



Assisted phytoremediation of lead contaminated soil using *Atriplex halimus* and its effect on some soil physical properties

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Abstract

Lead is one of the most harmful trace elements found in soils. Despite reductions in environmental lead exposure, an historical lead pollution continues affecting soils in many areas. To mitigate the impact of lead, phytoremediation assisted with addition of chelating agents can be a suitable option. In order to evaluate the phytostabilization and/or phytoextraction capacity of *Atriplex halimus*, a pot assay with six phytoremediation treatments was established using different combination of two chelating agents (citric acid and fulvic acids), a composted substrate as organic amendment and a soil highly contaminated with lead (2952 ± 280 mg Pb kg⁻¹, pH 6.30 ± 0.13) collected in the Valparaíso Region, Central Chile. Chelating agents were applied to irrigation water considering a concentration of 4 g L⁻¹, while the organic amendment was applied at the beginning of the assay (dose of 5% v/v). After 180 days, the addition of the chelating agents significantly increased the Pb concentrations in tissues, an effect that was enhanced by application of the organic amendment, reaching concentrations of 32, 42, and 360 mg Pb kg⁻¹ in leaves, stems, and roots, respectively. The treatments considered the addition of fulvic acids showed higher total plant dry weight levels (16.4 and 18.2 g, respectively) compared to the rest, which ranged from 8.3 to 10.0 g. With respect to soil physical properties, chelating agents increased microaggregate stability, but fulvic acids addition significantly decreased the soil available water. In general, *Atriplex halimus* demonstrated good potential for the phytostabilization of lead contaminated soils and a limited phytoextraction capacity.

Keywords Amendment management · *Atriplex halimus* · Dry weight · Lead · Phytoremediation

Introduction

Soil contamination by trace elements (TEs) is one of the most severe ecological problems in developing industrialized countries, in large part due to their non-biodegradability (Gupta et al. 2019; Madejón et al. 2018). In particular, lead (Pb) is one of the most common pollutants and is widely distributed in soils. It is typically found in fuels, paints, industrial products from foundries and mining, as well as shooting ranges, and battery recycling plants (Ahmad et al.

2016; Gottesfeld et al. 2018, Surriya et al. 2014). In the last 30 years, this metal has been reduced or eliminated in most gasoline and paints. However, because Pb does not degrade, these former uses leave their legacy as higher concentrations of Pb in the environment (Carocci et al. 2016), with a historical Pb pollution that continues affecting soils in many areas and an impact that can persist for centuries (Mariet et al. 2017).

The bioavailability of TEs in soils is affected by many chemical processes as sorption/desorption on/from soil components (e.g., organic matter, variable charge minerals), soil complexation, oxidation–reduction, and precipitation–dissolution reactions (Caporale and Violante 2016). In the case of Pb, only a small fraction of the total soil Pb is available to plants; instead, it is concentrated in the superficial horizons, with a low proportion of the metal in solution (Pourrut et al. 2011; Saifullah et al. 2009). In general, Pb in soils may occur as a free metal ion, complexed with inorganic constituents, or may exist as organic ligands (e.g., amino acids, fulvic acids, and humic acids); alternatively Pb may be

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adsorbed onto particle surfaces, such as Fe oxides, organic matter, and clay particles (Kushwaha et al. 2018). A high content of cation exchange capacity (CEC), clay minerals, carbonates, Fe and Mn oxides, phosphates, organic matter, and neutral pH contribute to low Pb mobility. Conversely, low content of these soil properties and alkaline or acidic soils contribute to increased Pb mobility (Egendorf et al. 2020).

The effects of Pb soil contamination can be mitigated by using phytoremediation, which employs plants to convert soil contaminants into non-toxic forms through accumulation in their harvestable tissues (phytoextraction) or stabilization in their roots (phytostabilization) (Pilon-Smits 2005; Ismail 2013). In the case of Pb phytoextraction, because only a small fraction of the metal is available, research has focused on improving the absorption of the element by applying various chelating agents to mobilize it and increase its availability (Butcher 2009; Htwe and Kawamoto 2018; Saifullah et al. 2009; Shakoor et al. 2014). Despite the above, many researchers have demonstrated the inadequacy of Pb phytoextraction approach (Egendorf et al. 2020). Otherwise, Pb phytostabilization is considered suitable because is known the low mobility and persistence of Pb in soils (Manzoor et al. 2019). Phytostabilization technique can be greatly enhanced by treating the soil with organic amendments (OA) in order to amplify the availability of TEs to the plants (Shackira and Puthur 2019) and contributing to improve Pb stabilization in soils (Pichtel and Bradway 2008), although it is necessary to carry out specific evaluations to determine the final effect of these measures on Pb availability.

The use of phytoremediation can be hindered when working with TEs of low solubility. In the case of Pb, an option to improve the availability of the element is to bring the pH to values lower than 5.5. However, this approach increases aluminum solubility, which reduces plant growth via toxicity (Butcher 2009). A more feasible strategy involves chelate-assisted or induced phytoextraction (Pinto et al. 2015), in which chelates (low molecular weight organic ligands) are often added to increase the absorption efficiency of TEs by tolerant plants, since its use activate TEs from the surfaces of soil particles into soil solutions by form soluble complexes (Clemente and Bernal 2006; Huang et al. 2019; Hsiao et al. 2007). This approach makes it possible to use high biomass production crops and overcome the problem of low TEs availability in soils (Pinto et al. 2015). The chelate-assisted phytoextraction of Pb uses metal-binding agents which enhance its solubility and availability for plant uptake (Khan et al. 2019). One of the most widely used chelating agents is citric acid (CA), which in addition to being natural and biodegradable, has proven to be a good complement for phytoextraction in soils contaminated with TEs (Freitas et al. 2014; Sarwar et al. 2017). Fulvic acids (FA) are another

important chelating agent less studied in phytoremediation processes. They are organic acids that contains more functional groups compared to humic acids and have shown to play an important role in the alleviation of abiotic stress in plants (Van Oosten et al. 2017). By also having the capacity to form complexes with TEs, FA contribute to increasing their mobility in the soil (Hooda 2010; Kabata-Pendias 2011; Zhou et al. 2005).

According to Zaier et al. (2014), halophytic species may be promising in phytoremediation. These plants, which can survive and reproduce in high salt environments, are potentially ideal candidates for phytoremediation of saline soils contaminated with TEs. In this regard, Liang et al. (2017) have suggested that halophytes can accumulate large amounts of salt while extracting TEs from saline soils (Anjum et al. 2014). In Chile, plants of the genus *Atriplex* are present with a great diversity of native and introduced species (Brignone et al. 2016). Among them, *Atriplex halimus* L. (*A. halimus*), a native of Mediterranean arid and semi-arid zones and their surroundings, is a vigorous perennial shrub that can reach between 1.0 and 2.5 m in height, with high resistance to both drought and salinity (Ingelmo et al. 1998; López 2002). The use of *A. halimus* in phytoremediation has been reported by several authors in recent years (Eissa 2019; El-Bakatoushi et al. 2015, Vromman et al. 2011; Tapia et al. 2016, 2020), but there is no enough information about their behavior in soils highly contaminated with Pb.

The main objective of this study was to evaluate the phytoremediation capacity of a highly Pb contaminated soil using *A. halimus* plants, chelating agents, and an organic amendment, while also examining their effect on soil physical properties. We hypothesized that the use of chelating agents and an organic amendment increases Pb bioavailability in the soil, favoring *A. halimus* phytoremediation capacity and improving soil physical properties. This research work was carried out at the Faculty of Agronomic Science of the University of Chile, Chile, in the period from December 2014 to December 2015.

Materials and methods

Sampling of lead contaminated soil

A Pb contaminated soil (S_{Pb}) was collected from a recreational area located in Puchuncaví, Valparaíso Region, Chile (273.017 m E, 6.386.884 m S) (Fig. 1). The sampling sector was in a hillside, in a bounded area measuring 12 m². Normal Pb levels in soil were verified in the surroundings (more information is unknown). A superficial extraction of soil was carried out (0–10 cm); then, the material was transported to the Faculty of Agricultural Sciences of the University of Chile (Santiago, Chile). A sieve was used to discard the

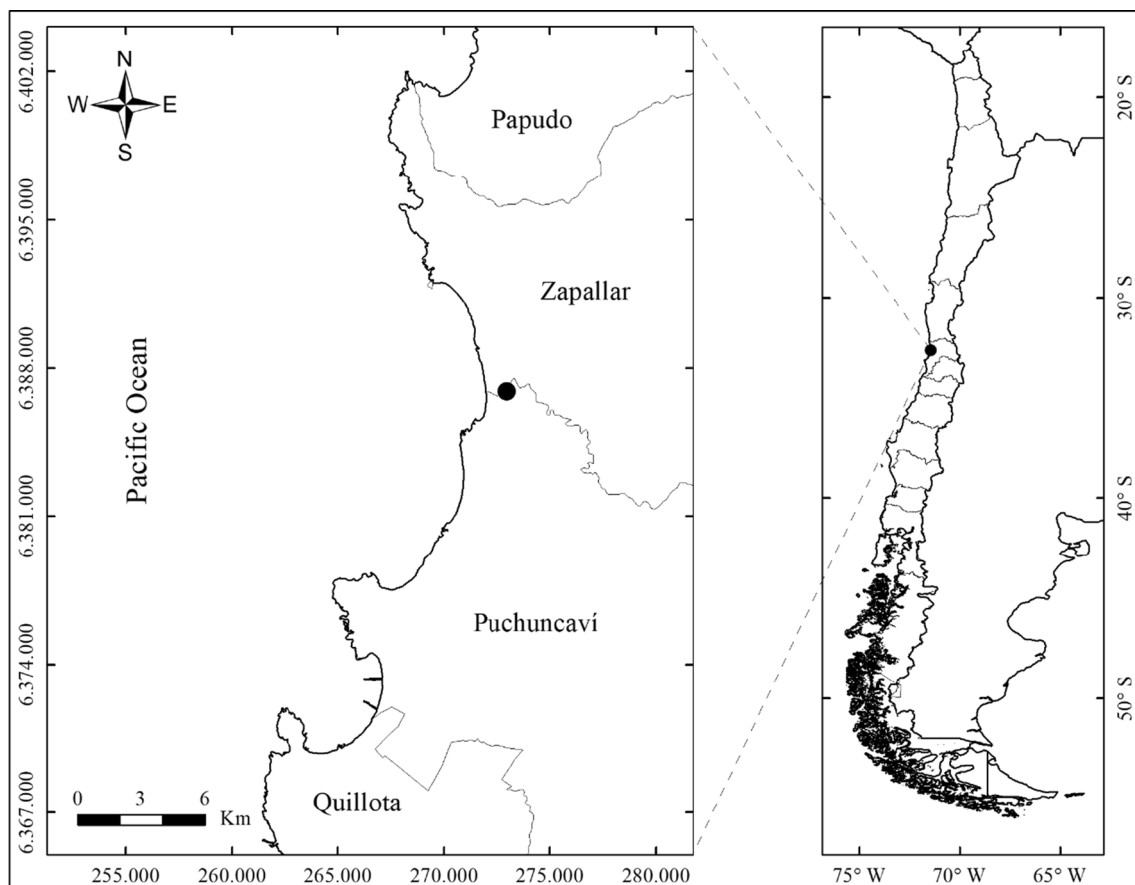


Fig. 1 Location of the sampling zone of soil contaminated with lead (Central Zone, Chile)

material with a diameter greater than 0.5 cm. Then, the soil was homogenized and an assay in 3 L pots was established. The pots were filled with 2.7 L of soil (4 kg per pot).

***Atriplex halimus* plants propagation**

Atriplex halimus plants were propagated from seeds (Euroseeds Seeds Montaraz, Spain). In order to foster the germination process, seed bracts were cut according to the methodology proposed by Lailhacar and Laude (1975); then, seeds were germinated in petri dishes placed in a chamber for 72 h. Subsequently, seedlings were transplanted to plastic cups with compost and kept under greenhouse conditions for 8 months. Plants were irrigated 1–2 times per week until before their final transplant to pots, with a plant height of 5–7 cm.

Treatments and pot assay establishment

The pot assay was conducted during 180 days in a research greenhouse. While the assay was conducted, the mean minimum temperature was 9.9 °C, and the mean maximum temperature was 27.6 °C. In order to create different

Pb availability conditions in S_{Pb} to assess *A. halimus* performance, 6 phytoremediation treatments were established using different combinations of two chelating agents: Citric Acid (CA, SIGMA-ALDRICH) and Fulvic Acids (FA, Plus Fulvital[®], HUMINTECH) and an Organic Amendment (OA). The treatments applied in pots ($n=3$) with *A. halimus* plants were: (1) S_{Pb} as control treatment, (2) $S_{Pb} + CA$, (3) $S_{Pb} + FA$, (4) $S_{Pb} + OA$, (5) $S_{Pb} + OA + CA$, and (6) $S_{Pb} + OA + FA$. The OA consisted in a commercial composted substrate based on pine bark, sawdust, and algae (Acidic earth, Roots[®]), considering an application dose of 5% v/v in pots.

All the treatments were irrigated with well water (Table 1) in order to imitate high salinity conditions in arid and semi-arid zones of Chile. The irrigation was performed considering 2 weekly applications of 300 mL from December 2014 to March 2015; then, 250 mL per week were applied from April 2015 to May 2015. The S_{Pb} and $S_{Pb} + OA$ treatments were only irrigated with well water. For the $S_{Pb} + CA$ and $S_{Pb} + OA + CA$ treatments, CA was added through irrigation water considering a concentration of 4 g L⁻¹ throughout the evaluation period (180 days). This addition is equal to half of the concentration of tartaric acid present in a standard



Table 1 General chemical properties of irrigation water used in the assay. EC: Electrical conductivity

Parameter	Unit	Value
pH	–	7.50
EC	(dS m ⁻¹)	1.39
Ca ²⁺	(mg L ⁻¹)	161.52
K ⁺	(mg L ⁻¹)	21.51
Mg ²⁺	(mg L ⁻¹)	1.64
Na ⁺	(mg L ⁻¹)	89.47
Cl ⁻	(mg L ⁻¹)	116.31
HCO ₃ ⁻	(mg L ⁻¹)	40.27
SO ₄ ⁻	(mg L ⁻¹)	235.83

nutrient solution of Hoagland and Arnon (1950). This concentration of CA has been used by Tapia et al. (2013) in previous phytoremediation assays. Similarly, the S_{Pb} + FA and S_{Pb} + OA + FA treatments considered the addition of FA in all irrigations, with the same product concentration being utilized throughout the evaluation period.

Characterization of lead contaminated soil and added products

An initial chemical and physical characterization of the contaminated soil were carried out ($n=4$). The pH was determined in suspension (1:2.5, W/V) by the potentiometric method using a pH meter (HANNA Instruments, HI3221); the electrical conductivity (EC) was determined in the saturated extract using a conductivity meter (Oakton, CON 510). Available N was determined by KCl extraction followed by NH₄ distillation and titration (Mulvaney 1996), while available P was extracted with sodium bicarbonate (0.5 M, pH 8.5) by the Olsen method (Kuo 1996). The cation exchange capacity (CEC) of the material was determined using Na acetate and NH₄ acetate at pH 7.0 (Chapman 1965), while the amount of Na present was measured by atomic absorption spectrophotometry (AAS) using a PerkinElmer 3110 instrument (Boston, MA, USA). The amount of organic matter was determined by combustion at 360 °C for 16 h, and available cations (Ca, K, Mg, Na) were determined using an

extraction with 1 M NH₄ acetate at pH 7.0 (Sadzawka et al. 2006). The total concentrations of Pb and other trace elements (Cu, Cr, Mn, and Zn) were determined by acid digestion in an autoclave (HIRAYAMA, HL30 E) with HNO₃ and H₂O₂ at 125 °C and 1.5 kg cm² for 35 min (Moreno-Jiménez et al. 2010) and subsequent analysis by AAS. An initial sequential extraction of Pb was performed according to the methodology of Tessier et al. (1979) with slight modifications. The procedures for each fraction are described in Table 2. After each extraction, the soil samples were centrifuged at 5000 rpm for 5 min, the supernatant was filtered, and the solid residue was reused in the following fraction. The recovery percentage of the method was 87.6%. At the end of the assay, the soils of each treatment were characterized ($n=3$) in terms of pH, EC, and sequential extraction of Pb.

Lead concentration, pH, and EC in the irrigation solutions with chelating agents were determined at the beginning of the assay. These initial evaluations found no Pb in the chelating agents or in the OA used in the assay (lower than detection limit of the measuring apparatus). Both chelating agents were dissolved in irrigation water in a concentration of 4 g/L, showing acid pH levels (CA: 2.5; FA: 4.1) and a high EC (CA: 2.9 dS m⁻¹; FA: 3.3 dS m⁻¹). It should be noted that the label of “Plus Fulvital[®]” reports the presence of low molecular weight organic matter (fulvic acids: 75%) and nutrients (Cu: 1.0%, Fe: 4.0%, Mg: 6–7%, Mn: 2.5%, S: 5–6% and Zn: 2.5%). The organic matter content of the OA used in the assay was 20.61% (combustion at 550 °C for 2 h), and its pH level (1:5 W/V) was 6.6.

In relation to soil physical properties, an initial characterization of texture was performed at the beginning of the assay using the Bouyoucos hydrometer method, soil bulk density by clod method, field capacity (FC), and permanent wilting point (PWP) by pressure plate as gravimetric water content at 33 and 1500 kPa, respectively (Sandoval et al. 2012). At the end of the assay, microaggregate stability was determined by the dispersion ratio (DR) method (Berryman et al. 1982). In addition, soil bulk density, FC, PWP, and available water content (AWC, calculated as the difference

Table 2 Procedure for sequential extraction of lead in soil contaminated

Fractions	Lead form	Extractant solution	Operational conditions
F I	Soluble and exchangeable	8 mL MgCl ₂ (1 M y pH 7)	Shake for 2 h
F II	Bound to carbonates	8 mL NaOAc (1 M y pH 5)	Shake for 5 h
F III	Bound to iron-manganese oxides	20 mL NH ₂ OH–HCl (0.04 M) en CH ₃ COOH (25%)	Water bath to 96 °C (6 h) and periodic shake
F IV	Bound to organic matter	3 mL HNO ₃ (0.02 M) + 5 mL H ₂ O ₂ (30%) 3 mL H ₂ O ₂ (30%) 5 mL 3.2 M NH ₄ OAc en 20% HNO ₃	Water bath to 85 °C (2 h) and periodic shake Water bath to 85 °C (3 h) and periodic shake Shake for 30 min
F V	Residual	4 mL H ₂ O + 4 mL 30% H ₂ O ₂ (30%) + 6 mL HNO ₃ (65%)	Autoclave digestion to 125 °C for 35 min



between FC and PWP) were again determined for each treatment ($n = 3$).

Plant characterization and phytoremediation assessment

The pot experiment considered an evaluation 180 days after the start of the assay in order to determine the dry weight (DW) and Pb concentration in leaves, stem, and roots for all treatments. After tissue separation, the plant material was dried at 65 °C in an oven until constant weight was reached ($n = 3$). Subsequently, the plant material (0.5 g) was finely ground and digested with 10 mL of distilled water, 3 mL of HNO₃, and 2 mL of H₂O₂ in an autoclave at 125 °C and 1.5 kg cm⁻² for 35 min (Moreno-Jiménez et al. 2010). Based on the scientific literature (Egbuchunam et al. 2010; Ghosh and Singh 2005), information obtained was utilized to calculate the following Pb phytoremediation indexes: transport index to the aerial part “*Ti*” (Eq. 1), bioconcentration factor “*BCF*” (Eq. 2), and phytoextraction percentage “*PP*” (Eq. 3). Additionally, following Xia (2004) and considering shoots as the sum of leaves + stem tissues, the root-shoot DW ratio (“*RS*”) was calculated according to Eq. 4.

$$Ti = \frac{\text{Shoot Pb concentration (mg kg}^{-1}\text{)}}{\text{Root Pb concentration (mg kg}^{-1}\text{)}} \quad (1)$$

$$BCF = \frac{\text{Shoot Pb concentration (mg kg}^{-1}\text{)}}{\text{Initial Pb concentration in soil (mg kg}^{-1}\text{)}} \quad (2)$$

$$PP = \left[\frac{\text{Pb content in shoot (mg)}}{\text{Initial Pb content in soil (mg)}} \right] \times 100 \quad (3)$$

$$RS = \frac{\text{Root dry weight (g)}}{\text{Shoot dry weight (g)}} \quad (4)$$

Statistical analysis

The results of Pb concentration in tissues, DW, *Ti*, *BCF*, *PP*, *RS*, and soil physical properties obtained at the end of the assay were compared according to a completely randomized design, using analysis of variance (ANOVA) through general linear models provided by INFOSAT, a statistical software package (2008 version). Whenever necessary, data were converted to square roots to ensure normal distribution. If significant differences were observed, a comparison among means was made with post hoc Fisher testing; significance was reported at $p \leq 0.05$.

Results and discussion

Initial characterization of lead contaminated soil

According to the guidelines established by Jones (2003), the initial S_{pb} soil characterization showed a high EC (saline soil), a weakly acidic pH, and a high level of organic matter (Table 3). In relation to soil fertility, without being a good indicator, high levels of available N were found (Riquelme et al. 2004), while the levels of available P and K ranged from medium to adequate, as did the levels of Ca, Mg, Na, and CEC (Bernier and Bortolameolli 2000). The textural class of the soil was sandy loam.

Pb concentration in soil exceeded the maximum permission concentrations established by soil environmental quality standards in several countries, such as Canada, Italy, Germany, England, and others (Prasad 2008). Concentrations of copper, manganese, and zinc are typically found in central Chile, while chromium concentration is higher than normal for Chilean conditions, exceeding the maximum permission concentration established for most of countries (Prasad 2008). Regarding the sequential extraction of Pb performed

Table 3 Chemical and physical characteristics of soil contaminated with lead (values are mean ± standard deviation, $n = 4$)

Parameter	Unit	Mean value
EC	(dS m ⁻¹)	4.58 ± 0.53
pH _{water (1:2.5)}	–	6.30 ± 0.13
OM	(%)	5.28 ± 0.05
N _{available}	(mg kg ⁻¹)	55.23 ± 2.63
P _{available}	(mg kg ⁻¹)	15.44 ± 0.42
K _{available}	(cmol ₊ kg ⁻¹)	0.59 ± 0.02
Ca _{available}	(cmol ₊ kg ⁻¹)	18.65 ± 0.68
Mg _{available}	(cmol ₊ kg ⁻¹)	1.88 ± 0.04
Na _{available}	(cmol ₊ kg ⁻¹)	2.06 ± 0.66
CEC	(cmol ₊ kg ⁻¹)	18.00 ± 6.59
Cu _{total}	(mg kg ⁻¹)	117.88 ± 5.11
Cr _{total}	(mg kg ⁻¹)	297.50 ± 52.04
Mn _{total}	(mg kg ⁻¹)	727.50 ± 26.30
Pb _{total}	(mg kg ⁻¹)	2952.25 ± 279.50
Zn _{total}	(mg kg ⁻¹)	77.50 ± 5.00
Clay	(%)	19.07 ± 2.05
Silt	(%)	21.78 ± 4.00
Sand	(%)	59.15 ± 5.21
Bulk density	(Mg m ⁻³)	1.46 ± 0.05
FC	(%)	17.5
PWP	(%)	8.8

EC electrical conductivity, OM organic matter, CEC cationic exchange capacity, FC field capacity as gravimetric water content at 33 kPa, PWP permanent wilting point as gravimetric water content at 1500 kPa



at the beginning of the assay (Fig. 2), Pb was found mainly bounded to carbonates (FII) and iron-manganese oxides (FIII), with both fractions representing 82% of the total Pb concentration in the soil. The low Pb availability stands out, which is represented by the low percentage of the FI fraction (1.02%).

Lead concentration in *Atriplex halimus* tissues

The evaluations carried out at the end of the assay in different tissues of *A. halimus* showed Pb concentrations in leaves and stem ranging from 13.7–32.0 to 12.5–41.5 mg kg⁻¹, respectively (Table 4), while high concentrations of Pb were observed (63.5–360.1 mg kg⁻¹) in root tissues. These results show that levels in root tissues were 4–7 times higher than those in aerial tissues. This is typical of plants that belong to the excluder group, which accumulate Pb in their underground tissues (Seregin and Ivanov 2001). In general, it has been observed that Pb concentrations in roots increase

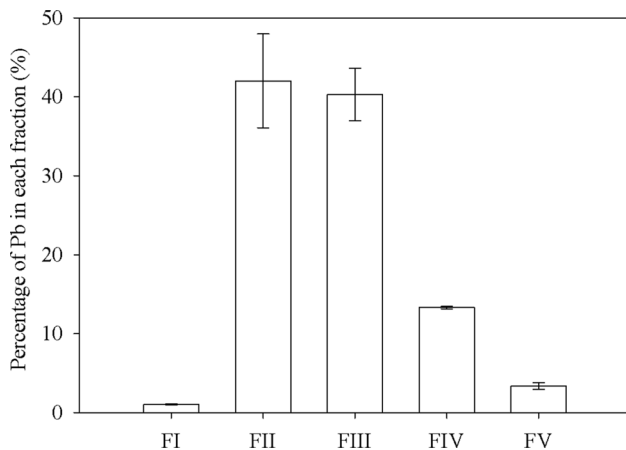


Fig. 2 Distribution of lead (%) in the different soil fractions according to sequential extraction performed at the beginning of the assay. FI, soluble and exchangeable (1.02%); FII, bound to carbonates (42.01%); FIII, bound to iron-manganese oxides (40.29%), FIV, bound to organic matter (13.31%); FV, residual (3.37%). The lines within the bars indicate the standard deviation; $n=4$

consistently with exposure to Pb (Mohtadi et al. 2012; Zaier et al. 2014), with a similar behavior being observed in aerial tissues (Geebelen et al. 2002; Kachout et al. 2012). The Pb concentrations in tissues observed in this assay are comparable with those reported by Manousaki and Kalogerakis (2009), who found a range of 5–25 mg kg⁻¹ for shoots of *A. halimus* plants that grew during 70 days in a soil enriched with Pb (Pb concentration in soil: 800 mg kg⁻¹). Other studies have reported a Pb concentration of 28 and 10 mg kg⁻¹ for *A. halimus* leaves and stem, respectively (Acosta et al. 2011).

In general, a high Pb concentration in root is expected, because in most plants Pb is primarily accumulated in root cells due to the blockage by Casparian strips within the endodermis (Pourrut et al. 2011). Pb is also trapped by the negative charges that exist on root cell walls, and only a limited amount of Pb is translocated from roots to shoot tissues (Pourrut et al. 2011). These mechanisms cause most plants to accumulate Pb in their underground tissues (Seregin and Ivanov 2001). In this regard, Yoon et al. (2006) studied the dynamics of Pb in 17 plant species exposed to high concentrations of this element in the soil, finding that in 95% of cases the concentrations of Pb in roots were much higher than those found in shoots.

Concerning the effect of treatments on Pb concentration in tissues, the statistical analysis showed significantly higher Pb concentrations in leaves, stems, and roots for the $S_{Pb} + OA + CA$ and $S_{Pb} + OA + FA$ treatments, while the rest of the treatments showed similar levels of Pb in tissues. For most plants, excessive Pb concentrations are toxic, leading to an inhibition of chlorophyll biosynthesis and decreasing biomass production (Cenkci et al. 2010; Kaur 2014; Shaikoor et al. 2014). Considering that the toxicity range of Pb for mature leaves ranges from 30 to 300 mg kg⁻¹ for most plants (Kabata-Pendias 2011), the maximum Pb concentrations in leaves and stem in this assay, observed in treatment $S_{Pb} + OA + CA$ (32.0 and 41.5 mg kg⁻¹), are within this toxicity range. Other authors have reported that Pb toxicity begins in the 100–200 mg kg⁻¹ range (Trakal et al. 2015). However, according to the observations made in this assay,

Table 4 Lead concentration in different tissue of *Atriplex halimus* according to phytoremediation treatments (values are mean \pm standard deviation, $n=3$)

Treatment	Lead concentration (mg kg ⁻¹)					
	Leaves	Stem	Root			
S_{Pb}	17.2 \pm 3.3	b	12.5 \pm 5.9	b	63.5 \pm 18.2	c
$S_{Pb} + CA$	19.2 \pm 4.2	b	21.1 \pm 4.1	b	143.3 \pm 34.2	c
$S_{Pb} + FA$	15.1 \pm 8.0	b	19.3 \pm 6.7	b	107.5 \pm 37.8	c
$S_{Pb} + OA$	13.7 \pm 3.2	b	13.6 \pm 6.7	b	95.4 \pm 19.4	c
$S_{Pb} + OA + CA$	32.0 \pm 6.9	a	41.5 \pm 11.0	a	360.1 \pm 91.6	a
$S_{Pb} + OA + FA$	23.2 \pm 7.6	ab	26.9 \pm 15.1	ab	146.6 \pm 55.2	b

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



the 30 mg kg⁻¹ limit could be more accurate for *A. halimus* plants as some nonspecific symptoms of Pb toxicity reported in the literature were observed in plants of treatment S_{Pb} + OA + CA, such as fewer and more brittle leaves (Islam et al. 2007), a stunted growth and chlorosis (Ghori et al. 2019).

Dry weight of *Atriplex halimus* tissues and phytoremediation indexes

As shown in Table 5, the S_{Pb} + FA and S_{Pb} + OA + FA treatments showed significantly higher total DW levels (16.4 g and 18.2 g, respectively) compared to the rest, whose total DW ranged from 8.3 to 10.0 g. These differences are consistent with the mean DW values observed for leaf, stem, and root tissues, with significantly higher values being observed for the S_{Pb} + FA and S_{Pb} + OA + FA treatments in all cases. With respect to the *RS* index, it was observed that the S_{Pb} + OA + CA treatment presented a significantly higher value (0.50) compared to the rest. Although in terms of total DW no statistical differences were attributed to CA (with respect to S_{Pb}), the *RS* index for treatment S_{Pb} + OA + CA was significantly higher than the rest. Chiu et al. (2006) indicate that the *RS* index is a highly representative indicator of the environmental stress encountered by plants. According to Xia (2004), the *RS* index increases when plants are

stressed by hostile environments. In this assay, the higher *RS* value is consistent with a higher concentration of Pb in aerial and root tissues, probably due to higher Pb availability in soil and uptake by plants of treatment S_{Pb} + OA + CA.

Phytoremediation indexes (Table 6) showed low *Ti*, ranging from 0.10 to 0.24, while *BCF* and *PP* factors showed extremely low values, which is explained by the wide difference between the Pb concentrations observed in the aerial part of *A. halimus* plants and the initial soil Pb concentration. Regarding the effects of treatments on phytoremediation efficiency of *A. halimus*, a significant increase was noted for *BCF* and *PP* in treatments S_{Pb} + OA + CA and S_{Pb} + OA + FA, which could be attributed to chelating agents added in conjunction with OA. Low phytoremediation indexes are common for Pb; for example, in two-month hydroponic assays in which plants were exposed to increasing doses of Pb, it has been found that Pb accumulated mostly in roots, presenting low translocation rates to aerial tissues (*Ti* < 0.06) (Amer et al. 2013). This behavior has been confirmed for plants of the same genus in pot assays, since after 4 weeks of exposure to a soil highly contaminated with Pb (1333.5 mg kg⁻¹), the element was mostly accumulated in roots, presenting *Ti* values between 0.07 and 0.85 and a bioconcentration factor referred to roots between 0.54 and 2.32 (Kachout et al. 2012). However, longer evaluations periods need to be considered because, according to Lam

Table 5 Dry weight of *Atriplex halimus* tissues according to phytoremediation treatments (values are mean ± standard deviation, *n* = 3)

Treatment	Dry weight (g)					<i>RS</i>
	Leaves	Stem	Root	Total		
S _{Pb}	3.9 ± 0.7 b	3.6 ± 0.8 bc	2.1 ± 0.4 b	9.6 ± 1.8 b	0.28 ± 0.01 cd	
S _{Pb} + CA	2.9 ± 0.9 b	3.5 ± 0.7 bc	1.8 ± 0.2 b	8.3 ± 1.8 b	0.29 ± 0.04 bc	
S _{Pb} + FA	6.1 ± 0.7 a	7.0 ± 0.9 a	3.2 ± 0.2 a	16.4 ± 1.7 a	0.24 ± 0.02 d	
S _{Pb} + OA	3.1 ± 0.6 b	4.4 ± 0.6 b	2.5 ± 0.5 b	10.0 ± 1.6 b	0.33 ± 0.02 b	
S _{Pb} + OA + CA	3.4 ± 0.7 b	3.0 ± 0.3 c	3.2 ± 0.4 a	9.6 ± 1.3 b	0.50 ± 0.02 a	
S _{Pb} + OA + FA	6.5 ± 1.8 a	8.2 ± 1.0 a	3.6 ± 0.5 a	18.2 ± 3.0 a	0.25 ± 0.02 cd	

Different letters in column indicate significant differences between treatments, separately, according to the Fisher test at *p* ≤ 0.05. *RS* root-shoot dry weight ratio

Table 6 Phytoremediation indexes for *Atriplex halimus* according to phytoremediation treatments (values are mean ± standard deviation, *n* = 3)

Treatment	<i>Ti</i>	<i>BCF</i>	<i>PP</i>
	–	–	(%)
S _{Pb}	0.24 ± 0.06 a	0.0050 ± 0.0013 bc	0.0009 ± 0.0001 b
S _{Pb} + CA	0.15 ± 0.04 b	0.0069 ± 0.0006 bc	0.0011 ± 0.0003 b
S _{Pb} + FA	0.17 ± 0.05 ab	0.0059 ± 0.0013 bc	0.0020 ± 0.0005 b
S _{Pb} + OA	0.15 ± 0.05 b	0.0046 ± 0.0010 c	0.0009 ± 0.0002 ab
S _{Pb} + OA + CA	0.10 ± 0.03 b	0.0123 ± 0.0027 a	0.0020 ± 0.0006 ab
S _{Pb} + OA + FA	0.10 ± 0.04 b	0.0085 ± 0.0036 b	0.0032 ± 0.0019 a

Different letters in column indicate significant differences between treatments, separately, according to the Fisher test at *p* ≤ 0.05. *Ti* transport index to the aerial part, *BCF* bioconcentration factor, *PP* phytoextraction percentage

et al. (2017), plants belonging to the same genus (*Atriplex nummularia*) established for 11 months on mining tailings showed *Ti* values for Pb ranging from 0.96 to 2.74 and *BCF* values between 0.06 and 0.21, although the Pb concentration in mine tailings was lower (183 mg kg^{-1}) compared to the soil of this assay.

Effects of treatments on soil chemical properties

Lead uptake by plants is controlled by the characteristics of the soil, such as particle size, pH, and CEC (Pourrut et al. 2011; Seregin and Ivanov 2001), with soil pH being the most important factor influencing the mobility of TEs in soils, because it is directly related to the formation of soluble complexes and adsorption/precipitation phenomena (Adriano 2001; Alloway 2010). In this assay, the soil pH increased for all treatments, from 6.3 (initial characterization) to a range of 6.90–7.34, probably due to the well water irrigation utilized in this assay (pH 7.5). It should be noted that, despite the low pH values of the irrigation solutions with chelating agents, no acidification of the soil was observed in the treatments. The $S_{Pb} + FA$ and $S_{Pb} + OA + FA$ treatments showed significantly lower soil pH values at the end of the assay (Table 7) compared to the rest of the treatments, which showed slightly alkaline pH values without significant differences compared to the control treatment (S_{Pb}). At the end of this assay, the pH in soil treatments was similar, except for those in which FA were added through irrigation water, as a slightly acidifying effect was observed on these. This behavior can be attributed to the high amount of acidifying functional groups in FA, particularly carboxylic groups (Hooda 2010; Li et al. 2018). Differently, since simple organic acids such as CA are rapidly mineralized by soil microorganisms (Jones 2003; Römkens et al. 2002), these small changes in soil pH indicate that the acidifying effect of chelating agents is buffered by the soil and that the effect of irrigation water pH ultimately predominates. In similar assays, the effect of

chelating agents and that of pH on Pb mobility could not be differentiated (Tapia et al. 2013); in contrast, in the present assay, these effects were separated: although the pH of soils were similar in the S_{Pb} , $S_{Pb} + CA$, and $S_{Pb} + OA + CA$ treatments, the treatments that involved CA addition displayed higher concentrations of Pb in leaf, stem, and root tissues.

In relation to soil salinity, although the soil was already saline at the beginning of the assay (4.58 dS m^{-1}), a strong increase was observed in the EC after the application of treatments, showing extremely saline values in the range of $10\text{--}12 \text{ dS m}^{-1}$, except for the $S_{Pb} + OA + CA$ treatment, which showed a significantly lower value of 6.74 dS m^{-1} . Considering that the use of irrigation waters with an EC between 0.7 and 3.0 dS m^{-1} entails a moderate risk of soil salinization (Ayers and Westcot 1985), the irrigation applied in this assay may not have been enough to wash excess salts away, leading to high EC levels and a large increase in soil salinity: from 4.58 dS m^{-1} at the beginning of the assay to $10\text{--}12 \text{ dS m}^{-1}$ at the end of the assay. A high soil salinity can result in a competition of salt-derived cations with positively charged TEs for sorption sites on the solid phase and complexation capacity of salt-derived anions with TEs (Zhou et al. 2019). However, according Haghghi et al. (2016), an increase in soil salinity enhanced Pb absorption by plants, a behavior reported in the literature that can increase Pb phytostabilization (Nawaz et al. 2016; Yu-lin 2008). In this regard, halophytic plants such as *A. halimus* have mechanisms that allow them to tolerate high salinity conditions (ion compartmentalization and osmotic adjustment, among others) (Lokhande and Suprasanna 2012) and successfully grown in contaminated soils. This makes them useful for the phytoremediation of saline soils, as was verified in this assay.

According to the sequential extraction of Pb performed at the end of the assay, Pb was found to be mostly associated with the FII and FIII fractions (Pb bound to carbonates and iron-manganese oxides), at an average of 20% and 61%, respectively (Fig. 3). In general terms, Pb distribution changed for these fractions compared to measurements made at the beginning of the assay, where FII represented 42.01% and FIII 40.29%. These Pb distribution changes suggest that an increase in soil pH fostered the precipitation of hydroxides. With respect to the FI fraction, which represents Pb in available form (soluble and exchangeable), at the end of the assay it remained around or lower than 1% for all treatments, except for the $S_{Pb} + OA + FA$ treatment, which showed the highest Pb percentage for this fraction (2.30%). According to Sinegani et al. (2015), FA form strong complexes with Pb and a high concentration of dissolved organic carbon improves their mobility in the soil. In general, it has been mentioned that the interaction of dissolved organic carbon of low molecular weight (such as FA) with trace elements can foster mobilization processes by chelation (Antoniadis

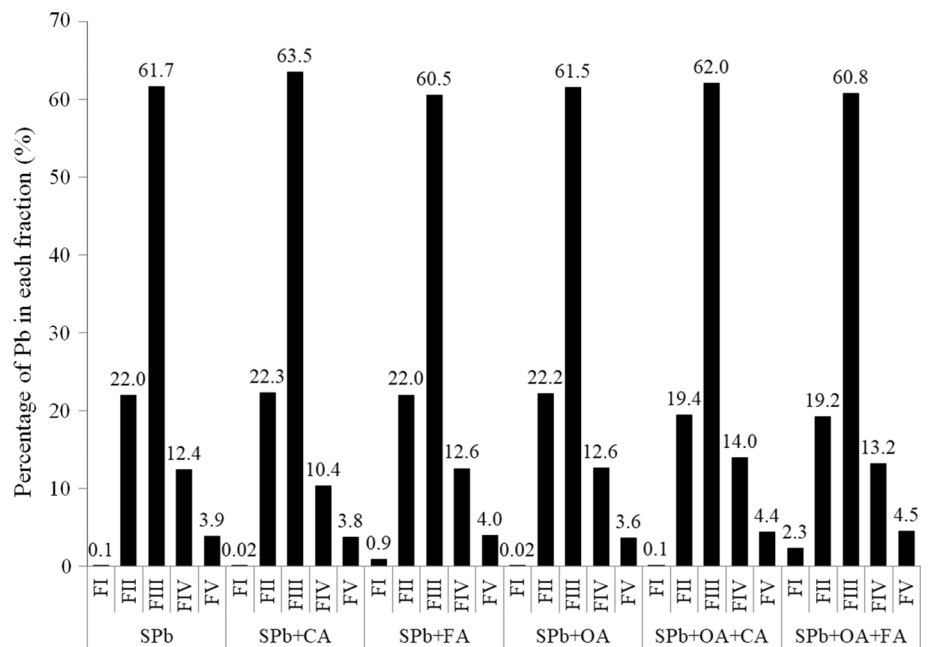
Table 7 pH and electrical conductivity of soil according phytoremediation treatments at the end of the assay (values are mean \pm standard deviation, $n=3$)

Treatment	pH		EC	
	–		(dS m^{-1})	
S_{Pb}	7.21 ± 0.06	a	11.43 ± 1.03	a
$S_{Pb} + CA$	7.28 ± 0.04	a	10.02 ± 0.96	a
$S_{Pb} + FA$	6.91 ± 0.15	b	11.17 ± 1.50	a
$S_{Pb} + OA$	7.32 ± 0.03	a	12.01 ± 1.81	a
$S_{Pb} + OA + CA$	7.34 ± 0.03	a	6.74 ± 0.68	b
$S_{Pb} + OA + FA$	6.90 ± 0.06	b	11.65 ± 0.46	a

Different letters in column indicate significant differences between treatments, separately, according to the Fisher test at $p \leq 0.05$. EC electrical conductivity



Fig. 3 Distribution of lead (%) in the different soil fractions according to treatments and sequential extraction performed at the end of the assay. FI, soluble and exchangeable; FII, bound to carbonates; FIII, bound to iron-manganese oxides; FIV, bound to organic matter; FV, residual. Values are means ($n = 3$)



and Alloway 2002). This was demonstrated by Brown et al. (1997), who attributed greater Pb mobilization in soils to complex formation with FA. In this regard, Hooda (2010) mentions that FA are soluble in acid and alkaline media and improve the mobility of TEs.

Ponizovsky and Mironenko (2001) reported for weakly acidic soils, values for the FI fraction range from 0 to 1%, which is in line with the well-known fact that only a small proportion of the total Pb is available (Pourrut et al. 2011; Saifullah et al. 2009). In this assay, higher percentages of Pb found in FI fraction for $S_{Pb} + FA$ (0.87%) and $S_{Pb} + OA + FA$ (2.29%) treatments indicates that the effect of FA on Pb availability was improved by the OA. Considering surface runoff and ground leaching from soil is one of the major routes of transport of soil TEs to water bodies (Sahu and Basti 2020), a special concern should be taken with the use of chelating agents to increase Pb available in soils. However, low molecular weight organic acids (such as CA and FA used in this assay) form complexes of low to moderate stability, and due its easy biodegradability in soil (Sarwar et al. 2017), a minimum environmental risk is expected.

Effects of treatments on soil physical properties

Organic amendments produce beneficial changes in the physical properties of the soil, improving its structure, aeration, and water retention (Murphy 2015; Diacono and Montemurro 2010). Although chelating agents are commonly used in assisted phytoremediation, there is insufficient

information about their effect on soil physical properties. In this assay, the treatments differed significantly in all soil physical properties analyzed (Table 8).

Bulk density is not an intrinsic soil property, because it depends on external conditions and various natural and artificial processes (Dexter 1987). In this assay, soil bulk density was significantly higher in the $S_{Pb} + CA$ and $S_{Pb} + OA + CA$ treatments (1.65–1.67 $Mg\ m^{-3}$), and the CA effect on soil bulk density was a phenomenon that may have been strengthened by the high root DW found for treatment $S_{Pb} + OA + CA$ (see Table 5). In this regard, Guidi et al. (1985) have mentioned that root growth causes compaction of the surrounding soil against the root apex, which increases bulk density and reorganizes the distribution and continuity of the soil pores. Furthermore, it has been observed that the flow of a CA solution in high concentrations decreases hydraulic conductivity due to peptization phenomena (Mizoguchi et al. 2006), which have been described by Kedziorek and Bourg (2000) as the obstruction of the porous soil medium resulting from the formation of colloids and their movement in depth. The increase in soil bulk density caused by the addition of CA may have a positive effect on soil physical properties, because greater micro-aggregate stability was observed when CA was added with or without applying OA, compared to the S_{Pb} and $S_{Pb} + OA$ treatments. With respect to the OA, the S_{Pb} and $S_{Pb} + OA$ treatments showed the typical beneficial effect of adding organic matter to soil, exhibiting lower soil bulk density values for $S_{Pb} + OA$ (1.43 $Mg\ m^{-3}$) and differing significantly from the rest of treatments, except for $S_{Pb} + OA + FA$. Moreover, the



Table 8 Physical properties in the soils according to treatments at the end of the assay (values are mean \pm standard deviation, $n=3$)

Treatment	Bulk density (Mg m ⁻³)	FC (%)	PWP (%)	AWC (%)	DR (%)					
S _{Pb}	1.56 \pm 0.07	b	15.3 \pm 0.8	b	10.5 \pm 0.2	bc	4.8 \pm 0.7	b	80.2 \pm 5.1	a
S _{Pb} + CA	1.65 \pm 0.11	ab	14.9 \pm 0.3	bc	10.0 \pm 0.2	c	4.9 \pm 0.5	ab	68.2 \pm 4.8	b
S _{Pb} + FA	1.52 \pm 0.05	b	13.0 \pm 0.1	d	10.2 \pm 0.4	bc	2.8 \pm 0.5	c	41.0 \pm 3.4	c
S _{Pb} + OA	1.43 \pm 0.09	c	15.9 \pm 1.0	ab	10.7 \pm 0.3	b	5.1 \pm 1.0	ab	79.3 \pm 0.8	a
S _{Pb} + OA + CA	1.67 \pm 0.04	a	16.7 \pm 0.2	a	10.5 \pm 0.4	bc	6.1 \pm 0.4	a	63.3 \pm 5.8	b
S _{Pb} + OA + FA	1.45 \pm 0.12	bc	13.7 \pm 1.2	cd	11.5 \pm 0.2	a	2.1 \pm 1.2	c	42.5 \pm 4.6	c

Different letters in column indicate significant differences between treatments, separately, according to the Fisher test at $p \leq 0.05$. FC field capacity as gravimetric water content at 33 kPa, PWP permanent wilting point as gravimetric water content at 1500 kPa, AWC available water content, RD dispersion ratio

consecutive application of FA through the irrigation water produced a similar effect to that observed in the S_{Pb} + FA and S_{Pb} + OA + FA treatments.

Microaggregate stability was significantly higher (lower RD values) for treatments which consider the addition of chelating agents compared to treatments S_{Pb} and S_{Pb} + OA, while the highest microaggregate stability was observed for the treatments which consider FA addition. Blankinship et al. (2016) have mentioned that stable aggregates are beneficial for several reasons, including carbon sequestration, nutrient retention, and erosion control. However, despite these apparent benefits, in this assay was observed a decreased AWC in the treatments where FA were added, which can be attributed to particle reordering and the clogging of the soil pores that contribute to water retention. In this regard, a significantly lower AWC was observed for treatment S_{Pb} + OA + FA, with 56% less AWC than treatment S_{Pb}. A similar result was observed for treatment S_{Pb} + FA, which yielded 42% less AWC, while treatment S_{Pb} + OA + CA showed a 27% higher AWC value than treatment S_{Pb}. In contrast, the addition of only OA at the beginning of the assay (S_{Pb} + OA) did not differ significantly from treatment S_{Pb} in terms of AWC. These phenomena have been reported for other soil amendments, such as polyacrylamide solutions, which clog soil pores due to their viscosity while also increasing aggregate stabilization (Lee et al. 2015), as well as for sandy soils amended with biochar, where saturated hydraulic conductivity is reduced due to the clogging of pores or structural collapse (Obia et al. 2017). These effects were not observed for treatments S_{Pb} + CA and S_{Pb} + OA + CA, which could be due to the rapid mineralization of CA in the soil.

Conclusion

The continuous application of chelating agents through irrigation water promoted higher Pb concentrations in *A. halimus* tissues, an effect that was enhanced by the prior application of an organic amendment. While the application of fulvic acid led to higher plant dry weight, citric acid addition with an organic amendment promoted higher Pb concentrations in aerial tissues, though some visual toxicity symptoms were observed. Regarding Pb fractionation in the soil at the end of the assay, the addition of fulvic acids was associated with a greater proportion of Pb in the available fraction of the soil. The application of fulvic acids increased soil stability and decreased soil bulk density. However, a reduction in available water content in soil was observed. The use of citric acid produced an increase in soil bulk density; also, moderately higher microaggregate stability was observed compared to the control treatment.

Preferential Pb accumulation in root tissues was observed, indicating that *A. halimus* is a suitable species for the phytostabilization of highly Pb contaminated soils. Though the management approach proposed could be an effective way of promoting greater Pb in aerial tissues, low values for *Ti*, *BCF*, and *PP* were determined and Pb phytoextraction capacity of *A. halimus* is limited.

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