pH of 6.3 ± 0.1, electrical conductivity of $3.84 \pm 0.10 \, dS \, m^{-1}$, extractable S of $2209 \pm 102 \, mg \, kg^{-1}$, SO_4^{2-} of $1243 \pm 27.8 \, mg \, L^{-1}$ and low contents of N, P and organic matter. Among the trace metals and metalloids (As, Cd, Cr, Mn, Mo, Ni, Pb and Zn), only Cu was found at high levels ($1999 \pm 223 \, mg \, kg^{-1}$) in these mine tailings, but the metal availability is low (1.25%). Potassium humates showed a strongly alkaline pH, conditions that favour the formation of stable dissolved metal humates. The plant was cultivated in pots containing mine tailings (control) and mine tailings with the addition of potassium humates at doses of 30 and $60 \, kg \, ha^{-1}$ dissolved in irrigation water. The plants were adapted to this substrate with low fertility and high levels of SO_4^{2-}. At 120 days of cultivation, plants mine tailings containing $60 \, kg \, ha^{-1}$ of potassium humates showed significantly higher concentrations in aerial part of Cu and Fe ($29.3 \pm 7.5 \, mg \, kg^{-1}$ and $546 \pm 181 \, mg \, kg^{-1}$, respectively), than control. The concentration of Mn, S and Zn in the plant was not affected by the application of potassium humates. However, the plant showed accumulation capacity of Mn in leaves ($930 \pm 255 \, mg \, kg^{-1}$). Except for Fe and Mn, the concentrations of metal in this plant do not exceed the levels of metals considered sufficient or normal indicating a low metal availability in these mine tailings. With the addition of potassium humates, the rate of transport to the aerial part of the plant was lower for Cu, indicating that the species increased the absorption of this metal preferentially in the roots, which is favourable for phytostabilization.

1. Introduction

In several countries, deposits of mine tailings cover large areas of land and significantly impact the landscape (Mendez and Maier, 2008a; Kossoff et al., 2014). Chile, the leading copper producer in the world (U.S. Geological Survey, 2016), is one of the primary regions that contain deposits of mine tailings, along with Peru, northern Mexico, the eastern United States, southwestern Spain, western India, South Africa, Australia and Canada (U.S. Geological Survey, 2016; Mendez and Maier, 2008a). In many of these regions, problems associated with the management of mine tailings have been reported (Schoenberger, 2016). In Chile, there are approximately 723 tailing deposits, of which 124 are active, 436 are inactive, 132 are abandoned and 31 are in an unknown state (SERNAGEOMIN, 2015). In 2011, a law regulating the closure of mining operations was established mandating the chemical stabilization of tailings to control their impact on air, water and soil (DOE, 2011).

The chemical composition of mine tailings varies widely depending on the characteristics of the rock (Lottermoser, 2010); generally, they consist mainly of Si, S, Fe, Al, Ca, Mg, K, Na and Mn (Tordoff et al., 2000; Hossner and Shahandeh, 2005; Kossoff et al., 2014). The N and P levels present in mine tailings are very low, and they are devoid of organic matter, structure and support for microorganisms (Ye et al., 2002; Wong, 2003; Mendez and Maier, 2008a,b). Mine tailings are a source of metallic elements; however, new mineral extraction technologies have decreased the total concentration of metals present in tailings, whereas the concentration of bioavailable metals in mine tailings is relative and may or may not be sufficient to cause phytotoxicity (Tordoff et al., 2000). Tailings from copper mining in Chile may contain high concentrations of Cu and S (Ginocchio, 1996), elements that can easily contaminate soil, water and air (Lottermoser, 2010). In general, the physical, chemical and biological characteristics of mine

tailings negatively affect seed germination and plant growth (Ginocchio, 1996; Mendez et al., 2007), however, with a good management the objective of developing a vegetal cover is achieved.

Several studies recommend the use of phytostabilization as a sustainable strategy for the management of mine tailings (Tordoff et al., 2000; Conesa et al., 2006; Conesa et al., 2007a,b; Madejón et al., 2012; Doronila et al., 2014). The development of vegetation at contaminated sites can reduce the mobility of the elements present in the tailings (Robinson et al., 2005) and provide a chemical and physical barrier to metal transport (Tordoff et al., 2000). By phytostabilization, the metals can be adsorbed, precipitated, complexed with organic matter and accumulated within root tissues (Cunningham et al., 1995). The establishment of vegetation in areas containing mine tailings progressively increases the level of organic matter that is present and improves the level of nutrients (Shen et al., 2014).

In arid and semi-arid climates, phytostabilization involves the use of metal-tolerant plants in conjunction with the addition of organic amendments and irrigation management (Wong, 2003; Mendez and Maier, 2008a; Doronila et al., 2014). Studies show that the quantity and type of amendments required depend on the types of plants and the physical and chemical characteristics of the tailings (Mendez and Maier, 2008a). However, the phytostabilization of mine tailings is limited by the lack of knowledge regarding the minimum amount of amendments needed to establish and maintain plants over time and to stabilize the pollutants that are present (Mendez and Maier, 2008b). The addition of calcite decreases the concentration of the metal in solution by raising the pH, but this is usually a temporary solution if the tailings are still actively generating H^+ (pyrite oxidation). The amount of calcite needed to neutralize the acidity can be so great that adding calcite to remediate these soils is impractical; furthermore, if disposal of tailings occurs, the added materials can be washed away and can contaminate water and soil (Brady and Weil, 2008). Inorganic fertilizers are sometimes used, but generally these must be applied in combination with organic amendments or lime in order to reduce metal bioavailability. These amendments are not considered expensive, but the extensive tailings problem makes adding amendments one of the major costs associated with remediation (Mendez and Maier, 2008a). Although organic amendments are favoured for their ability to immediately decrease metal bioavailability, weathering and decomposition of organic residues may ultimately enhance metal mobility (Mendez and Maier, 2008a). Therefore, the quality of the organic matter added must be considered so as to avoid undesirable effects such as decreased pH and increased mobility of trace metals. The addition of organic amendments with greater humification and higher content of inorganic components, particularly Fe favours the adsorption of metals (Tapia et al., 2010). A number of researchers have used the Langmuir and Freundlich models to explain adsorption phenomena between metals and humic substances (Zhang et al., 2009; Yang et al., 2015). Humic and fulvic acids (humic substances) impart high chemical reactivity to organic matter because they increase its cation exchange capacity and thus the formation of relatively stable complexes of organic compounds with trace metals (Stevenson, 1994). However, it is unclear whether the complexes that form between soluble humic substances and metals increase or decrease their availability (Adriano, 2001). The pH and functional groups of the humic substances are the main factors that explain the behaviour of the humic substances of certain organic amendments with respect to Cd (Tapia et al., 2010).

Plant selection is an important factor, and plants with metal exclusion capacity may be useful in phytostabilization (Poschenrieder et al., 2001; Wong, 2003). In addition, the use of native plants is recommended because native plants often demonstrate tolerance to local environmental conditions and may favour natural ecological succession (Orchard et al., 2009; Tapia et al., 2013a; Liu et al., 2016). Other qualities that make plants useful in phytostabilization are high aerial biomass generation, deep roots, low rates of transport of metals, ease of handling, low water requirements, high salt tolerance and not edible by animals. However, the ultimate objective of the phytostabilization of mine tailings is the establishment of a succession of plant communities that promotes the development of soil processes, increases microbiological diversity, and finally restores the function of the sustainable ecosystem (Mendez and Maier, 2008a). During the first stage, it is necessary to cover mine tailings with fast-growing vegetation to avoid dispersion of pollutants by wind or water and, on the other hand, to generate organic matter and microbiological activity in a short time. *Carpobrotus aequilaterus* is a native plant of Chilean ornamental value (Riedemann and Aldunate, 2004); the genus to which it belongs is widely distributed throughout the world. The native species of Australia, *Carpobrotus rossi*, has been recommended for its potential in the phytoremediation of Cd (Zhang et al., 2014). Tissue from the Mediterranean species *Carpobrotus edulis* has been used as an anion and metal adsorbent (Chiban et al., 2011). *Carpobrotus aequilaterus* has been found to spontaneously colonize mineral tailings (Orchard et al., 2009) and is characterized as a succulent, creeping (saline-tolerant) growth species that naturally grows on sandy substrates on the seashore; it is drought-tolerant and has low nutrient requirements. However, there are no data on the metal levels or sulphur that are absorbed by this species. It is hypothesized that *Carpobrotus aequilaterus* is a species that adapts to mining tailings and its potential phytostabilization capacity will be favoured by the application of humic substances.

The objective of this work is to evaluate the phytostabilization of metals and sulphur by *Carpobrotus aequilaterus* in mine tailings with the addition of potassium humates.

2. Material and methods

2.1. Characterization of mine tailings and potassium humates

Samples of mine tailings were obtained from the Ovejería tailings dam (332,190, 30 m E; 6,341,395, 72 m S) from the CODELCO Andina Division located in the community of Til Til in the Santiago Metropolitan Region of Chile (Fig. 1). The dam is active and occupies approximately 900 ha of a total of 1900 ha projected at the end of its useful life (García, 2007). The wall of the dam is composed of the same tail (a coarse fraction of sand) by means of its deposit by the method of downstream. The tailings samples were obtained from the wall. Mineralogical analyses of mine tailings from the wall reported by SGS Chile Ltd. indicate the presence of quartz (50.74%), muscovite (19.04%), albite (16.01%), orthoclase (13.05%), pyrite (0.77%) and clinochlore (0.39%). The pH of the mine tailings was determined in suspension (1:2.5, W/V) and the electrical conductivity (EC) was determined in a saturated extract using Hanna HI 3222 and 4321 equipment (Woonsocket, Rhode Island, USA), respectively. Organic carbon (OC) was determined by the oxidation method with H_2SO_4 and $Na_2Cr_2O_7$ and determination of C and determination of C by colorimetric method comparing with a standard sucrose curve to 600 nm (Sims and Haby, 1971) using a Hatch DR500 spectrophotometer (Loveland, Colorado, USA). Total organic matter was calculated as MO (%) = $1.72 \times$ OC. The cation exchange capacity (CEC) of the material was determined using Na acetate and NH_4 acetate at pH 7.0 (Chapman, 1965), and the amount of Na present was measured by atomic absorption spectrophotometry (AAS) using a PerkinElmer 3110 instrument (Boston, Massachusetts, USA). Available N was determined by KCl extraction followed by NH_4 distillation and titration (Mulvaney, 1996). The available P was extracted with sodium hydrogen carbonate (0.5 M, pH 8.5) by the Olsen method (Kuo, 1996). Sulphate was determined in the saturated extract and by colorimetric determination with $BaCrO_4$ (Dewis and Freitas, 1970). The available S was determined with by extraction with 0.01 M $Ca(H_2PO_4)_2$ and turbidimetry (Sing et al., 1995). For the determination of the total concentration of the chemical elements As, Cd, Cr, Fe, Mn, Mo, Ni, Pb and Zn, the samples of mine tailings were digested with $HF\text{-}HClO_4\text{-}HCl\text{-}HNO_3$ (Dold and Fontboté, 2001), and metal levels were determined by AAS and As by flow-
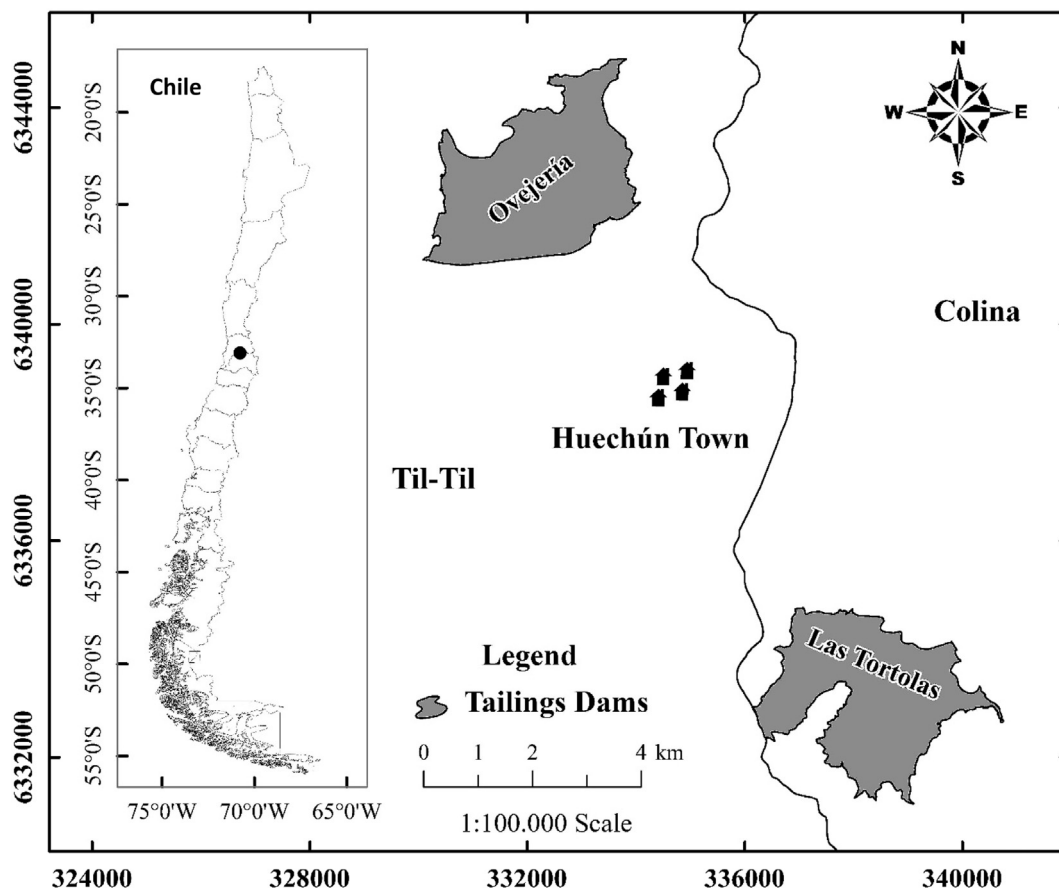
Fig. 1. Location of the Ovejería tailings dam from which mine tailings samples were obtained (Central zone, Chile).

injection hydride generation atomic absorption spectrophotometry (FI-HG-ASS) using a Perkin Elmer FIA 100 apparatus (detection limit: 0.01 μg As L^{-1}). For the determination of available metals, DTPA-CaCl$_2$-TEA at pH 7.3 was used (Baker and Amacher, 1982). For all analyses, 3 replicates were performed. The concentration of elements is expressed in mg kg^{-1} of dry matter.

As a source of humic substances, the commercial product Pow Humus® WSG 85 (Humintech GmbH, Germany) was used. This product is mixtures of salts of humic acids in granules that are obtained through alkaline extraction of German leonardite (highly oxidized lignite). The label indicates pH 9.0–10.0, a solubility in water of 100%, 80–85% potassium humates, 10–12% potassium (as K$_2$O dry matter), 1% Fe and a particle size of insoluble constituents > 100 μm. The product was used as a solution of potassium humates. The chemical characteristics of the product were determined in triplicate. The pH and EC were determined in a potassium humates:water suspension prepared at a ratio of 1:2.5 (W/V). The CEC was determined with ammonium acetate at pH 7.0 (Chapman, 1965). Total organic matter was determined by combustion at 550 °C (TMECC, 2001). The total extractable C (TEC) fraction was isolated by adding 25 mL of a solution 0.5 M NaOH to 30 mg of potassium humates. The mixture was shaken for 48 h. The supernatant solution was then separated from the residue by centrifugation and filtration. The supernatant solution containing the TEC fraction was acidified to pH ~ 1 with concentrated HCl to allow the precipitation of the humic acid-like (HAL) fraction. The fulvic acid-like (FAL) fraction remained in solution was then separated from the residue by centrifugation (Schnitzer, 1982). The TEC and content of C in the FAL fraction (FAC or fulvic acid C) were determined by oxidation with 10 mL of 1 N K$_2$Cr$_2$O$_7$ followed by addition of 3 to 4 drops of o-phenanthroline indicator and titration with 1 N FeSO$_4$·7H$_2$O (Nelson and Sommers, 1982). The content of C in the HAL fraction (HAC or

humic acid C) was calculated by difference: HAC = TEC − FAC (Senesi, 1989; Romero et al., 2007).

2.2. Cu sorption isotherms

For the Cu sorption experiments, a batch equilibration was conducted (Zhang et al., 2009). Thirty milligrams of potassium humates were precisely weighed and combined with 25 mL of solution in a plastic centrifuge tube. The solutions were prepared in 0.1 M NaNO$_3$ solution and contained increasing Cu^{2+} (as CuSO$_4$) concentrations of 0, 15, 25, 35 and 45 mg L^{-1}. All samples were measured in triplicate. The tubes were fitted with caps and shaken at 220 rpm for 24 h at 25 °C. The suspensions were then centrifuged at 5000 rpm for 15 min and passed through a 0.45-μm filter. The level of Cu in the suspension was analyzed by AAS. Cu in solution at 0 mg L^{-1} was not detected. The limit of detection of AAS for Cu^{2+} was 0.01 mg L^{-1}. Copper sorption amounts were calculated by determining the difference between the initial and the equilibrium concentrations of Cu^{2+}. The amount of adsorbed Cu^{2+} was calculated as follows:

$$Cs = \frac{v(ci - c)}{m} \tag{1}$$

where Cs is the amount of Cu^{2+} adsorbed (mg g^{-1}), v is the volume of the equilibrium solution, ci and c represent the initial and equilibrium concentrations of Cu^{2+} (mg L^{-1}), respectively, and m is the weighed amount of humic substances. The adsorption data were fitted to both the Langmuir and Freundlich equations, as follows:

$$\text{Langmuir equation } C_s = \frac{K_d C_{max} C_w}{1 + K_d C_w} \quad \text{linear equation } \frac{1}{C_s}$$
$$= \frac{1}{C_{max}} + \frac{1}{K_d C_{max} C_w} \tag{2}$$

Freundlich equation $C_s = K_d C_w^n$ linear equation $\log C_s$

$$= \log K_d + n \log C_w \tag{3}$$

where C_s is the Cu adsorption concentration (mg g^{-1}), C_{max} is the maximum Cu sorption value (mg g^{-1}), C_w is the equilibrium value for Cu in solution (mg L^{-1}), K_d is the adsorption constant that corresponds to the intercept of the equation of the line, and n is a constant that corresponds to the slope of the line.

2.3. Pot experiment

The pot assay was conducted from December 2014 to May 2015 in the research greenhouse of the Laboratory of Soil and Water Chemistry of the Faculty of Agronomic Sciences of the University of Chile. The mean minimum temperature of the test was 9.9 ± 3.1 °C, and the mean maximum temperature was 27.6 ± 3.4 °C. *Carpobrotus aequilaterus* plants were established in mine tailings using plastic pots of 1 L in volume. The initial dry weight of the plants before their cultivation in mine tailings was 2.14 ± 0.7 g of shoot (leaf + stem) and 0.34 ± 0.04 g of root, and the initial concentration of elements in the aerial part was, in mg kg^{-1}: Cu, 7.60 ± 0.63; Fe, 84.2 ± 30.6; Mn, 280 ± 80; Ni, 8.4 ± 3.1; S, 5537 ± 964; and Zn, 7.47 ± 3.54. The concentrations of Cd, Cr, and Pb were lower than the detection limit of the measuring apparatus. Three weeks after transplantation, the following treatments were began: 1) mine tailings (MT); 2) mine tailings with application of potassium humates at 30 kg ha^{-1} of mine tailings (or 25 mg of potassium humates kg^{-1}) (MT + humate30); and 3) mine tailings with application of potassium humates at 60 kg ha^{-1} of mine tailings (or 50 mg of potassium humates kg^{-1}) (MT + humate60). Each treatment included 6 plants. At 60 days were analyzed 3 plants and at 120 days another 3 plants. The pH and EC in suspension MT + potassium humates:water (1:2.5 W/V) was pH 7.8 ± 0.1 and 1.39 ± 0.4 dS m^{-1} in MT + humate30 and pH 7.8 ± 0.1 and 1.80 ± 0.1 dS m^{-1} in MT + humate60.

In the water of irrigation (pH 7.5 and EC 1.39 dS m^{-1}) the doses of potassium humates dissolved was applied gradually. At 60 and 120 days after the start of the assay, the dry weight and the Cd, Cr, Cu, Fe, Mn, Ni, Pb, S and Zn concentrations in shoots and roots were determined (in triplicate). The samples were dried at 60 ± 5 °C and passed through a 0.5-mm sieve. Subsequently, vegetal samples (0.5 g) were digested in an autoclave Speedy WM-341 (MinChuan W. Rd. Taipei, Taiwan) with 3 mL of 65% HNO$_3$, 2 mL of 33% H$_2$O$_2$ and 5 mL of distilled water at 125 °C at a pressure of 125 kPa for 30 min; the samples were then filtered and diluted to 15 mL with distilled water (Lozano-Rodriguez et al., 1997). The metal content of the samples was determined by AAS. The levels of Cd, Cr, Ni and Pb were lower than the detection limits of the measuring apparatus at 60 and 120 days and therefore the results are not presented. To determine the amount of sulphur in plant tissue, the samples were calcined with Mg(NO$_3$)$_2$ and dissolved in HCl (AOAC, 1995) and the turbidity produced by the addition of BaSO$_4$ was measured (Lachica et al., 1973). The Ti = [element in aerial part] / [element in roots] was used to calculate the transport index to the metal part (Ti) of metals and S, using the average values of concentrations measured in three replications. The limit of detection of AAS for metals was 0.01 mg L^{-1}. Montana Soil SRM 2711 for trace element concentrations was employed to evaluate accuracy. The certified values are: As 105 ± 8 µg g^{-1}; Cd 41.7 ± 0.25 µg g^{-1}; Cu 114 ± 2 µg g^{-1}; Mn 638 ± 28 µg g^{-1}; Zn 350.4 ± 4.8 µg g^{-1} and Fe 2.89 ± 0.06%. The values obtained were As 98.19 ± 0.03 µg g^{-1}; Cd 38.5 ± 0.8 µg g^{-1}; Cu 110.0 ± 3.3 µg g^{-1}; Mn 589 ± 29 µg g^{-1}; Zn 323.3 ± 25.9 µg g^{-1} and Fe 2.54 ± 0.1%. Standard reference of vegetative material 1573a of the National Institute of Standards and Technology metal concentrations was employed. The certified values are: Cu 4.70 ± 2 µg g^{-1}; Fe 368 ± 7.0 µg g^{-1}; Mn 246 ± 8.0 µg g^{-1} and Zn 30.9 ± 0.7 µg g^{-1}.

The values obtained were Cu 4.23 ± 0.70 µg g^{-1}; Fe 331.2 ± 55.2 µg g^{-1}; Mn 221.4 ± 36.9 µg g^{-1} and Zn 27.81 ± 3.09 µg g^{-1}. All chemicals were of high quality, and certified standard solutions of metals Merck (Darmstadt, Germany) were used.

2.4. Statistical analysis

Trace metals and sulphur concentration (mg kg^{-1}) in the aerial part and roots of *C. aequilaterus* between treatments (MT, MT + humate30 and MT + humate60) at 60 and 120 days, separately, were compared using one-way analysis of variance (ANOVA). The dry weight of the plants after 120 days was also compared between treatments. To test for statistically significant differences between treatments, the Duncan test at $p \leq 0.05$ was used. All statistical tests were conducted using the SPSS 13.0 software package.

3. Results

3.1. Chemical characteristics of mine tailings

Mine tailings were characterized by high sand content, a slightly acid pH, low salinity and high concentrations of Cu, soluble (SO$_4^{2-}$) and extractable S (Table 1). As is common in mine tailings, fertility parameters such as CEC, available NPK and organic matter were low. The total concentration of trace metals (loid)s in the mine tailings was not - high relative to the published reference ranges for mine tailings in other parts of the world (Hossner and Shahandeh, 2005) and with respect to the metal levels in soils, except for Cu. The availability with respect to the total concentrations of the metals in the mine tailings was 9.2% for Cd, 3.7% for Pb, 2.15% for Zn and 1.25% for Cu (Table 2). For the remaining metals, the availability was < 1.0%. The availability levels of Cu, Ni, Pb and Zn are in the range of the levels found in non-contaminated soils (Kelly, 1979).

3.2. Potassium humates and Cu sorption curve

Potassium humates in water display strongly alkaline pH (Table 3). Their high electrical conductivity is due to their significant content of the product of K$_2$O. Their cation exchange capacity is less than the range of 100–300 cmol$_c$ kg^{-1} that has been reported for organic matter (Porta et al., 2003). Potassium humates possess a total organic carbon content of 27.7%, indicating that they also include a considerable proportion of inorganic components, which are probably associated with Fe and K. The total extractable carbon of potassium humates is formed per 89% of humic acid-C whereas fulvic acid-C is 11%. The Cu sorption curve (Fig. 2) is presented in linear form for the Langmuir and Freundlich models. The correlation coefficient (R^2) for the linear adjustment of the adsorption isotherms of Langmuir and Freundlich was similar indicating that that there is no single model that defines the adsorption behaviour of potassium humates used in this assay. For Langmuir model C_{max} (Eq. (2)) is 2.59 mg Cu g^{-1} of potassium humates.

3.3. Trace metals and sulphur concentration in plants

In the aerial part of *C. aequilaterus* plants at 120 days of cultivation (Table 4), the concentration of Cu and Fe in the MT + humate60 was significantly higher than in the MT, and there were no differences in Mn, S and Zn, whereas at 60 days of cultivation only Fe in the MT + humate60 showed significantly higher than in the MT. However, only the metals Fe and Mn exceed the levels considered normal or sufficient in leaves (Kabata-Pendias, 2011). In the roots at 120 days (Table 5), as leaves, the concentration of Cu and Fe in the MT + humate60 was significantly higher than in the MT, without differences in Mn, S and Zn. The concentrations of Cu and Fe did not show differences in the MT at 60 and 120 days, indicating that without the addition of

Table 1

Main chemical and physical characteristics of mine tailings Ovejería. Values are mean $\pm$ standard deviation (n = 3).

	Mine tailings Ovejería	Range of reference mine tailing[a]	References of total levels in non-contaminated soil
pH	6.3 $\pm$ 0.1	1.8–9.4	–
Electrical conductivity (dS m^{-1})	3.84 $\pm$ 0.10	0.1–22.4	–
Organic matter (%)	0.31 $\pm$ 0.05	0.02–25	–
CEC (cmol$_c$ kg^{-1})	6.35 $\pm$ 1.51	0.19–46.5	–
Available N (mg kg^{-1})	6.55 $\pm$ 1.80	–	–
Available P (mg kg^{-1})	0.40 $\pm$ 0.01	1.0–400	–
Available K (mg kg^{-1})	9.32 $\pm$ 0.41	1.0–564	–
SO$_4^{2-}$ (mg L^{-1})	1243 $\pm$ 27.8	–	–
Extractable S (mg kg^{-1})	2209 $\pm$ 102	–	–
Total As (mg kg^{-1})	19.7 $\pm$ 0.01	–	0.1–55[b]
Total Cd (mg kg^{-1})	2.17 $\pm$ 0.56	2.0–280	0.01–2.0[b]
Total Cr (mg kg^{-1})	85.6 $\pm$ 16.7	–	10–150[b]
Total Cu (mg kg^{-1})	1999 $\pm$ 223	1.0–750	2.0–250[c]
Total Fe (mg kg^{-1})	46,300 $\pm$ 2500	4000–570,000	2000–550,000[c]
Total Mn (mg kg^{-1})	647 $\pm$ 76	10–4000	100–4000[b]
Total Mo (mg kg^{-1})	164 $\pm$ 22	100–800	0.1–40[c]
Total Ni (mg kg^{-1})	24.5 $\pm$ 9.2	10–546	2.0–750[c]
Total Pb (mg kg^{-1})	19.3 $\pm$ 3.0	0.3–2810	2.0–300[b]
Total Zn (mg kg^{-1})	109 $\pm$ 7.8	1.0–5000	1.0–900[b]
Bulk density (kg L^{-1})	1.57	0.2–3.1	–
Sand (%)	85	1.0–97	–
Silt (%)	13	0–96	–
Clay (%)	2.0	0–40	–

[a] Hossner and Shahandeh (2005).
[b] Adriano (2001).
[c] Sparks (2003).

Table 2

Metal concentration (mg kg^{-1}) by DTPA extraction in mine tailings (value are means $\pm$ standard deviations, n = 3).

	Mine tailing Metal DTPA-extraction	References of available levels in non-contaminated soil[a]
	mg kg^{-1}	mg kg^{-1}
As	–	0–30
Cd	0.20 $\pm$ 0.01	–
Cr	0.20 $\pm$ 0.01	–
Cu	20.8 $\pm$ 2.5	0–100
Fe	9.20 $\pm$ 0.63	–
Mn	1.55 $\pm$ 0.10	–
Ni	0.20 $\pm$ 0.10	0–20
Pb	1.40 $\pm$ 0.16	0–200
Zn	2.35 $\pm$ 0.45	0–250

[a] Kelly (1979).

Table 3

Chemical characteristics of potassium humates (value are means $\pm$ standard deviations; n = 3).

pH (1:2.5 W/V)	9.6 $\pm$ 0.05
Electrical conductivity (1:2.5 W/V) (dS m^{-1})	24.3 $\pm$ 0.09
Total organic C (%)	27.8 $\pm$ 2.1
Exchange cationic capacity (cmol$_c$ kg^{-1})	69.9 $\pm$ 5.0
Total extractable C (g kg^{-1})	131.2 $\pm$ 27.2
Humic acid C (g kg^{-1})	116.8 $\pm$ 4.3
Fulvic acid C (g kg^{-1})	14.4 $\pm$ 0.5

potassium humates there is no effect on the absorption of these metals in these mine tailings. The Mn level of 439 and 930 mg kg^{-1} in leaves exceeds the toxic range between 400 and 1000 mg kg^{-1} (Kabata-Pendias, 2011). Despite the high soluble and extractable concentrations of S in the plants in the MT (Table 1), its level did not exceed the normal levels of 2000–5000 mg kg^{-1} (Havlin et al., 2014). The concentration of S in the roots showed high variability.

With respect to the transport of metals to the aerial portion of the plant (Ti), Mn was the only element that showed a value $>$ 1 for all

treatments (Fig. 3), indicating that *C. aequilaterus* shows phytoextraction capacity for Mn in these mine tailings. For Cu, Fe, S and Zn, Ti was $<$ 1, indicating the phytostabilization capacity of *C. aequilaterus* for these elements. For Fe and Mn, with the addition of potassium humates, there was a tendency to increase Ti, indicating that the concentration of these metals increased more in the aerial part of the plants than in the roots (Fig. 3). However, for Cu, with the addition of potassium humates there was a tendency to decrease Ti (Fig. 3); that is, the concentration of this metal increased preferably in the roots.

3.4. Dry weight of plants

Dry shoot weight (Fig. 4) was significantly higher in the MT than in the MT + humate60. It is likely that the high concentration of Mn in the aerial part of the plants in the MT + humate60 (930 $\pm$ 255 mg kg^{-1}, Table 4) caused this decrease. Neither of the treatments resulted in significant differences in the dry weight of roots.

4. Discussion

4.1. Mine tailings

Soil pH is the most important factor influencing the mobility of metals in soils; it is directly related to the formation of soluble complexes and the phenomena of adsorption and precipitation (Adriano, 2001; Hoda, 2010; Alloway, 2010). It is known that acid conditions increases the concentration of metals in solution and increase their availability; therefore, the slightly acid pH of mine tailings in this study could favour these processes. The electrical conductivity of mine tailings did not indicate salinity. Salinity is common in mine tailings. If the electrical conductivity is low, it may be due to the recent origin of the mine tailings (Ye et al., 2002).

Mine tailings of this study showed very low concentrations of essential nutrients such as available N, P and organic matter, a feature that is common to most mine tailings throughout the world (Tordoff et al., 2000). This is demonstrated by the low natural revegetation of these substrates (Conesa et al., 2007b). The high levels of Al, Si and Na
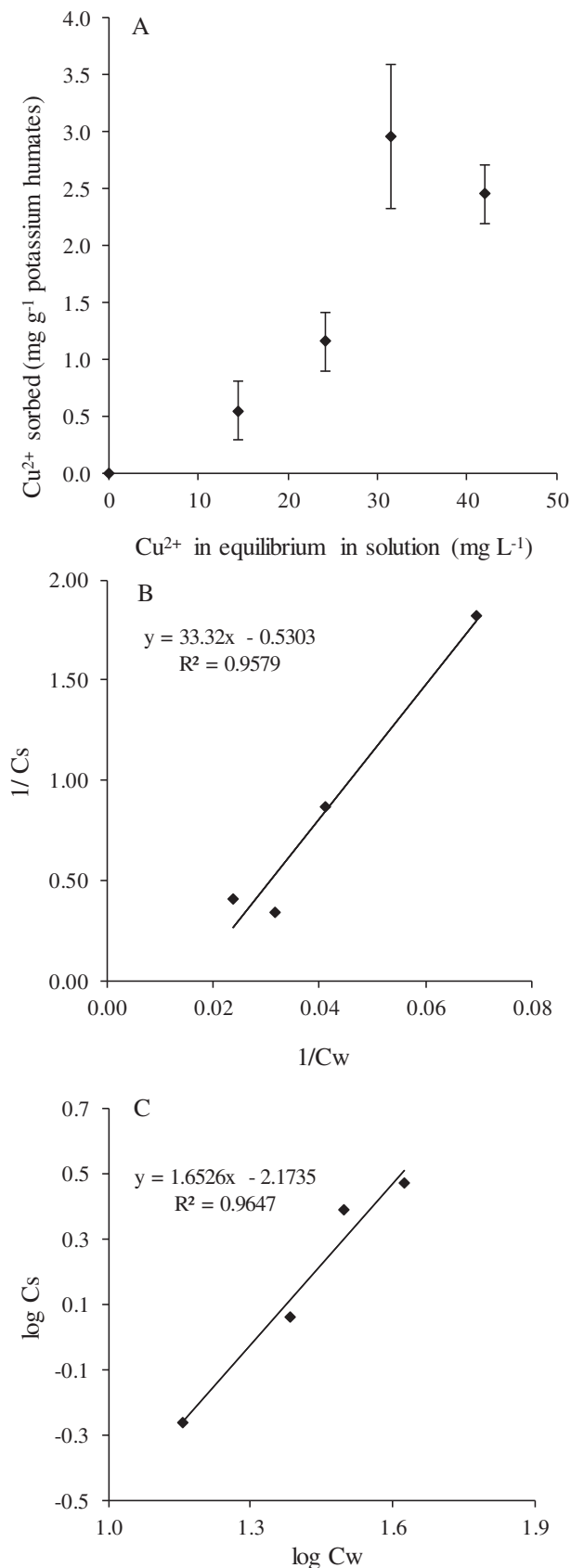
Fig. 2. Cu^{2+} adsorption isotherms for potassium humates (A) and fitting curves derived from Langmuir (B) and Freundlich (C) equations (the lines within the bars indicate standard deviation; n = 3).

in tailings and the low content of organic matter, CEC, N and P are responsible for the lack of fertility of mine tailings, which requires the incorporation of amendments (Conesa et al., 2007b, Mendez and Maier, 2008a,b). Many investigations have been conducted to evaluate the ability of various organic amendments to add nutrients to mine tailings and/or decrease the mobility of metals present in tailings (Ye et al., 2002; Santibañez et al., 2012; Gil-Loaiza et al., 2016; Alvarenga et al., 2004; Doronila et al., 2014; Lee et al., 2014).

Copper is present in higher total and available concentrations than other metals in mine tailings because it comes mainly from chalcopyrite ($CuFeS_2$), which is the main ore in Chile (Alloway, 2010). A similar concentration of Cu (approximately 2000 mg kg^{-1}) as is found in other mine tailings of the central zone (Dold and Fontboté, 2001) was found in mine tailings in this study. Other researches on areas in the north and central zones of Chile reported levels of total Cu of 485 mg kg^{-1} (Santibáñez et al., 2008) and 4393 mg kg^{-1} (Santibañez et al., 2012). As a reference, the total concentration of Cu in agricultural soils in the north zone of Chile was found to fall within the range of 78–349 mg kg^{-1} (Carkovic et al., 2016) and in soils of the central zone near to the coast to be 60 mg kg^{-1} (Muena et al., 2010). In other countries, higher levels of total Cu have been found in soils, for example 1508 mg kg^{-1} in England and Wales, 1836 mg kg^{-1} in the United States, and 3088 mg kg^{-1} in northern California, the latter after mining activity (Alloway, 2010).

Sulphides constitute a major proportion of the rocks found in metallic ore deposits as Cu. Consequently, the concentrations of soluble S (SO_4^{2-}) and extractable S in the mine tailings in this study were high. Santibañez et al. (2012) found sulphate levels of 2912 mg L^{-1} in mine tailings in the north and central zones of Chile. When sulphur is exposed to the atmosphere or to oxygenated groundwater, sulphides are oxidized, producing sulphate-laden acid water, trace metals and metalloids (Lottermoser, 2010). Pyrite oxidation with release of Fe^{2+}, Cu^{2+}, Mn^{2+}, Zn^{2+} cations and $HMoO_4^-$, $H_2AsO_4^-$ and SO_4^{2-} oxyanions has been described in the central Mediterranean zone of Chile (Dold and Fontboté, 2001). In this locality, sulphide concentrations on the order of 1.0–1.7% as pyrite equivalent, levels that are considered low, have been found in tailings (Dold and Fontboté, 2001).

With respect to the total concentration of As, although Chile is a geographical area that is known worldwide for its high concentration of this metalloid in soils (Bundschuh et al., 2012; Tapia et al., 2013a), the mine tailings in this study did not exceed 40 mg kg^{-1} in total As, a level that is commonly found in soils (Sparks, 2003; Moreno-Jiménez et al., 2012).

The total concentrations of Cd and Cr in mine tailings are similar to the levels commonly found in soils (Adriano, 2001). The total concentration of Fe is comparable to the levels found in agricultural soils in northern Chile, which vary between 35,000 and 43,257 mg kg^{-1} (Carkovic et al., 2016). The total concentration of Mn in these mine tailings is also comparable to the levels found in agricultural soils in Chile of approximately 1000 mg kg^{-1}, a common level in soils (Adriano, 2001) that can vary between 736 and 1178 mg kg^{-1} (Carkovic et al., 2016).

The total concentration of Mo is higher in mine tailings of this study (164 ± 22 mg kg^{-1}) than the reference levels in soils (1.8–3.0 mg kg^{-1}) because Cu mining is associated with this element (Alloway, 2010). In another study of mine tailings in central Chile (a semiarid zone), levels of Mo of 109 mg kg^{-1} were found (Santibáñez et al., 2008). Maximum concentrations of Mo of approximately 74 mg kg^{-1} have been found in soils (Alloway, 2010), whereas Mo concentrations of 5–100 mg kg^{-1} have been found in phosphorite. The total Ni concentration in mine tailings in this study did not exceed the range of Ni concentrations commonly found in soils, which ranges from 20 mg kg^{-1} (Adriano, 2001) to 80 mg kg^{-1} (Alloway, 2010). The total Pb concentration in the mine tailings in the present study is low compared to that found in most soils. The total Pb concentration in soils is variable with a mean of 25 mg kg^{-1} (Alloway, 2010) and 90 mg kg^{-1}

Table 4

Trace metals and sulphur concentration (mg kg^{-1}) in the aerial part of *Carpobrotus aequilaterus* cultivated in mine tailings (MT) with the application of potassium humates at doses of 30 (MT + humate30) and 60 kg ha^{-1} (MT + humate60) at 60 and 120 days (value are means ± standard deviations, n = 3).

Days	Treatments	Cu	Fe	Mn	S	Zn
		Leaves (mg kg^{-1})				
60	MT (mine tailing)	19.6 ± 6.4ab	209 ± 70a	497 ± 79ab	5439 ± 1043a	23.9 ± 10.8a
60	MT + humate30	13.6 ± 2.0a	258 ± 24a	671 ± 163b	5317 ± 344a	31.6 ± 2.8a
60	MT + humate60	24.3 ± 4.0b	368 ± 10b	439 ± 107a	4919 ± 801a	27.9 ± 17.3a
120	MT	19.1 ± 3.1a	209 ± 90a	693 ± 310a	3897 ± 1246a	31.6 ± 10.2a
120	MT + humate30	23.3 ± 3.7ab	280 ± 51a	700 ± 79a	4592 ± 91a	38.2 ± 7.6a
120	MT + humate60	29.3 ± 7.5b	546 ± 181b	930 ± 255a	4441 ± 373a	57.4 ± 31.8a
Sufficient or normal levels in leaves[1]		5–30[1]	50–250[2]	30–300[1]	2000–5000[2]	25–150[1]

Different letters in column indicate significant differences between treatments at 60 and 120 days, separately, according to the Duncan test at $p \leq 0.05$.

[1] Kabata-Pendias, 2011.

[2] Havlin et al., 2014.

(Adriano, 2001).

The extractable Cu, Ni, Pb and Zn concentrations in DTPA in the mine tailings is low in our study and did not exceed the levels reported for uncontaminated soils (Kelly, 1979). DTPA solution is assumed to extract both carbonate-bound and organically bound metal fractions in calcareous soils, and indicates the amount of metals potentially available for plant uptake (Walter et al., 2006). The Risk Assessment Code (RAC) gives an indication of the possible risk by applying a scale to the percentage of metals present in the exchangeable and carbonate fractions (Perin et al., 1985). Accordingly, if this value is < 1% there is no risk, 1–10% indicates low risk, 11–30% medium risk, 31–50% high risk, and > 50% very high risk (Mileusnić et al., 2014). Consequently, the Cu available (1.25%) is low risk in these mine tailings and probably the plant will not uptake it in high levels. In the semi-arid zone of Chile, the concentrations of available metals in soils may reach 70 mg kg^{-1} for Cu, 120 mg kg^{-1} for Fe, 120 mg kg^{-1} for Mn and 70 mg kg^{-1} for Zn (Casanova et al., 2013); these values are higher than the values found in mine tailings in the present study. In other studies of high-sulphur mine tailings (pH 7.6), extractable Cu was present at 4.6 mg kg^{-1} and total Cu was present at 85 mg kg^{-1}, whereas extractable and total Pb were present at 178 mg kg^{-1} and 1453 mg kg^{-1}, respectively (Ye et al., 2002).

In general, the soils of Chile contain metal concentrations that surpass the reference levels for the rest of the world. This is due to the characteristics of Chilean geography, which include a series of mountains and volcanoes that form the Andean mountain range and narrow valleys in which soils accumulate. The Andean segment at 27–35° S latitude has been described as rich in metals, including world-class porphyry copper deposits (Oyarzún et al., 2016). However, in the mine tailings in the present study, the concentrations of metals that cause toxicity, such as As, Cd, and Pb, were not high. In Chile, there are no soil quality guidelines that dictate permissible levels of metals or other elements. Any comparison with international standards is relative because the background levels of metals in Chile may be higher than those

in other countries due to Chile's particular lithological characteristics and the presence of different types of climate. In the absence of soil quality guidelines, it is difficult to establish the level of risk or contamination associated with tailings deposits that are adjacent to populated areas (Carkovic et al., 2016).

4.2. Potassium humates and sorption of Cu

Metal complexation by organic matter such as humic substances can promote or reduce metal mobility depending on the solubility of the organic ligand (Adriano, 2001; Robinson et al., 2005). Humic substances are deprotonated over a wide pH range, leaving a negative charge that attracts the cations and have greater or lesser degrees of affinity for specific metallic elements (Zhou et al., 2005). It has been shown that Cu and Fe bind with higher affinity to humic acids than other metals such as Mn and Zn as the concentration of the metal in solution and the pH increase (Kerndorff and Schnitzer, 1980). The potassium humates used in this study, is a commercial product, that show a higher content in humic acids-C than fulvic acids-C. The potassium humates is soluble and strongly alkaline, therefore are expected to predominantly form complexes with Cu and Fe, which are present in higher, total and extractable concentration, in the mine tailings than Mn or Zn.

The adsorption of Cu has been extensively investigated using the Langmuir model (Gondar and Bernal, 2009; Pereira et al., 2001; Li et al., 2010; Paradelo et al., 2012). The maximum adsorption of Cu (C_{max}) for the potassium humates in this assay is lower than that obtained in similar assays with humic substances; for example, 3.57 mg g^{-1} was reported by Gondar and Bernal (2009) and 23.04 mg g^{-1} was reported by Li et al. (2010). Copper adsorption curves were also obtained at different pHs, yielding C_{max} values of 44.17 mg g^{-1} at pH 5.0 (Jordão et al., 2001) and values ranging from 36 to 190 mg g^{-1} when the pH was increased from 5.0 to 7.0 (Paradelo et al., 2012). The lower value of C_{max} Cu of potassium humates

Table 5

Trace metals and sulphur concentration (mg kg^{-1}) in the roots of *Carpobrotus aequilaterus* cultivated in mine tailings (MT) with the application of potassium humates at doses of 30 (MT + humate30) and 60 kg ha^{-1} (MT + humate60) at 60 and 120 days (value are means ± standard deviations, n = 3).

Days	Treatments	Cu	Fe	Mn	S	Zn
		Roots (mg kg^{-1})				
60	MT (mine tailing)	110 ± 41a	894 ± 345a	478 ± 182a	6851 ± 2212a	42.8 ± 12.7a
60	MT + humate30	161 ± 4.2ab	1230 ± 274a	437 ± 101a	7892 ± 2548a	63.2 ± 12.5a
60	MT + humate60	148 ± 35ab	2030 ± 679b	423 ± 45.4a	6814 ± 2200a	45.9 ± 18.5a
120	MT	128 ± 27a	1340 ± 310a	550 ± 254a	6161 ± 2873a	51.6 ± 25.6a
120	MT + humate30	199 ± 7.4b	1670 ± 118ab	509 ± 22a	4949 ± 2308a	91.5 ± 27.5a
120	MT + humate60	231 ± 14b	2320 ± 602b	672 ± 133a	6552 ± 3055a	94.0 ± 34.1a

Different letters in column indicate significant differences between treatments at 60 and 120 days, separately, according to the Duncan test at $p \leq 0.05$.
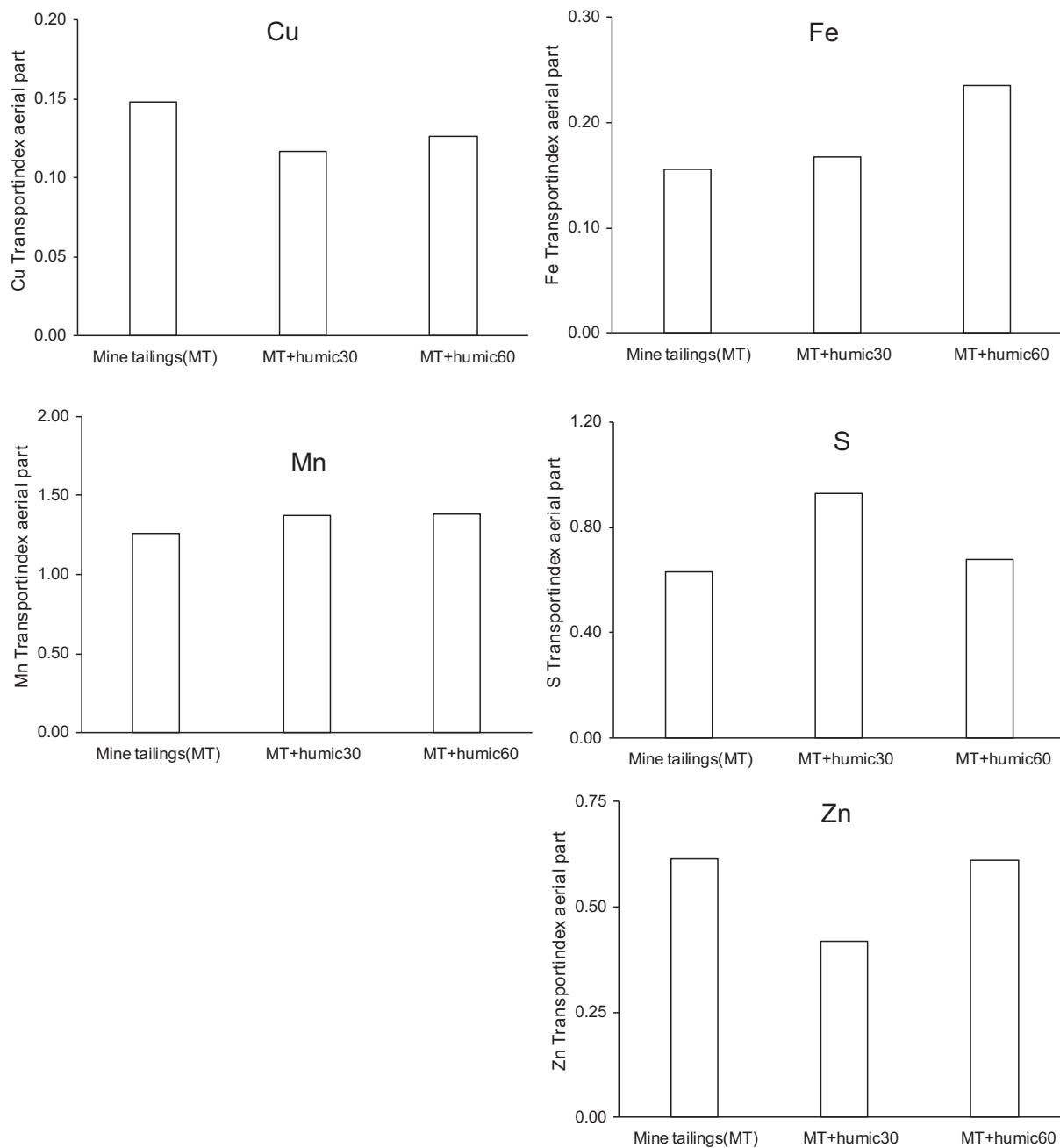
Carpobrotus aequilaterus

Fig. 3. Transport index of metals and S ([element in aerial part] / [element in roots in mg kg^{-1}]) of *Carpobrotus aequilaterus* at 120 days cultivated in mine tailings with addition of potassium humates.

obtained in this study (2.59 mg g^{-1}) can be attributed to the strongly alkaline pH of the product (pH 9.6 ± 0.05, Table 3), which generates a dissolution effect that allows the formation of soluble Cu complexes or dissolved metal humates (Waller and Pickering, 1990; Tipping, 2002). Beveridge and Pickering (1980) found a marked increase in the concentration of Cu in solutions of commercial humic acids from 0 mg L^{-1} at pH 7.0 to 8.8 mg L^{-1} at pH 9.0, indicating that at pH values close to neutral, Cu exists as soluble metal humates.

4.3. Metals and sulphur in plants; biomass

In general, the observed increase in the concentrations of Cu, Fe, Mn and Zn in *C. aequilaterus* plants grown in mine tailings to which

potassium humates were added is consistent with the function of these humic substances as chelating agents. The formation of soluble complexes of humic substances with micronutrients, metal-humic complexes, is often reported as a strategy to enhance plant nutrition through trace elements, as metals may be prevented from leaching and become more bioavailable to plants under these conditions (Canellas et al., 2015). The highly alkaline pH of the potassium humates in this study favoured the formation of soluble complexes with the metals and therefore increased their availability to the plant and therefore the retention of metals in their biomass. The presence of plant cover can reduce the infiltration and leaching of metals in mine tailings (Conesa et al., 2007a) because the metals absorbed by the plants are retained as part of the biomass, preventing their transport to water, soil and air.
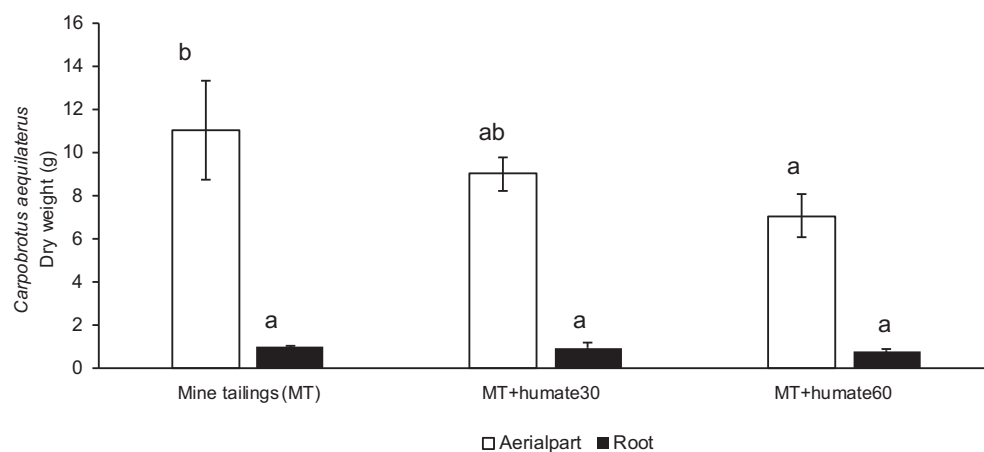
Fig. 4. Dry weight (g) of *Carpobrotus aequilaterus* after 120 days of cultivation in mine tailings with the addition of potassium humates. The lines within the bars indicate the standard deviation; n = 3. Different letters indicate significant differences between treatments for the aerial part and the roots according to the Duncan test at $p \leq 0.05$.

In this study, the level of Cu found in the aerial part of *C. aequilaterus* after the addition of potassium humates was greater than in other assays of mine tailings. Solis-Dominguez et al. (2012) obtained Cu concentrations ranging from 9.88 to 18.5 mg kg^{-1} in the aerial part of trees, shrubs and grasses cultivated in mine tailings with 10% compost (total Cu: 127 mg kg^{-1} and pH 3.6) after 60 days. Santibáñez et al. (2008) measured 21 mg kg^{-1} Cu in the aerial part of *Lolium perenne* (total Cu: 485 mg kg^{-1} and pH 8.1) after 6 months growth in mine tailings, whereas Alvarenga et al. (2004), in a contaminated pyrite mining area (total Cu 164–1444 mg kg^{-1}; pH 3.9–6.6), found concentrations of Cu ranging from 8.3–29.7 mg kg^{-1} in the aerial part of *Cistus ladanifer* and 5.8–80.6 mg kg^{-1} in roots. The higher level of Cu in *C. aequilaterus* is attributable to the higher concentration of total and available Cu in the mine tailings in our study and the addition potassium humates. Most plants accumulate more Cu in their roots, and the restriction of transport of Cu to the aerial portion of the plant seems to be a common feature of most plants that are resistant to Cu and have developed mechanisms for exclusion of the metal (Poschenrieder et al., 2001). Nevertheless, in sites with high availability of Cu, drought tolerance is insufficient to permit colonization (Poschenrieder et al., 2001). Recently, in China, very high levels of Cu have been found in native plants colonizing mine tailings (total Cu 74–3251 mg kg^{-1}; pH 7.4–8.2); these levels range from 20.4 (*Medicago falcata* L.) to 873 mg kg^{-1} (*Polygonum aviculare* L.) in the aerial part and from 52.5 (*Lactuca undulata* Ledeb) to 554 mg kg^{-1} (*Achnatherum splendens* Nevski) in the roots (Liu et al., 2016).

Regarding the Fe concentration in mine tailings, Fe has been studied less in plants than other metals, probably because toxicity of this element to plants is uncommon; Fe deficiency is more widely studied. However, Fe is found naturally in high concentrations in mine tailings and is related to reactions that generate acidity (Lottermoser, 2010). The levels of Fe in the leaves of *Cistus ladanifer* L. found in a contaminated pyrite mining area (pH 3.9–6.6) ranged from 645 to 3800 mg kg^{-1}, compared to 260 mg kg^{-1} in the leaves of plants growing in uncontaminated soil (Alvarenga et al., 2004). In our study, the Fe concentration in *C. aequilaterus* increased significantly with the addition of potassium humates. As reference, the natural Fe concentration in grasses can vary widely from 43 to 376 mg kg^{-1} (Kabata-Pendias and Mukherjee, 2007). Iron toxicity symptoms generally can occur with > 300 mg kg^{-1} (Havlin et al., 2014). It is likely that an antagonistic effect between the absorption of Fe and Mn occurred; the latter showed a high concentration in *C. aequilaterus* that exceeded the normal level in plants. The presence of excess Mn causes a reduction in the absorption and translocation of Fe, which in turn results in a decrease in chlorophyll levels (Kabata-Pendias and Mukherjee, 2007). It has been found that Fe and Mn are highly sensitive to the application of chelating agents and pH. In pine bark substrate of acid pH significantly increased the concentration of manganese in *Rosmarinus officinalis*

(Tapia et al., 2014). The soluble concentrations of Fe and Mn relative to Cu and Zn increased notably with the application of citric acid in plants grown on a substrate of biosolids (Tapia et al., 2013b). Note that the initial Mn concentration in *C. aequilaterus* was high and there was a progressive increase in the concentration of this metal over time. The application of other chelating agents in other plants has also been shown to generate a significant increase in Mn. In *Atriplex halimus*, the Mn level increased from 25 to 42 mg kg^{-1} with continuous application of citric acid for 60 days in biosolids (total Mn 192 mg kg^{-1}; pH 6.4) (Tapia et al., 2013b).

The stability of metal-chelate complexes is determined by factors such as the number of atoms that form a bond with the metal ion, the number of rings that are formed, the nature and concentration of metal ions and the pH (Stevenson, 1994). At any given pH and ionic strength, trivalent cations such as Fe^{3+} are bound in greater amounts than divalent cations. The stability sequence for some select divalent cations is Cu^{2+} > Ni^{2+} > Co^{2+} > Zn^{2+} > Fe^{2+} > Mn^{2+} (Stevenson, 1994); however, although Mn is the last in the sequence of metal-chelate complex stability and displayed lower available concentrations (DTPA) than Cu, Fe and Zn in the mine tailings in this study, it was the element that reached the highest levels in *C. aequilaterus*, showing that this species possesses the capacity for Mn accumulation. Manganese levels in the leaves of *C. ladanifer*, which has been reported to be a Mn accumulator, were shown to fluctuate between 347 and 1200 mg kg^{-1} in a contaminated pyrite mining area (total Mn: 252–1200 mg kg^{-1}) (Alvarenga et al., 2004). The high bioavailability of Mn has been associated with high acidity (pH < 5.5), anaerobic conditions, and poor aeration. Manganese toxicity to plants can also occur in highly alkaline soils at a pH of approximately 8.0; under these conditions, complex Mn anions can be formed (Kabata-Pendias and Mukherjee, 2007).

The Zn levels found in *C. aequilaterus* are much lower than those of another species within the same genus (*C. rossi*); the Zn concentration in the latter species was 203 mg kg^{-1} in the aerial part and 66 mg kg^{-1} in roots after 70 days growth in soil (total Zn: 119 mg kg^{-1}, pH 5.4) (Zhang et al., 2014). Therefore, it is expected that *C. aequilaterus* will absorb Zn if the pH is more acidic than in the present assay (pH 6.3). The levels of Zn of *C. aequilaterus* are comparable to those in *Lolium perenne* in mine tailings with low total Zn concentration (41 mg kg^{-1}, pH 8.1); there, Zn levels in leaves reached 33 mg kg^{-1} after 6 months (Santibáñez et al., 2008). However, high concentrations of Zn in the range of 99–314 mg kg^{-1} in the aerial part and 18.2–92.5 mg kg^{-1} in the roots were reported in *Cistus ladanifer* growing in a contaminated pyrite mining area (total Zn 140–871 mg kg^{-1}; pH 3.9–6.6), indicating that the plant absorbs a certain amount of Zn independent of its concentration in soils. Toxic levels of Zn (750 mg kg^{-1} in the aerial part) have been found in *Zygophyllum fabago*, which naturally colonizes Zn-contaminated tailings (total Zn 13,000 mg kg^{-1}, pH 7.4–7.6) (Conesa et al., 2007a) and in *Jatropha dioica* with 6249 mg kg^{-1} (Carrillo and

González, 2006). In general, Zn does not display high affinity for humic acids, and in bioavailability assays it has been shown not to be associated with humic acids (Kang et al., 2011).

The absorption of S by plants growing in mine tailings has been little investigated. However, some research has studied the potential of plants for use in sulphur phytoremediation (Ernst, 1998). In mineral tailings, S is of importance because of its abundance (FeS_2) and its relationship to acidity. The increase in sulphate concentration in water is a common problem related to mining activities (Lottermoser, 2010; van der Perk, 2012). A phytostabilization strategy for S involving retaining the element through plants and microflora may be a good option and may prevent it from passing to nearby waters as sulphates. In gypsum soils with sulphate toxicity, phytoremediation has been recommended as an effective strategy (Ruiz et al., 2003). The effectiveness of phytoextraction for remediation of sulphur-enriched soils is highly dependent on the availability of S to plants (Ernst, 1998). Sulphur can accumulate organically in plants, and the presence of organic S seems to be a key factor in determining the potential of plants to remediate soils with high contents of this element (Ruiz et al., 2003). Organic S in soil is an important link in the overall cycle of S in nature. When there is a lack of organic matter, sulphate is susceptible to leaching, and organic forms of S are incorporated into microbial tissue and hence into humus (Stevenson, 1994). Therefore, the application of humic substances to mine tailings could be an effective strategy for retaining S in organic form. In the present study involving *C. aequilaterus*, there was no increase in leaf or root S after the addition of potassium humates; this is probably due to the repulsion of negative electrical charges. In the potassium humates used in this study, OH^- charge (high pH value) predominates, and plants normally absorb sulphur from the soil as SO_4^{2-} (Ernst, 1998; Anderson and Fitzgerald, 2003). In general, solution SO_4^{2-} concentrations of 3.0–5.0 mg L^{-1} are sufficient for most crops (Havlin et al., 2014). However, high concentrations of SO_4^{2-} were tolerated by *C. aequilaterus* in mine tailings of this study (1243 ± 27.8 mg L^{-1}). In a germination assay in which increasing levels of SO_4^{2-} from 515 to 3315 mg L^{-1} were tested, Ginocchio (1996) found increased seedling productivity in *Senna candolleana*, indicating that elevated levels of SO_4^{2-} have a fertilizing effect and are not toxic. Note that sulphate levels in the oceans are approximately 2700 mg L^{-1} (Havlin et al., 2014). Plants with S contents of 2.5–8.0% have been classified as thiophores (Ernst, 1998). The level of S in *C. aequilaterus* in the present study is interesting because it is comparable to the 4800 mg S kg^{-1} obtained in *Gypsophila fastigiata* grown on sulphur-enriched soil (Ernst, 1998).

With respect to the rate of transport of metals to the aerial part of the plant (Ti), values < 1 indicate phytostabilization capacity and lower risk of metal entry into the food chain when these plants are consumed (Conesa et al., 2006). The clear tendency to decrease Ti for Cu with the addition of potassium humates is a desired effect for the phytostabilization of these mine tailings. The high affinity of Cu for potassium humates favoured the formation of stable complexes that remain in the roots. Note that increasing the dose of potassium humates increased the Ti value for Fe and Mn but not for Zn; the latter metal had a higher available concentration (DTPA) and has a higher affinity for humic substances than Mn (Stevenson, 1994). It is likely that other factors also determine the affinity (mobility) of metals for chelating agents. In mine tailings, Fe and Mn are present in higher total concentrations than Zn and are of lower molecular weight; therefore, it is presumed that the former metals are more available for transport to the aerial part of plants. Regarding the Ti of S, no trend was found with the addition of potassium humates. Thus, increasing the dose of potassium humates must be carefully studied so as not to increase the salinity of the mine tailings. Also a key factor in the phytostabilization or phytoextraction of S is the selection of the plant (Ernst, 1998).

With respect to the biomass of *C. aequilaterus*, the addition of potassium humates to the mine tailings did not stimulate plant growth; on the contrary, a dose of 60 kg ha^{-1} decreased the dry weight of the aerial part of the plants by approximately 36%. However, current evidence suggests that the biostimulating effects of humic substances are associated with structural and physiological changes in roots and buds that are related to nutrient uptake, assimilation and distribution (nutrient use efficiency traits). In addition, humic substances can induce changes in the primary and secondary metabolism of the plant that are related to tolerance to abiotic stress, which collectively modulates plant growth and promotes fitness (Canellas et al., 2015). Therefore, it was expected that the use of humic substances would increase the dry weight of the plants. The decrease in dry weight with the addition of potassium humates is attributed to the high levels of Mn in the aerial part of the plant.

A remarkable feature of *C. aequilaterus* is its tolerance to nutrient deficiency, particularly P and N deficiency, and to high concentrations of sulphates. Halophytic character of the genus has been reported for the species *C. edulis* (Flowers et al., 1977). The use of native plants that are tolerant to metals may represent a long-term solution, and in semi-arid areas in which tailings are present, the establishment of vegetation also requires species adapted to drought (Conesa et al., 2006). Once phytostabilization is established, although the landscape can never be completely restored to its original appearance, the aesthetics of revegetation with tolerant metal species harmonize with the surrounding landscape (Tordoff et al., 2000). *Carpobrotus aequilaterus* is a self-sustaining plant that thrives with no human attention or artificial management, as recommended for phytostabilization (Li, 2006). *Carpobrotus aequilaterus* generates an abundant aerial biomass that is incorporated as organic matter in mine tailings; thus, together with its particular creeping growth and its tolerance of metals and SO_4^{2-}, it offers an interesting option for the phytostabilization of mine tailings.

5. Conclusions

The total concentration of trace metals and metalloids in the mine tailings of this study was not high (As, Cd, Cr, Mn, Mo, Ni, Pb and Zn), only Cu was found at high levels (1999 ± 223 mg kg^{-1}), but the availability is low (1.25%). The addition of potassium humates at a dose of 60 kg ha^{-1} a mine tailings increased the concentration of Cu and Fe in aerial part of *C. aequilaterus* after 120 days of cultivation. The levels of Cu, and Zn in leaves do not exceed the levels of metals considered sufficient or normal indicating a low metal availability in these mine tailings. However the concentration of Fe in leaves was high and the plant showed accumulation capacity of Mn. The high pH value of potassium humates favoured the formation of soluble complexes with the metals that increased their bioavailability. The addition of potassium humates to mine tailings caused an increase in Cu concentration in the roots of the plants and generated a lower rate of transport to the aerial part, indicating that the species preferentially increased the absorption of Cu in the roots, a characteristic that is favourable for phytostabilization. The concentration of sulphur in the plants was not affected by the addition of potassium humates; however, the species was adapted to the high levels of sulphates present in the mine tailings.

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