Phytostabilization of trace elements and ^{13}C isotope composition of *Atriplex atacamensis* Phil. cultivated in mine tailings treated with organic amendments

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Abstract Mining generates large quantities of mineral processing wastes that are typically stored in mine tailings (MT) ponds. Long-term exposure of the surrounding areas to the material from the tailings ponds has been reported to have adverse effects on both human health and the environment. The purpose of this study was to evaluate the ability of *Atriplex atacamensis* Phil. to phytostabilize metals (Cu, Fe, Mn, and Zn) and sulfur (S) when grown directly on mine tailings with and without compost (C) and humic substance (HS). The stress status of *A. atacamensis* Phil. was also evaluated through the ^{13}C isotopic composition of bulk leaves. A 120-day greenhouse experiment was conducted and three treatments were evaluated: (i) MT without any amendments (control), (ii) MT+C (dose: 89 ton ha^{-1}), and (iii) MT+HS (0.72 ton ha^{-1}). Mine tailings material exhibited low salinity, alkaline pH, high extractable S–SO$_4$ concentrations, and low fertility; total Fe, Mn, and Zn concentrations were within the reference range for mine tailings, but total Cu concentrations were high at 1860 ± 236 mg kg^{-1}. The HS had higher pH, EC, CEC, and available concentrations of N, P, and K than compost, while S-SO$_4$ concentrations were similar in both amendments. ^{13}C NMR analysis showed that the HS contained more alkyl, aromatic, and phenolic groups, while the compost was dominated by O-alkyl and carboxyl groups. At the end of the experiment, the MT+C treatment achieved a significant decrease in Cu, Fe, and Mn concentrations in the roots and aboveground parts of *A. atacamensis* Phil. and an increase in Zn values in both tissues. Both amendments increased the sulfur content in the aboveground parts, while metal concentrations under the HS treatment proved similar to control. Furthermore, the δ^{13}CV-PDB values obtained in this study indicate that the organic amendments did not cause additional physiological stress to the plants compared to the MT treatment. Overall, *A. atacamensis* Phil. was shown to have the ability to phytostabilize metals and sulfur, making it a potential candidate species for in situ evaluation of the phytostabilization process on mine tailings.

Keywords Phytostabilization · Organic amendments · Heavy metals · Sulfur · Nuclear magnetic resonance · ^{13}C isotope composition

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Introduction

The mining industry generates large quantities of mineral processing wastes, which are typically stored in tailings ponds. The wastes may consist of a mixture of water, fine solids, sand, and multiple chemical elements in high concentrations, such as metals, metalloids, and sulfur (Santibañez et al., 2011). It is estimated that the global production of mining wastes exceeds 10 billion tons per year (Adiansyah et al., 2015). Chile, the world's largest copper producer and the second-largest molybdenum producer, currently has 24 billion tons of tailings deposited in the country's 746 tailings storage facilities (102 operating, 471 idle, and 173 abandoned), with an average of 530 million tons of mining wastes added annually (SERNAGEOMIN, 2018). Prior to 2011, the country did not have laws regulating mining operation closure and rehabilitation practices, which led to the emergence of many unregulated tailings storage sites. Law 20.551, passed in 2011, authorized such regulation to protect the life, health, and safety of people, ensure the chemical and physical integrity of tailings facilities and reduce their impact on water, soil, and air (DOE, 2011).

Areas subjected to long-term exposure to mine tailings have been widely reported to pose a risk to human health and the environment due to leaching losses of toxic metal(loid)s and aeolian transport and dispersion of waste materials (Sanchez-Lopez et al., 2015; Wang et al., 2017; Xie & van Zyl, 2020). The magnitude of these effects depends on the physicochemical properties of the tailings, including metal(loid) mobility, pH, concentration, bioavailability, composition, and organic content (Alloway, 2010). In general, pH is the crucial factor related to metal(loid) mobility since it is associated with adsorption, desorption, and soluble complexes formation of them (Hooda, 2010). For instance, soils and mine tailings under acidic conditions, low content of cation exchange capacity (CEC), and organic matter favor increased trace elements mobility (Adriano, 2001; Alloway, 2010; Hooda, 2010). In contrast, alkaline pH and high concentrations of those properties contribute to decreasing trace element's mobility in those environments. Several studies over the last decade have recommended phytoremediation as a form of bioremediation strategy that consists in putting a vegetative cover over contaminated soil or tailings sites containing high concentrations of potentially toxic substances

(Acuña et al., 2021; Parraga-Aguado et al., 2014; Pérez et al., 2021; Tapia et al., 2020). This technique uses select plant species to stabilize and reduce the mobility of chemical elements by accumulating them in plant organs, immobilizing them in the rhizosphere, reducing leaching, and depleting substrate moisture through transpiration (Wang et al., 2017). Phytoremediation can be separated into two types: phytostabilization and phytoextraction. Phytostabilization is associated with plants that preferentially accumulate the elements in roots. On the other hand, in phytoextraction, the elements are accumulated in the aboveground parts and then cut to remove the pollutant. In arid and semiarid zones, the phytostabilization technique is more recommended for mine tailings or soils since a permanent vegetation cover can prevent erosion in the zone (Méndez & Maier, 2008). However, due to the low biological activity and nutrient concentrations of tailings substrates under arid and semi-arid conditions, the use of organic amendments was found to be crucial to improve substrate quality, fertility, and plant growth rates (Menares et al., 2017). Furthermore, organic amendments are known to affect the adsorption and complexation of metal(loid)s by reducing their mobility and availability in soil and mine waste substrates, in part due to the presence of alkyl, phenol, carboxyl, and aromatic functional groups that are involved in and control the metal complexation mechanism (Senesi, 2018; Teixeira et al., 2011). Therefore, the physicochemical properties and the dosage of the amendments should be carefully analyzed to ensure they are compatible with the chosen phytostabilization strategy and do not lead to increased mobility of the elements or a decrease in pH. In this regard, commercial humic substances and amendments made from organic waste, such as compost, have been recommended as suitable substrate improvers for mine tailings' treatment (Duarte et al., 2019; Manzano et al., 2016; Tapia et al., 2017). However, regardless of the type of organic amendments used, their effectiveness will always depend on the plant species selected for the phytostabilization process.

The selection of appropriate plant species for a phytostabilization project must ensure that plants can grow in highly saline or alkaline conditions, tolerate high concentrations of metals, adapt to dry periods in a semi-arid climate, and grow relatively quickly (Marques et al., 2009; Wu et al., 2013). The genus *Atriplex*, widely distributed in arid and

semi-arid regions of the world, are halophyte plants that have been studied and reported as a promising candidate species for the phytostabilization of soils and mine tailings with variable metal(loid) content and salinity (Acosta et al., 2018; Fernandez et al., 2016; Tapia et al., 2013, 2020). Hydroponic experiments with *Atriplex atacamensis* Phil. (*A. atacamensis*), a Chilean endemic from the world's driest Atacama desert, have shown that it can stabilize metalloids such as arsenic (Orrego et al., 2020) and thrive under high concentrations of copper and salinity in an arid environment (Fernandez et al., 2016; Tapia et al., 2013). However, no studies have investigated the phytostabilizing potential of *A. atacamensis* grown directly on mine tailings.

Carbon isotopic composition ($\delta^{13}C$) has been used to provide insights related to metabolic, chemical, and physical processes associated with carbon transformation processes in plants (Robinson et al., 2000). In that sense, several studies have utilized this analysis as an integral measure to evaluate environmental stress on plants (Merchant et al., 2012), responses to salt stress on physiological mechanisms (Araus et al., 2021; Borzouei et al., 2020), and drought effect (Gouveia et al., 2019; Mariotte et al., 2013; Mårtensson et al., 2017). However, there are also no studies that have evaluated the stress level of plants grown on mine tailings using the ^{13}C isotope as a physiological indicator, considering that mine tailings can have chemical and physical conditions adverse to plant growth. Therefore, the objectives of this study were (i) to evaluate the phytostabilizing ability of *A. atacamensis* for copper (Cu), iron (Fe), manganese (Mn), zinc (Zn), and sulfur (S) when grown on mine tailings with two different organic amendments, and ii) to determine whether the ^{13}C isotopic composition can be used as a potential stress indicator for the species under study.

Materials and methods

Sampling and characterization of mine tailings and organic amendments

The tailings samples were obtained from the wall of the Ovejería tailings pond of the Codelco Andina Division (33°2′23,25″S 70°47′29,02″W) in Til Til,

Santiago Metropolitan Region, Chile. The climate of the tailings pond area is semi-arid and rainy in winter, classified as BSk according to Köppen-Geiger (Peel et al., 2007). The mean annual precipitation is 219 mm and the mean annual minimum and maximum temperatures are 6.7 °C and 27.6 °C, respectively (Santibañez, 2017). Compost and a commercial humic substance (HS) (Pow Humus® WSG 85, Humintech GmbH, Germany) were used as amendments. The humic substance was a mixture of water-soluble humates obtained by alkaline extraction from German leonhardite; Tapia et al. (2017) characterized the German product and reported total extractable carbon (TEC), humic acid carbon (HAC), and fulvic acid carbon (FAC) concentrations (g kg^{-1}) of 131.2 ± 27.2, 116.8 ± 4.3 and 14.4 ± 0.5, respectively. The compost used for the experiment consisted of vegetable residue and branch trimmings composted at the Department of Environmental Management, La Pintana District, Santiago de Chile.

Electrical conductivity (EC) and pH of the compost were measured in a 1:5 w/v compost:water suspension using a Hanna HI 3222 m (Woonsocket, Rhode Island, USA). For mine tailings, pH was evaluated in a 1:2.5 w/v suspension and EC was measured in a saturated extract. Total organic carbon (TOC) in all three types of samples was determined according to Walkley and Black (1946) by oxidation with $K_2Cr_2O_7$-H_2SO_4 and determination using $FeSO_4 \cdot 7H_2O$ as titrant. Organic matter (OM) was calculated using the following equation: OM $(\%) = TOC \times 1.724$. Samples were digested with HF-$HClO_4$-HCl-HNO_3 (Dold & Fontboté, 2001), and total Cu, Fe, Mn, and Zn concentrations were subsequently determined using atomic absorption spectrophotometry (AAS) (PerkinElmer 3110). DTPA solution (0.005 M DTPA, 0.01 M $CaCl_2$, and 0.1 M triethanolamine adjusted to pH 7.3) was used to measure available Cu, Fe, Mn, and Zn, and concentrations were determined by AAS (Baker & Amacher, 1982). Available sulfur (S-SO_4) was extracted with $Ca(H_2PO_4)_2$ and measured by turbidimetry using a UV–Vis spectrophotometer (Hach DR5000, USA) (Sing et al., 1995). Available nitrogen (N) was obtained by extraction with KCl followed by distillation and NH_4 titration (Mulvaney, 1996). The Olsen method was used to extract available phosphorus (P) from samples using sodium hydrogen carbonate (0.5 M and pH 8.5) (Kuo, 1996). In addition,

available potassium (K) was extracted using ammonium acetate (1 M and pH 7.0) with the concentration determined by the AAS method.

To determine the TEC of the compost, 0.5 g compost was mixed with 25 mL of 0.5 M NaOH and shaken for 48 h. The solution was centrifuged and the supernatant was separated from the precipitate. Concentrated HCl was added to the supernatant containing TEC until pH ~ 1.0 to precipitate the humic acid fraction (HAF) from the solution. The fulvic acid fraction (FAF) was separated from the HAF by centrifuge (Schnitzer, 1982) and the concentrations of TEC and C in FAF were determined by oxidation with 1.0 N $K_2Cr_2O_7$ and phenanthroline as indicator followed by titration with 1.0 N $FeSO_4 \cdot 7H_2O$ (Nelson & Sommers, 1982). HAC concentration was calculated using the following equation: HAC = TEC − FAC (Romero et al., 2007; Senesi, 1989; Tapia et al., 2017).

^{13}C-NMR solid-state

Solid-state ^{13}C NMR spectroscopy was performed on a Bruker 400 MHz AVANCE III HD spectrometer equipped with a 4 mm triple resonance probe. A cross-polarization spinning method was used with a magic angle at a spinning speed of 14 kHz and a contact time of 1 ms. The pulse delay was 500 ms, which was proven to be long enough to avoid saturation. The ^{13}C chemical shifts were referenced to external tetramethylsilane (= 0 ppm) and adjusted with glycine (= 176.04 ppm) as an external standard. The spectra were subdivided into the following chemical shift regions: alkyl C (0–45 ppm), N-alkyl C (45–60 ppm), O-alkyl C (60–93 ppm), Di-O-alkyl C (93–110), aromatic C (110–140 ppm), carboxyl C (160–190 ppm), and amide-ketonic C (190–220 ppm) (Stevenson, 1994). The relative intensity distribution of ^{13}C was determined by integrating the signal intensities in the various chemical shift regions using an integration routine supplied with the instrument software.

Corrections were made to the intensity distribution of the spinning sidebands (Knicker & Lüdemann, 1995).

Experimental assay under controlled conditions

The pot experiment was conducted under greenhouse conditions at the Faculty of Agricultural Science of the University of Chile (33°34′11,20″S 70°37′54,50″W). from February to May 2018 (summer and fall seasons) The mean temperature in the greenhouse during the study period was 21.7 °C, which was similar to the mean temperature (18.1 °C) in the tailings pond area reported by the weather station of the mining company. The plants of the Chilean endemic species *A. atacamensis* (20 – 25 cm height) were planted directly into the Ovejería tailings pond using polyethylene pots with a 1 L capacity. Prior to transplanting, *A. atacamensis* plants had been grown in pearlite and irrigated with tap water. The treatments were: mine tailings with no added amendment (MT); mine tailings with added HS at a dose of 0.72 ton ha^{-1} (1.30 g pot^{-1}) (MT + HS); and mine tailings with added compost at 89 ton ha^{-1} (158 g pot^{-1} or 5% w/w) (MT + C). Each treatment had four replicates. The HS dose was calculated based on the results of Tapia et al. (2019) (360 kg ha^{-1}) and the product cost of 12 USD kg^{-1}.

Total Cu, Fe, Mn, Zn, S (mg kg^{-1}), and TOC (g kg^{-1}) concentrations, dry weight (g), and δ^{13}CV-PDB values of the plants before transplanting are given in Table 1. The amendments were applied in solid form, and each treatment studied was repeated four times. The trial was conducted in a randomized block design. In the first month of the experiment, plants were watered with a nutrient solution consisting of $NH_4H_2PO_4$ (0.12 g L^{-1}), KNO_3 (0.5 g L^{-1}), $Ca(NO_3)_2$ (0.49 g L^{-1}), and $MgSO_4 7H_2O$ (0.49 g L^{-1}), three times per week, 200 mL per pot, to ensure proper plant establishment in the first days of the experiment. From

Table 1 Initial characterization of *Atriplex atacamensis* Phil. (values are mean ± standard deviation, $n = 4$)

Structure	Dry weight	δ^{13}C^{V-PDB}	TOC	S	Cu	Fe	Mn	Zn
	g	‰	g kg^{-1}	%		mg kg^{-1}		
Leaves + stems	1.9 ± 0.7	− 19.390 ± 0.15	367 ± 22.4	0.25 ± 0.07	10.2 ± 4.38	65.7 ± 21.9	22.4 ± 17.1	7.60 ± 1.32
Roots	0.7 ± 0.1	-	-	0.38 ± 0.11	74.9 ± 10.0	507 ± 67.6	54.8 ± 7.60	76.1 ± 14.8

March to May, treatments were irrigated with tap water (pH: 7.92; EC: 1.51 dS m^{-1}; Cu: 0.03 mg L^{-1}; Fe: 0.39 mg L^{-1}; Mn: 0.01 mg L^{-1} and Zn: 0.02 mg L^{-1}) twice a week with a dose of 200 mL per pot.

Analysis of plants

At 120 days into the trial, four plants per treatment were harvested and washed with distilled water. The aboveground parts (leaves + stems) and roots were separated and dried in an oven at 70° ± 5 °C for 24 h to determine dry weight. Roots and aboveground parts were ground and 0.5 g of each replicate were autoclaved with 6 mL 65% HNO$_3$, 4 mL H$_2$O$_2$, and 6 mL distilled water at 125 °C and 1.5 kPa for 40 min (Moreno-Jiménez et al., 2012). The concentrations of Cu, Fe, Mn, and Zn were determined by AAS. For sulfur content, 0.5 g of each sample was calcined in Mg(NO$_3$)$_2$, dissolved in 2 M HCl, and BaSO$_4$ turbidity was measured using a UV–Vis spectrophotometer (Lachica et al., 1973). Results were expressed in mg kg^{-1} dry matter. All reagents used were of analytical grade quality (Merck, Darmstadt, Germany), and deionized water (18.2 MΩ.cm at 25 °C) obtained using a Milli-Q system (Merck, Darmstadt, Germany) was used for the preparation of reagents and standards. The detection limit of the AAS (PerkinElmer 3110) for Cu, Fe, Mn, and Zn is 0.05 mg L^{-1}. The Soil and Water Chemistry laboratory from the Faculty of Agricultural Science, University of Chile, is accredited by the Servicio Agricola y Ganadero (SAG), an official government organization associated with the Agriculture Ministry of Chile (RE 7849, 2020). Internal samples reference from the Soil and Water Chemistry Laboratory were used to verify the accuracy and precision of the samples measured. In general, for metal-enriched samples (tailings, soils, plants, and waters) the recovery rate is between 98 and 102%.

Total carbon concentration and δ^{13}CV-PDB determination in plants

Representative leaf samples of the upper, middle, and lower parts of *A. atacamensis* were collected at each treatment iteration and placed in pre-labeled envelopes. The samples were oven-dried (Memmert, model UF 160, Germany) at 65 °C to constant weight and ground to a fine powder in a mill (IKA, model MF10 basic, Germany) (Cook et al., 2018). Approximately 1.0 mg of each sample was weighed on an analytical balance (Precisa, model 125A, Switzerland) and sealed in a tin capsule. Capsules were placed in a 96-well microtiter plate and used for isotopic analysis. Leaf carbon isotopic composition and total carbon were measured using an isotope ratio mass spectrometer (Sercon, model Integra2, UK). Results were expressed as ‰ for δ^{13}C and g kg^{-1} dry matter for leaf total carbon. The accuracy of the instrument was 0.2‰ for δ^{13}C.

Phytoremediation indexes and metals uptake

The translocation index (TI) was used to evaluate the migration of elements from soils or mine tailings to plant tissues (Acuña et al., 2021; Dede & Ozdemir, 2016; Tapia et al., 2019). The TI of Cu, Fe, Mn, Zn, and S in plant samples was calculated as follows (Eq. 1):

$$TI = {}^{C_{AP}}\!/_{C_R} \tag{1}$$

where C_{AP} is the concentration of the element in the aboveground parts (mg kg^{-1}) and C_R is the concentration of the element in the roots (mg kg^{-1}). The values obtained were interpreted as follows: TI > 1 indicates phytoextraction of the element by *A. atacamensis,* and TI < 1 indicates the species' phytostabilization ability.

The bioconcentration factor (BCF) was calculated to evaluate the trace elements' uptake of *A. atacamensis* and its potential hyperaccumulation efficiency in the roots (Lam et al., 2017; Taeprayoon et al., 2022). The BCF of Cu, Fe, Mn, and Zn in roots was calculated according to Eq. 2.

$$BCF = {}^{C_R}\!/_{C_{MT}} \tag{2}$$

where C_R is the concentration in roots (mg kg^{-1}) and C_{MT} is the trace element total concentration in the mine tailings (mg kg^{-1}). According to Kamari et al. (2014) and Lam et al. (2017), BCF values < 1.0 indicate plants are excluder, between 1.0 and 10, are accumulator, and BCF > 10 indicate a potential trace elements hyperaccumulation by *A. atacamensis*.

Additionally, the metals and S uptake contents were calculated in roots and the aboveground parts by *A. atacamensis* (Eq. 3). The metals and S uptake contents indicate the concentration in plant tissues that were taken by the root system based on plant biomass; this analysis evaluates the potential phytostabilization capacity of plants studied for bioremediation in soils and tailings (Meeinkuirt et al., 2016; Taeprayoon et al., 2022; Tapia et al., 2020).

$$E_{uptake} = C_{AP/R} x B_{AP/R} \qquad (3)$$

where $C_{AP/R}$ is the concentration in roots or aboveground parts (mg kg^{-1}) and $B_{AP/R}$ is the roots or aboveground parts dry biomass (kg). The metals and S uptake contents were expressed in mg tissues^{-1}.

Statistical analyses

Concentrations (mg kg^{-1}) and uptake (mg) of Cu, Fe, Mn, and Zn, percent S, dry matter content, total carbon (g kg^{-1}), δ^{13}CV-PDB, TI, and BCF at 120 days were compared across treatments by one-way analysis of variance (ANOVA). Tukey's test ($p \leq 0.05$) was used to evaluate statistically significant differences among treatments. Statistical analyses were performed using InfoStat v.2017 software.

Results and discussion

Chemical characterization of mine tailings and organic amendments

The results of the chemical characterization (Table 2) showed that mine tailings had low salinity, alkaline pH, and low fertility, consistent with the detected N, P, K, CEC, and OM levels (Tapia et al., 2017). As expected, high concentrations of extractable S-SO$_4$ were detected, which is in agreement with the findings of Dold and Fontboté (2001), who showed that pyrite oxidation reactions result in the release of metal(loid)s and sulfates into solution. Total Fe, Mn, and Zn concentrations were within the global reference range for tailings reported by Hossner and Shahandeh (2005) (Fe: 0.4–57%; Mn: 10–4000 mg kg^{-1} and Zn: 1.0–5000 mg kg^{-1}); however, total Cu concentration was more than twice the maximum content reported by these authors (1–750 mg kg^{-1}). In Chile, soils and mine

Table 2 Chemical characterization of the mine tailings and organic amendments evaluated. Values are mean ± standard deviation ($n = 4$)

Parameter	Unit	Mine tailings	Humic substance	Compost
pH		8.10 ± 0.06	10.1 ± 0.03	8.10 ± 0.10
EC	dS m^{-1}	3.84 ± 0.10	11.4 ± 0.06	4.50 ± 0.05
Organic matter	%	0.15 ± 0.02	28.3 ± 0.04	26.1 ± 0.50
Total organic carbon	%	-	16.4 ± 0.02	13.3 ± 0.40
CEC	cmol$_c$ kg^{-1}	4.01 ± 0.03	144 ± 2.00	59.3 ± 5.40
Available N	mg kg^{-1}	6.15 ± 0.43	612 ± 350	121 ± 59.9
Available P	mg kg^{-1}	0.67 ± 0.12	3,150 ± 961	326 ± 56.9
Available K	mg kg^{-1}	9.32 ± 0.41	116,127 ± 1,219	11,776 ± 154
Available S-SO$_4$	mg kg^{-1}	765 ± 10,2	593 ± 43,8	634 ± 38,2
Bioavailable Cu	mg kg^{-1}	34.7 ± 2.10	6.98 ± 0.14	2.21 ± 0.22
Bioavailable Fe	mg kg^{-1}	428 ± 62,1	59.6 ± 5.40	48.4 ± 5.09
Bioavailable Mn	mg kg^{-1}	5.50 ± 0.10	2.23 ± 1.61	8.46 ± 0.05
Bioavailable Zn	mg kg^{-1}	15.0 ± 1.17	2.46 ± 0.08	8.50 ± 1.10
Total Cu	mg kg^{-1}	1,860 ± 236	9.81 ± 1.86	36.2 ± 5.25
Total Fe	mg kg^{-1}	34,731 ± 3,419	6,557 ± 289	13,690 ± 1,450
Total Mn	mg kg^{-1}	640 ± 45.8	5.53 ± 0.29	200 ± 3.55
Total Zn	mg kg^{-1}	247 ± 12.8	2.67 ± 0.58	32.8 ± 0.63
Total humic—C	g kg^{-1}	-	131 ± 27.2[a]	19.5 ± 0.55
Humic acid—C	g kg^{-1}	-	117 ± 4.30[a]	15.6 ± 2.50
Fulvic acid—C	g kg^{-1}	-	14.4 ± 0.50[a]	4.15 ± 0.05

tailings typically exceed background Cu concentrations reported by other authors, such as 2–250 mg kg^{-1} according to Sparks (2003), and 14–109 mg kg^{-1} according to Kabata-Pendias (2011). The reason is that some geographic areas of the country are naturally enriched in Cu (Oyarzún et al., 2016). Values ranging from 436 to 2564 mg kg^{-1} Cu in mine tailings in central Chile (Dold & Fontboté, 2001), 4393 mg kg^{-1} in the north-central zone (Santibañez et al, 2011), and 1.6% Cu in northern Chile (Lam et al., 2018) have been reported.

For regulatory purposes, the use of a risk assessment code (RAC) has been recommended to assess the level of risk associated with the available concentrations of metal(loid)s in sediments, soils, and mine tailings that may potentially migrate from the exchangeable and carbonate fractions (Mileusnić et al., 2014; Singh & Lee, 2015; Tapia et al., 2017). According to the five levels of RAC, available concentrations <1% pose no risk of migration; concentrations of 1 to 10% are low risk; 11 to 30% are medium risk; 31 to 50% are high risk; and above 75% are very high risk (Sun & Chen, 2018). According to the RAC matrix, the available Cu, Fe, and Zn concentrations found in this study, 1.86%, 1.23%, and 6.07%, respectively, indicate a low risk of migration, while the Mn concentration of 0.85% indicates no risk.

Turning to organic amendments, we found that the HS had higher pH, EC, CEC, and available N, P, and K concentrations than the compost, while both amendments had similar TOC percentages and S-SO$_4$ concentrations. The observed chemical parameters of the amendments were favorable for improving the fertility of the tailings substrate to ensure adequate growth and development of *A. atacamensis*. In addition, the alkaline pH of the compost and the HS reduced the mobility of the metals studied, while the salinity provided by both amendments stimulated plant growth (Pardo et al., 2017; Tapia et al., 2020). For metallic elements, we found that both total and available concentrations of Cu, Fe, Mn, and Zn were higher in the compost than in the HS; however, none of the metallic elements in either amendment exceeded their respective concentrations in the tailings. The main difference between the two amendments was the higher concentration of HA-C and FA-C in the HS compared to the compost, which was due to the higher moisture content in the HS. This

difference indicates the higher reactivity of the HS to cationic elements, which may reduce the solubility and mobility of metallic elements through adsorption or the formation of less mobile complexes (Boruvka & Drábek, 2004; Clemente et al., 2006).

The ^{13}C NMR spectra shown in Fig. 1 indicate that the chemical structures of the amendments are dissimilar, presumably due to the different source materials of the compost and HS (plant matter vs. leonardite). As for the main spectral peaks, the relative areas of the C-groups are shown in Table 3. The analysis showed that the alkyl-C group is the dominant spectral region for both amendments and is linked to humic acids with aliphatic structures, recalcitrant short-chain components such as cutin and suberin, and volatile fatty acids (Lin et al., 2018; Tambone et al., 2019). Meanwhile, the content of O-alkyl groups in the compost was higher than that of the HS fraction, indicating the presence of labile organic compounds such as carbohydrates, cellulose, and hemicellulose, which are usually abundant in plant-based amendments and soils with plant residues (Bonanomi et al., 2018; Lin et al., 2018; Paetsch et al., 2016).

The proportion of aromatic C in the HS in this study was 1.95 times higher than in the compost, due to the longer degradation time of leonardite OM and the tendency of O-alkyl groups to decompose into aromatic, phenolic, and carboxyl groups (Wang et al., 2015). Our observations were consistent with that trend, as the O-alkyl region of the HS was three times smaller than that of the compost, but had more aromatic and phenolic groups. The abundance of aromatic and phenolic compounds in the HS may also indicate the predominance of structures derived from recalcitrant or organic compounds such as lignin and polyphenols (Lin et al., 2018) involved in the reactions between metals and humic substances. Taking into account additional factors, such as the degree of alkylation and maturity, this factor may be considered responsible for the metal adsorption behavior (Li et al., 2015). The relative region of the carboxyl group in this study was fairly similar in both amendments but slightly larger in the compost. This group plays an important role in the binding process of metal ions due to its high affinity to metallic elements (Senesi, 2018), facilitating the formation of heavy metal complexes and the stabilization of metallic elements in

Fig. 1 Solid state ^{13}C
NMR spectra of the organic
amendments
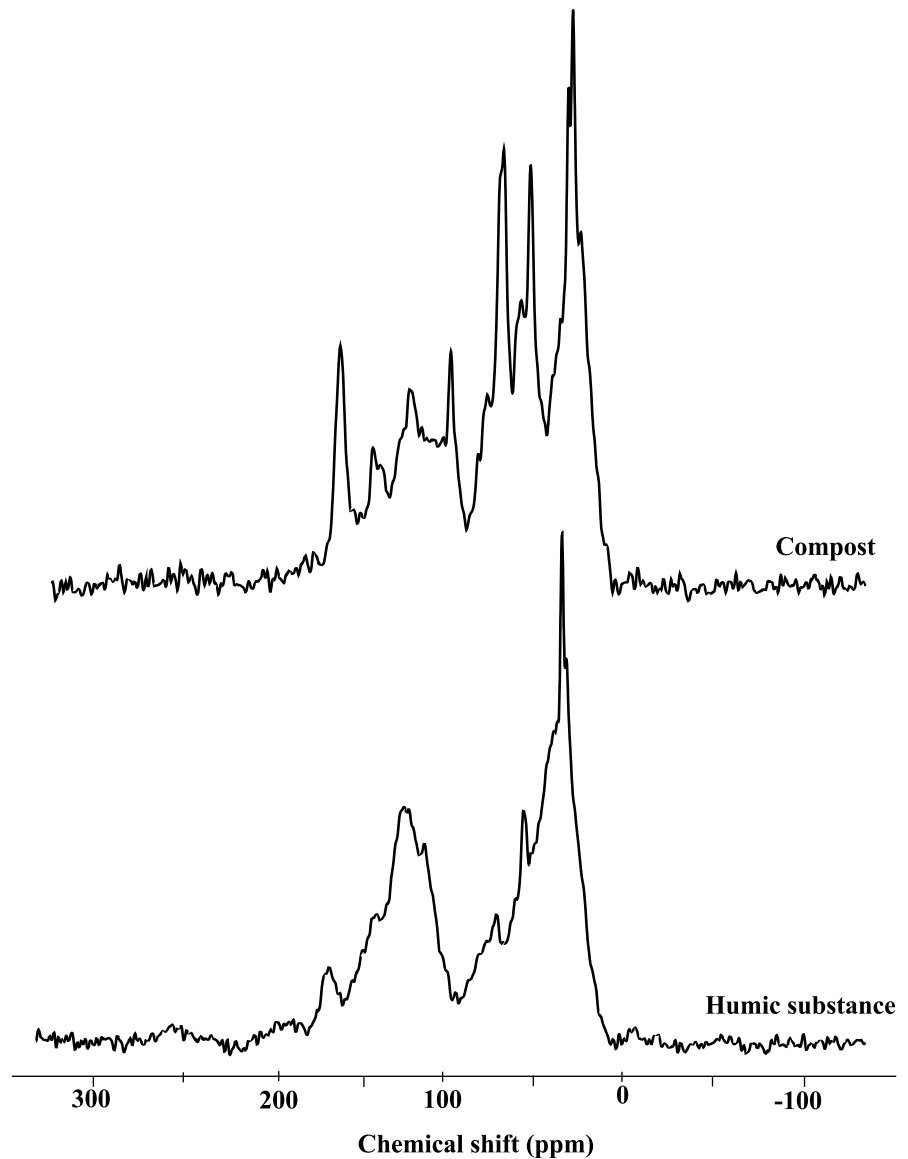
mine tailings or contaminated soils. These findings are consistent with those of Mayans et al. (2019), who conducted a chemical analysis of commercial HS derived from leonardite and found a greater relative area of alkyl-C, aromatic-C, and phenolic-C groups compared to composts derived from plant matter or animal manure. These authors also found that HS had a greater ability to complex with Cu due to its phenolic and carboxylic groups and was more thermostable due to its high recalcitrant OM.

Metallic elements and plant sulfur

Overall, the addition of the HS did not significantly increase Cu, Fe, Mn, or Zn concentrations in the aboveground parts or roots of *A. atacamensis* compared to the MT treatment (Table 4). However, the slight increase in root Cu concentrations in the MT + HS treatment suggests that the alkalinity of both the HS and the tailings material and the predominance of aromatic and alkyl fractions in

Table 3 Relative areas (%) of the chemical shift regions in ^{13}C CP-MAS NMR spectra of organic amendments

Chemical shift (ppm)	Type of C bond	Compost	Humic substance
		% Total area	
$0 < \delta \leq 45$	Alkyl	28.5	33.7
$45 < \delta \leq 60$	N-alkyl	11.7	7.04
$60 < \delta \leq 93$	O-alkyl	22.4	7.11
$93 < \delta \leq 110$	Di-O-alkyl	7.33	4.73
$110 < \delta \leq 140$	Aromatic	14.1	27.5
$140 < \delta \leq 160$	Phenolic	6.18	7.14
$160 < \delta \leq 190$	Carboxyl	7.73	6.73
$190 < \delta \leq 220$	Amide-ketonic	1.46	2.66

the HS, as indicated by ^{13}C NMR results, reduced the mobility of metal complexes and facilitated their adsorption on *A. atacamensis* root tissues. A similar trend for Cu was reported by Tapia et al. (2020), who evaluated *Atriplex nummularia* Lindl. (*A. nummularia*) in the same tailings, reporting root concentrations of 155 ± 4.40 mg kg^{-1} without amendments and 179 ± 21.1 mg kg^{-1} with 5% w/w commercial humic substance. Our results for root Cu concentrations were higher compared to other studies in mine tailings using species of the genus *Atriplex*. Thus, in *Atriplex halimus* L. (*A. halimus*) grown in mine tailings (total Cu: 1,080

to 2,201 mg kg^{-1}), Midhat et al. (2019) reported maximum root Cu values of 51.5 ± 0.72 mg kg^{-1}, and Tapia et al. (2019) in tailings with humic substances (at a dose of 120 kg ha^{-1}) reported maximum Cu concentrations of 185 ± 37.8 mg kg^{-1}. Furthermore, Acosta et al. (2018) found root Cu concentrations of less than 20 mg kg^{-1} in *A. halimus* grown in unamended tailings (pH: 7.4 ± 0.2; DTPA-Cu: 2.3 ± 0.3 mg kg^{-1}). Meanwhile, these results may have one drawback, which has to do with the high solubility of HS, making it vulnerable to leaching losses, especially if only one low dose is applied. An alternative that could improve HS efficiency would be to use higher doses with piecemeal application over time; however, the high cost (12 USD kg^{-1}) of commercial humic substances makes them unsuitable for continuous use, given those areas covered by phytoremediation are usually extensive.

Interestingly, the MT+C treatment resulted in significantly lower concentrations of Cu, Fe, and Mn in both aboveground parts and roots compared to the MT and MT+HS treatments. These results suggest that compost application likely immobilized Cu, Fe, and Mn by forming stable organometallic compounds adsorbed on the OM, thereby reducing the phytoavailability of these metals. The higher amount of compost applied (158 g pot^{-1}) compared to the HS (1.30 g pot^{-1}) clearly played a role because the MT+C treatment contained proportionally

Table 4 Trace metal and sulfur concentrations of *Atriplex atacamensis* Phil. in roots and aerial parts at 120 days (values are mean ± standard deviation, $n = 4$)

Treatments	Structure	S	Cu	Fe	Mn	Zn
		%	mg kg^{-1}			
MT	Leaves + stems	0.21 ± 0.01 b	17.7 ± 0.30 a	164 ± 13.9 a	111 ± 5.10 a	13.3 ± 1.70 b
MT + HS		0.33 ± 0.04 a	19.1 ± 1.50 a	176 ± 7.20 a	112 ± 9.80 a	15.7 ± 0.90 b
MT + C		0.35 ± 0.04 a	11.3 ± 0.50 b	88.4 ± 11.0 b	40.3 ± 7.11 b	29.7 ± 5.96 a
MT	Roots	0.41 ± 0.05 a	261 ± 54.6 a	3,048 ± 527 a	193 ± 60.5 a	32.6 ± 5.30 b
MT + HS		0.42 ± 0.06 a	323 ± 28.7 a	3,126 ± 774 a	183 ± 26.7 a	48.5 ± 2.50 b
MT + C		0.38 ± 0.04 a	191 ± 15.5 b	1,050 ± 30.9 b	86.4 ± 3.80 b	78.8 ± 9.50 a
Excessive or toxic			20 – 100[a]	> 300[b]	400 − 1000[a]	100 − 400[a]
Domestic animal toxicity limit[c]		≤ 0.35	≤ 40	≤ 500	≤ 10,000	≤ 500

Different letters in column of each structure, indicate significant differences between treatments, according to the Tukey test at $p \leq 0.05$

[a]Kabata-Pendias (2011)

[b]Havlin et al. (2014)

[c]NRC (2005)

more aromatic, phenolic, and carboxyl groups that have higher metal complexing capacity than other functional groups (Mayans et al., 2019). Usually, metallic elements are firmly incorporated into the OM via metal-chelate compounds and are believed to persist as long as the properties of the OM do not change significantly over time (Hooda, 2010). Depending on the element, the number of atoms bound to the metal ion, the number of rings formed, pH, and metal concentration, the stability of metal-chelate complexes may vary to a greater or lesser extent ($Cu^{+2} > Ni^{+2} > Co^{+2} > Zn^{+2} > Fe^{+2} > Mn^{+2}$) (Stevenson, 1994). Duarte et al. (2019) evaluated the effect of progressively rising composted manure doses (from 1 to 12% w/w) on chemical fractionation and metal retention in mine tailings and reported a 28.5% decrease in EDTA-extractable Cu and a decrease in interchangeable metal fractions (bioavailable to plants) at the highest doses. A similar trend was observed by Khan and Jones (2009), who reported that a 10% dose of green manure compost in Cu mine tailings (Cu total: 1905 mg kg^{-1}) decreased Cu and Fe extractability over time.

The opposite situation was observed with the Zn content in *A. atacamensis*, however, as the MT + C treatment significantly increased the Zn concentration in the aboveground parts compared to the MT and MT + SH treatments. The increase was probably due to the formation of soluble complexes that increased Zn availability to plants. Manzano et al. (2016), who evaluated metal mobility in mine tailings supplemented with compost (4% w/w), reported an increase in exchangeable Zn due to the formation of soluble complexes between Zn and dissolved organic carbon supplied with the amendment. In addition, the compost used may itself be a significant source of metals such as calcium and magnesium, which can affect Zn mobility due to ionic competition (Branzini & Zubillaga, 2012). Furthermore, bioavailability tests showed weaker Zn binding to humic acids due to lower affinity (Kang et al., 2011).

The aboveground and root content of Zn in this study (Table 4) was lower compared to other studies of the genus *Atriplex* on mine tailings. For example, Acosta et al. (2018) reported leaf Zn range of 300 to 400 mg kg^{-1} and root Zn content of 250 to 300 mg kg^{-1} in *A. halimus* grown on mine tailings (pH 7.5 and available Zn: 402 mg kg^{-1}) supplemented with pig manure (dose 3 L m^{-2}). Similar results were published about *Atriplex*

lentiformis (Torr.) by Gil-Loaiza et al. (2016), who supplemented tailings with compost in doses of 15% and 20% w/w (pH 2.5 and extractable Zn: 1800 mg kg^{-1}), reporting shoot Zn content in the range of 506 ± 253 to 936 ± 305 mg kg^{-1}. The lower concentrations observed in the Chilean species may be related to the amount of available Zn in the tailings in our study (15.0 ± 1.17 mg kg^{-1}), considering that another species, *Carpobrotus aequilaterus* (Haw.), grown in the same tailings with the addition of HS (30 kg ha^{-1}), exhibited the same concentrations in the aboveground parts as in our experiment (38.2 ± 7.6 mg kg^{-1}) (Tapia et al., 2017).

As for S concentrations (Table 4), *A. atacamensis* had between 0.21 ± 0.01 and $0.35 \pm 0.04\%$ in the aboveground parts and between 0.38 ± 0.04 to $0.42 \pm 0.06\%$ in the roots, which is consistent with typical S concentrations in plants (0.20–0.50%), according to Havlin et al. (2014). In addition, the sulfur and metal concentrations obtained in this study were below the limit for livestock feed plants (NRC, 2005), which was set to prevent contaminants from moving up the food chain through livestock grazing on live or dead plants at the senescence stage (Pérez-de-Mora et al., 2011). In fact, the sulfur concentrations in our experiment were similar to the results reported by other authors who studied the genus *Atriplex* as livestock feed. Koning et al. (2019) evaluated *A. nummularia* and *Atriplex amnicola* Lindl. for free-range poultry feed and reported leaf sulfur contents of 0.60 and 0.20%, respectively. Similarly, Norman et al. (2004) investigated the nutritional value of two species of the genus *Atriplex* used in commercial grazing systems and found S concentrations in the range of $0.38 \pm 0.07\%$ and 0.48 ± 0.12. In addition, Mahipala et al. (2009) reported concentrations ranging from $0.09 \pm 0.01\%$ to $0.47 \pm 0.04\%$ in *Atriplex* species grown in the Mediterranean climate and investigated as potential ruminant feed.

Regarding S phytoremediation, the use of higher plants has been proposed as an effective strategy in gypsum soils with toxic concentrations of sulfate, considering the diversity of metabolism, accumulation, and role of S as a major nutrient in plants (Ruiz et al., 2003). In this regard, several species common in semi-arid and arid habitats and capable of accumulating leaf S concentrations of 2.5 to 8.2% dry matter have been classified as thiophore plants and

recommended as candidates for use in remediation strategies (Ernst, 1998). The global environmental issue related to S and mine tailings is the acid mine drainage phenomenon, which is promoted by bacterial oxidation of sulfide minerals such as pyrite (FeS_2). This process is characterized by increasing the acidity of tailings and the available concentration of metals and sulfate, which are more susceptible to leaching (Lottermoser, 2010). Therefore, adequate species are needed to remove metals and sulfate from mine tailings. In general, few studies have evaluated the sulfur content of plants grown directly on mine tailings. Parraga-Aguado et al. (2014) reported shoot/leaf S concentrations of $0.61 \pm 0.10\%$ in *A. halimus*, grown naturally on mine tailings in semi-arid climates in Spain, and concluded that this halophytic plant could be used for desalination of mine tailings. In Chile, Tapia et al. (2017) used *Carpobrotus aequilaterus* (Haw.) with the HS at a rate of 60 kg ha^{-1} and obtained aboveground S content of $0.44 \pm 0.03\%$ and root S of $0.65 \pm 0.30\%$ at 120 days, which is higher than the concentrations reported in this study. However, *A. nummularia* grown on the same mine tailings with and without amendments (compost 5% w/w and humic substance 5% w/w) by Tapia et al. (2020) exhibited concentrations in aboveground parts as high as 2%. The authors concluded that this species is a potential thiophore plant and that the addition of commercial humic substances improves its ability to phytostabilize sulfur.

In our experiment, the addition of organic amendments significantly increased the aboveground S concentrations in *A. atacamensis* compared to the MT treatment (Table 4). However, despite using the same genus as Tapia et al. (2020), our results were insufficient to recommend the Chilean endemic *A. atacamensis* as a potential sulfur accumulator. The significant increase in S content in the MT + C and MT + HS treatments can be attributed to the contribution of S–SO$_4$ from the organic amendments incorporated in the mine tailings (87% contribution in the MT + C treatment and 81% in the MT + HS treatment). Furthermore, the average temperature of 21.7 °C during the study period was within the optimal range (20–40 °C) for increased S mineralization due to OM influx from the amendments and probably contributed to S-SO$_4$ availability in solution (Havlin et al., 2014).

Phytostabilization indexes, metals uptake, and $\delta^{13}C^{V-PDB}$ in leaves

TI values (Fig. 2) for all elements and treatments evaluated were below unity, indicating that this species tends to concentrate Cu, Fe, Mn, Zn, and S in the roots rather than in the aboveground parts, with or without organic supplementation of the tailings' substrate. Furthermore, the values of Cu and Fe were less than 0.1, indicating that *A. atacamensis* has a greater ability to phytostabilize these elements compared to the other metals studied. Several studies (Acuña et al., 2021; Eissa & Almaroai, 2019; Lam et al., 2017; Rabier et al., 2014) reported TI values lower than 1.0 for the genus *Atriplex* for various metal(loid)s in tailings and contaminated soils and concluded that this genus has remarkable tolerance to high metal(loid) concentrations while accumulating metals primarily in its roots rather than in the shoots and stems. Regarding the BCF (Fig. 3), the values for all treatments and elements were different, indicating that the metal accumulation capacity in roots by *A. atacamensis* varies between elements. In addition, the BCF results showed values below unity, classifying this specie as an excluder plant for Cu, Fe, Mn, and Zn; however, these results are explained by the significant difference between the metal concentrations in the mine tailings and the root contents. The BCF results for Cu, Fe, and Mn in the MT + C treatment followed a trend to have significantly lower values than MT and MT + HS, which are also reflected in the concentration of those elements (Table 4). These results are attributed to the compost addition and its effect on metals, decreasing the bioavailability by forming organometallic compounds adsorbed on the OM. The BCF values < 1.0 are frequently found for Cu, Fe, Mn, and Zn in the genus *Atriplex*; for instance, Lam et al. (2017) studied *A. nummularia* in mine tailings and found similar BCF values for Cu (0.12–0.17), Fe (0.03–0.10), Mn (0.11–0.33), and Zn (0.11–0.27), concluding that *A. nummularia* can be considered as an excluder specie for those elements. However, although BFC classifies *A. atacamensis* as an excluder species, our TI results (Fig. 2) show that *A. atacamensis* was mainly an accumulator for Cu and Fe, and can have the potential for phytostabilization in its roots which agree with TI results reported by Tapia et al. (2020) for Cu (0.15 – 0.20) in *A. nummularia* cultivated in mine tailings, considered a phytostabilization species for Cu after 120 days of trial.
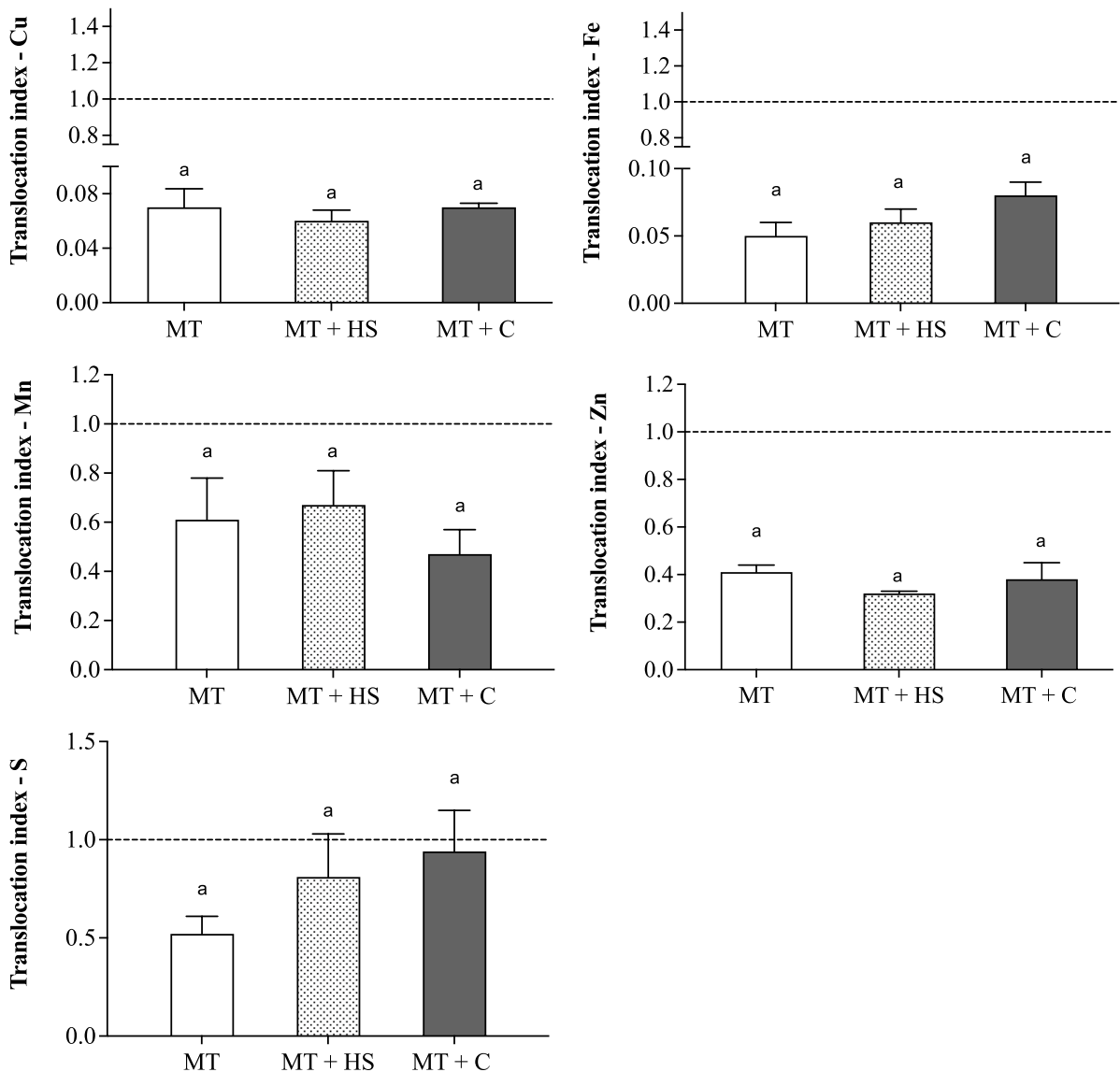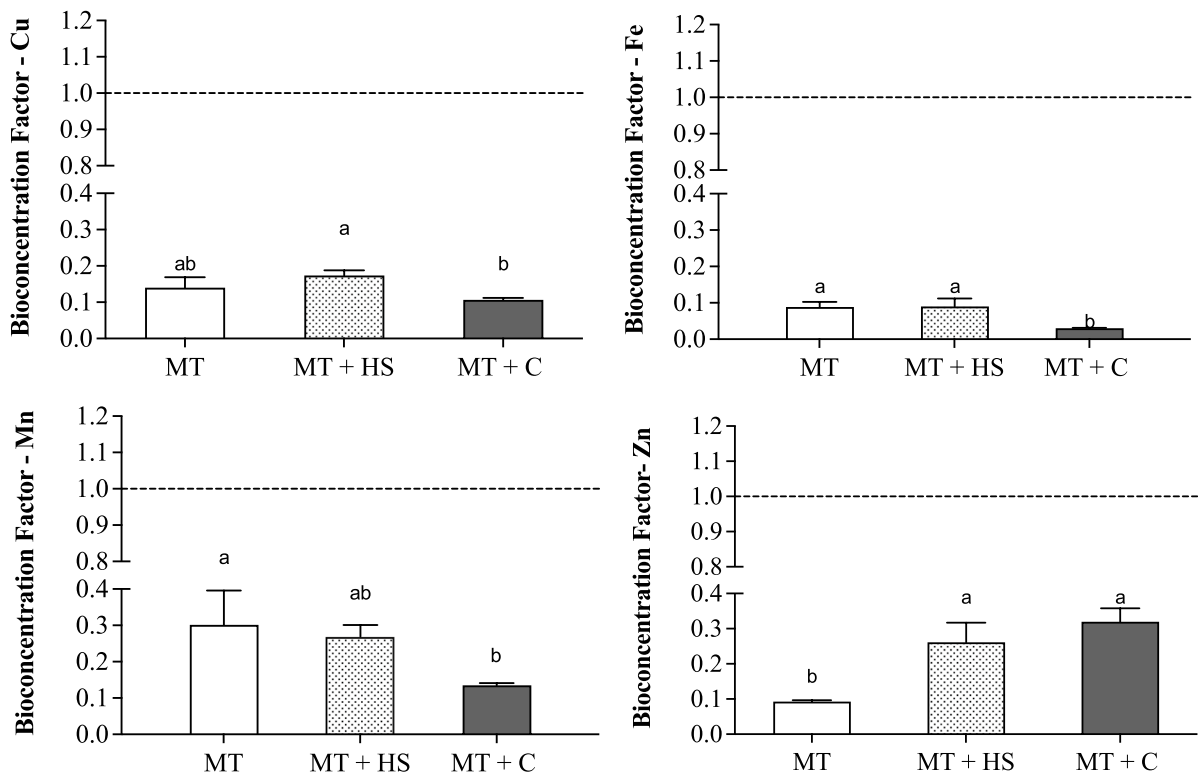
Fig. 2 Translocation index of Cu, Fe, Mn, Zn, and S for *Atriplex atacamensis* Phil. cultivated in mine tailing (MT), mine tailings + humic substance (MT + HS), and mine tailing + compost (MT + C). Values below the dotted line indicate the phyto-stabilization of elements. Values are mean ± standard deviation ($n = 4$). Different letters between treatments show statistically significant differences according to the Tukey test ($p \leq 0.05$)

The metals and S uptake values are shown in Fig. 4. The results indicate that *A. atacamensis* tends to accumulate more Cu and Fe in its roots than the aboveground parts, which agrees with the TI values (TI < 0.1) discussed above (Fig. 2). The treatments MT and MT + HS had Cu and Fe contents statistically higher than MT + C due to the compost influence on Cu and Fe stabilization in MT + C, which decreases the bioavailability for plants. Those results followed a similar pattern to the Cu and Fe concentrations (Table 4), which showed lower concentrations in MT + C than in MT and MT + HS. Adding compost reduces metal mobility as a result of precipitation and sorption processes as metal-chelate compounds formed are firmly incorporated into the OM of the substrate (Hooda, 2010).

Fig. 3 Bioconcentration factor of Cu, Fe, Mn, and Zn for *Atriplex atacamensis* Phil. cultivated in mine tailing (MT), mine tailings + humic substance (MT + HS), and mine tailing + compost (MT + C). Values are mean ± standard deviation ($n = 4$). Different letters between treatments show statistically significant differences according to the Tukey test ($p \leq 0.05$)

Mainly for Cu and Fe, the alkaline pH, high CEC, and the carboxyl groups associated with the high compost dose added could increase adsorption and precipitation processes in the mine tailings. Thus, adequate amendment doses are crucial to improving fertility, metal stabilization, growth rate, and elements' uptake in plants established in mine tailings under arid and semi-arid conditions. In our study, *A. atacamensis* showed a higher Cu uptake capacity for roots and aboveground parts than the *Atriplex nummularia* studied by Tapia et al. (2020) in the same mine tailings with compost and humic substance addition (5% w/w dose); indicating that *A. atacamensis* could be more suitable for Cu phytostabilization than *A. nummularia*. However, for S phytoremediation purposes, the species studied by Tapia et al. (2020) would be more suitable as significantly could accumulate higher S content in roots and aboveground parts than *A. atacamensis*.

Dry weight analysis (Fig. 5) showed that both organic amendments significantly reduced the dry weight of aboveground parts, while compost additionally reduced the dry weight of roots compared to the MT treatment. The reduction was attributed to metal immobilization by compost, which reduced Fe concentrations in *A. atacamensis* Phil. to deficiency levels (< 150 mg kg^{-1} leaves) inducing a series of physiological responses in plants, including reduced photosynthetic activity, inhibited root elongation, increased apical root diameter, and abundant root hair formation (Marschner, 2012). This conclusion is supported by the lower leaf TOC content in the MT + C treatment compared to the MT and MT + HS treatments (Table 5).

Measurements of carbon isotopic composition in leaf dry matter in C$_4$ plants can be used to assess changes in the ratio of intercellular and ambient CO$_2$ concentrations (Ci/Ca-ratio), photosynthetic and post-photosynthetic fractionations (Ellsworth & Cousins, 2016), as well as drought, salt, and nitrogen stresses (Walker and Sinclair, 1992; Fravolini et al., 2002; Monneveux et al., 2007). Our results (Tables 1 and 5)
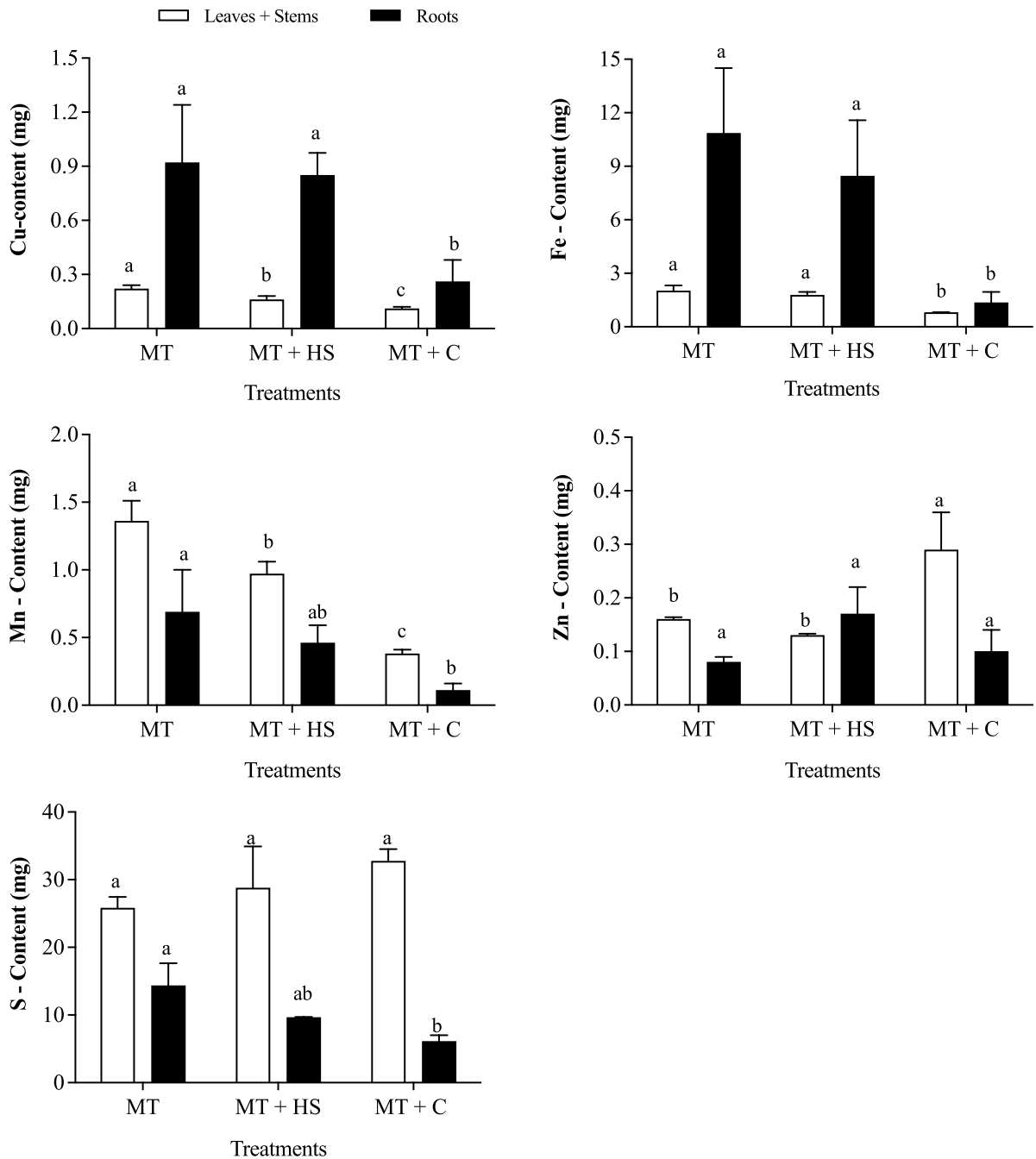
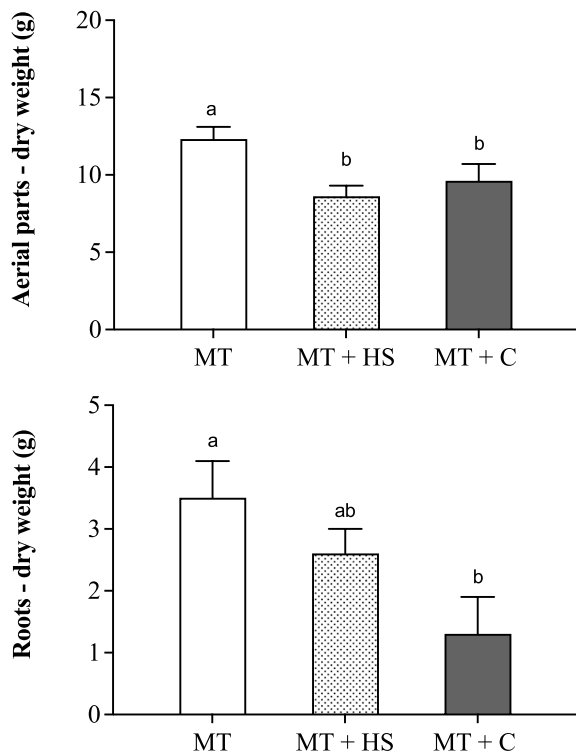**Fig. 4** Uptake content (mg) of Cu, Fe, Mn, Zn, and S for *Atriplex atacamensis* Phil. cultivated in mine tailing (MT), mine tailings+humic substance (MT+HS), and mine tailing+compost (MT+C). Values are mean±standard deviation ($n=4$). Different letters between treatments and tissues show statistically significant differences according to the Tukey test ($p \leq 0.05$)

showed that *A. atacamensis* leaf δ^{13}CV-PDB values at 120 days increased by 4.63%, 5.89%, and 3.09% in the MT, MT+HS, and MT+C treatments, respectively, compared to the values in plants established in pearlite. This was likely due to the salinity of the tailings' material used. Walker and Sinclair (1992) reported

Fig. 5 Dry weight of *Atriplex atacamensis* Phil. cultivated in mine tailing (MT), mine tailings + humic substance (MT + HS), and mine tailing + compost (MT + C). Values are mean ± standard deviation ($n = 4$). Different letters between treatments indicate statistically significant differences according to the Tukey test ($p \leq 0.05$)

a trend toward decreased discrimination in the leaf ^{13}C composition in the *Atriplex* species against ^{13}C in CO_2 in the air with increasing soil conductivity. In addition, Meinzer and Zhu (1999) observed that the decrease in discrimination against ^{13}C and other physiological parameters analyzed affected the efficiency of C_4 photosynthesis in *Atriplex lentiformis* under

Table 5 Total organic carbon concentration and carbon-13 discrimination of *Atriplex atacamensis* Phil. leaves at 120 days. (Values are mean ± standard deviation, $n = 4$)

Treatments	$\delta^{13}C^{V-PDB}$ ‰	TOC g kg^{-1}
MT	− 18.492 ± 0.34 a	372 ± 0.80 a
MT + HS	− 18.246 ± 0.24 a	367 ± 2.00 a
MT + C	− 18.789 ± 0.18 a	317 ± 3.40 b

Different letters in column indicate significant differences between treatments, according to the Tukey test at $p \leq 0.05$

salinity stress. The addition of organic amendments made no significant difference in the ^{13}C composition at the end of the experiment (Table 5), indicating that the amendments applied and their doses did not cause any additional salt stress beyond that exerted by the mine tailings material. The evaluation of this parameter may serve as a first approximation for the study of other physiological parameters of plants grown in tailings, such as stomatal conductance, photosynthesis, Ci/Ca ratio, leaf temperature, and leaf water potential, in order to elucidate the effect of organic amendments in mine tailings on stress in plants grown and evaluated under in situ conditions.

Conclusions

The study showed that *Atriplex atacamensis* Phil. grown in a tailings pond successfully fulfills the phytostabilizing function with or without the addition of organic amendments, resulting in higher concentrations of Cu, Fe, Mn, Zn, and S in the roots, but not in the aboveground parts. The addition of the humic substance did not make a significant difference compared to the MT treatment, but the compost was able to reduce the concentrations of Cu, Fe, and Mn in both roots and aboveground parts by immobilizing these metals, which also was demonstrated with the uptake values obtained. The opposite situation was observed in both plant tissues with respect to Zn content. These results are attributable, firstly, to the differences in the chemical structure of the amendments determined by ^{13}C NMR analysis and, secondly, to the dose of compost used, which provided a greater number of functional groups with a higher complexation capacity to metals. Our $\delta^{13}CV$-PDB values for the compost and HS treatments showed that *Atriplex atacamensis* Phil. experienced no greater salt stress at 120 days compared to the treatment without organic amendments. Overall, *Atriplex atacamensis* Phil. demonstrated the ability to phytostabilize metals and sulfur and may thus be considered a suitable candidate species for in situ evaluation of the phytostabilization process of mine tailings.

Declarations

Ethics approval All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors and are aware that with minor exceptions, no changes can be made to authorship once the paper is submitted.

Conflict of interest The authors declare no competing interests.

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