



The use of a halophytic plant species and organic amendments for the remediation of a trace elements-contaminated soil under semi-arid conditions

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ABSTRACT

The halophytic shrub *Atriplex halimus* L. was used in a field phytoremediation experiment in a semi-arid area highly contaminated by trace elements (As, Cd, Cu, Mn, Pb and Zn) within the Sierra Minera of La Unión-Cartagena (SE Spain). The effects of compost and pig slurry on soil conditions and plant growth were determined. The amendments (particularly compost) only slightly affected trace element concentrations in soil pore water or their availability to the plants, increased soil nutrient and organic matter levels and favoured the development of a sustainable soil microbial biomass (effects that were enhanced by the presence of *A. halimus*) as well as, especially for slurry, increasing *A. halimus* biomass and ground cover. With regard to the minimisation of trace elements concentrations in the above-ground plant parts, the effectiveness of both amendments was greatest 12–16 months after their incorporation. The findings demonstrate the potential of *A. halimus*, particularly in combination with an organic amendment, for the challenging task of the phytostabilisation of contaminated soils in (semi-)arid areas and suggest the need for an ecotoxicological evaluation of the remediated soils. However, the ability of *A. halimus* to accumulate Zn and Cd in the shoot may limit its use to moderately-contaminated sites.

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1. Introduction

In the region of Murcia (SE Spain), the area of land contaminated with trace elements (TEs), predominantly cadmium (Cd), copper (Cu), lead (Pb) and zinc (Zn), by mining activity in the Sierra Minera of La Unión-Cartagena, and the subsequent dispersal of the contaminants, exceeds 40 km² [1]. Elevated concentrations of As can be found as well in most of the soils of this former mining area [1–3]. Erosion of soils *via* wind and water is a phenomenon which affects areas such as this which have hot, dry summers and occasional heavy storms [4,5]. The establishment of a vegetation cover at the contaminated sites would physically stabilise the soils (phytostabilisation) and minimise the erosion and thus the dissemination of the contaminants [6].

The phytoremediation of contaminated sites under high-rainfall (>800 mm per year) conditions has been investigated greatly [7,8]. However, phytoremediation of contaminated, (semi-)arid sites requires species tolerant not only of poor soil conditions (elevated

TE concentrations, poor structure and nutrient availability and often salinity) but also of seasonal drought and high temperatures. Hence, halophytes have advantages due to their tolerance of heat, drought and soil salinity that may limit the appropriate growth of other plants in mining soils [6]. One example is the genus *Atriplex* (Amaranthaceae), several species of which have been investigated for this purpose around the world, with promising results [5,9–11]. *A. halimus* L. (Mediterranean Salt-bush) is a perennial halophytic shrub which grows throughout the Mediterranean basin in areas of low annual rainfall (R) and high potential evapotranspiration (PET), many of which can be classified as arid (R = 100–400 mm, R:PET = 0.06–0.28) or semi-arid (R = 400–600 mm and R:PET = 0.28–0.45) [12]. This species, used for stabilisation of soils against erosive forces since its deep root system reinforces the soil [4,12], has colonised some TE-contaminated sites in the Sierra Minera [13,14]. Previous work with *A. halimus*, with plants grown in pots of soil [15–17], in “spiked” compost substrate [18] or in hydroponic culture [14,19], showed that it is relatively tolerant of high concentrations of Cd, Cu, Pb and Zn in the external medium, in terms of growth. In the studies of Lutts et al. [14] and Tapia et al. [18], where TE availability to plants was much higher than in an aged, contaminated soil and the level of only a single TE was high (eliminating interactions amongst TEs), the shoot concentrations of TEs led the authors to suggest that *A. halimus* could be used for their phytoextraction from contaminated

Abbreviations: B_C, microbial biomass C; B_N, microbial biomass N; CM, compost; DOC, dissolved organic C; HL, hydrated lime; PET, potential evapotranspiration; PS, pig slurry; R, rainfall.

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soil, although Pérez-Esteban et al. [17] did not consider this as a viable proposition. Since the aim of our work is to restore the conditions of a contaminated site to something approaching those of the surrounding, non-contaminated soils (calcareous, pH > 7, low TE solubility and mobility), phytostabilisation, rather than phytoextraction, is a more-realistic remediation technique.

In Southern Spain, residues from the olive-oil extraction process and pig slurry are produced in vast amounts. One way of recycling them is as soil amendments for contaminated sites, as these sites often require the addition of organic matter (OM) and plant nutrients to improve fertility and plant growth conditions in the soil [20]. Employing these “waste” materials for this purpose represents an environmentally-beneficial way of recycling them. The organic amendments may affect the bioavailability of TEs in soils, and this will depend on the particular element, soil type and the characteristics of the amendments [20,21]. The beneficial effects of the application of these organic materials to soils from the Sierra Minera on soil microbial biomass and plant germination and growth have been recently reported from glasshouse and laboratory experiments [16,22]. However, field-scale work is required to test their suitability for phytoremediation.

The purpose of this study was to determine the ability of *A. halimus* to survive and grow at a site highly contaminated by TEs (As, Cd, Cu, Mn, Pb and Zn) and to influence contaminant availability in the soil (phytoimmobilisation). The phytostabilisation of this site is needed to prevent dissemination of contaminants by run-off water and wind erosion. The effects of pig slurry and composted olive-mill waste, and specifically of the organic matter in these materials through comparison with control soil receiving only lime, on the soil conditions, microbial population, soil solution (through *in situ* pore water extraction) and hence on the growth of *A. halimus*, were followed over a 2-year period. This is, to the best of our knowledge, the first report on the use of this species in a field phytoremediation experiment under semi-arid conditions.

2. Materials and methods

2.1. Experimental design: site and amendments

The experimental site was located in a mine spoil soil close to the village of El Llano del Beal (Cartagena, SE Spain; 37°37'12.40" N, 0°50'13.51" W) that can be considered as a sterile deposit of mine tailings with no edaphic development and completely bare of vegetation (Supporting Information, SI Fig. A1). The soil is a loamy sand with 5.2% clay, 15.3% silt and 79.5% sand, 2.1% CaCO₃, 0.2% OM and slightly acidic (pH 6.2). Pseudo-total As, Pb and Zn concentrations in this soil (Table 1) clearly exceed background concentrations estimated for soils from this area (As: 12–16 mg kg⁻¹, Pb: 43–57 mg kg⁻¹, Zn: 90–96 mg kg⁻¹ [23]). According to the Spanish legislation, this soil needs recovery measures (concentrations > 100 times generic reference levels [24]). Data from a meteorological station located 15 km from the site show that the average R and R:PET for the period 2000–2010 were 382 mm and 0.314, respectively. During the assay, the maximum and minimum temperatures were 35.9 °C and 0.8 °C, respectively, with a mean annual rainfall of 431 mm.

Two organic materials and an inorganic control with liming were used as soil amendments: mature compost prepared from solid olive mill waste, fresh pig slurry and hydrated lime. The compost is alkaline, has low electrical conductivity (EC), is rich in nutrients and OM (Table 1) and has a high degree of humification. The pig slurry was collected from a farm situated in Tallante (Cartagena, SE Spain). This material is slightly alkaline, with high EC, low OM content (Table 1) and rich in NH₄⁺-N (3.15 g kg⁻¹). Trace element concentrations in these residues were below the

Table 1

Characteristics of the soil and the organic amendments used in the experiment. EC: electrical conductivity, OM: organic matter, TOC: total organic carbon.

Characteristics	Soil	Compost	Pig slurry ^a
pH	6.2 ± 0.1	8.8 ± 0.01	7.8 ± 0.01
EC (dS m ⁻¹)	2.7 ± 0.01	6.1 ± 0.2	24.3 ± 0.01
OM (%)	0.2 ± 0.01	73.1 ± 0.3	1.05 ± 0.07
TOC (g kg ⁻¹)	1.3 ± 0.1	438.6 ± 7.9	7.9 ± 0.04
Total-N (g kg ⁻¹)	0.4 ± 0.05	31.7 ± 0.8	3.9 ± 0.01
Total-P (g kg ⁻¹)	0.07 ± 0.04 ^b	4.9 ± 0.1	0.5 ± 0.1
Available-As (mg kg ⁻¹)	0.39 ± 0.01	–	–
As (mg kg ⁻¹)	664 ± 28	<0.01	<0.01
Cd (mg kg ⁻¹)	19 ± 1	<0.01	<0.01
Cu (mg kg ⁻¹)	193 ± 8	48 ± 0.5	12 ± 0.4
Fe (g kg ⁻¹)	108 ± 1	1.62 ± 0.1	0.045 ± 0.004
Mn (mg kg ⁻¹)	4073 ± 368	92 ± 1	11 ± 1
Pb (mg kg ⁻¹)	10188 ± 97	36 ± 2	0.9 ± 0.2
Zn (mg kg ⁻¹)	9686 ± 251	141 ± 4	116 ± 2

^a Expressed on a fresh weight basis (moisture = 97.4%); –: not analysed.

^b Available-P (NaHCO₃-extractable).

limits established for agricultural use of compost and fertiliser products made from wastes in Spain (Cd < 0.7, Cu < 70, Pb < 45, Zn < 200 mg kg⁻¹ [24]). The hydrated lime (Ca(OH)₂ from limestone calcinations) used was acquired commercially (Cales Pascual S.L.).

The two organic treatments and the inorganic control, in a fully-randomised design with four replicates per treatment, were set up in experimental plots (2 m²) with 0.5 m between plots. The treatments were: hydrated lime as a control non-organic treatment (HL, applied at a rate of 2.4 t ha⁻¹), compost (CM, 60 t ha⁻¹) and pig slurry (PS, 60 m³ ha⁻¹). Untreated control plots were not considered, as plant growth in this soil is negligible if the soil pH and/or organic matter content are not raised [16]. The different amendments were added to the soil in one single dose with no further addition intended. The doses of compost and slurry were chosen in order to add similar amounts of plant-available N (inorganic and potentially mineralisable, calculated according to Bernal et al. [25]): 76 and 79 μg g⁻¹ soil, respectively. The dose of HL was calculated to increase soil pH to a similar value as the organic (alkaline) amendments. Compost was added a month before transplanting, whilst pig slurry and lime were added 17 days before. Each plot was divided into two sub-plots, one to be planted with *A. halimus* and the other to be left without plants. A composite sample consisting of two subsamples was taken from the surface 0–20 cm from each subplot in the different soil samplings.

Soil pore water was collected *in situ* from the plots at a depth of 15–20 cm, using FLEX type ‘Rhizon’ samplers (Eijkelkamp Agrisearch Equipment, The Netherlands [26,27]).

2.2. Plant material

Seeds of *A. halimus* obtained from plants growing at another contaminated site in the Sierra Minera [14] were sown in trays containing perlite, in a growth chamber. They were grown for 9 weeks in the chamber and then for 3 weeks outside before being transplanted in the field: 20 plants per sub-plot of 1 m². All sub-plots (with and without plants) were watered (4 l m⁻²) three times with tap water: at transplanting and one and two months later. Samples of young, fully-grown leaves were taken from the middle part of the plants after 4, 16 and 24 months. Samples of mature fruits were taken after 12 and 24 months. Leaves and fruits were rinsed with deionised water, washed with 0.1% Triton for 1 min to remove surface depositions and then rinsed four times with deionised water. They were oven-dried (65 °C, 3 d), milled to a fine powder and 250 mg samples were digested at 210 °C with a 1:1

mixture of nitric and perchloric acids. For each set of samples, a reference plant material (Bowen's Kale [28]) was digested also.

Four and 12 months after transplanting, the percentage plant ground cover for each sub-plot was estimated by ImageJ software (downloaded from <http://rsb.info.nih.gov/ij/>, 7 May 2009) processing of photographs taken 2 m above the soil surface. Above-ground biomass production was estimated after 16 months by harvesting three "typical" plants per sub-plot and measuring the oven-dry weight (65 °C, 3 d), which was scaled-up according to the number of surviving plants per sub-plot.

The timing of the experiment was as follows:

- November 2008: Addition of soil organic amendments;
- December 2008: transplanting of *A. halimus*;
- April 2009: Sampling 1 (soil, plant leaves, plant cover);
- December 2009: Sampling 2 (soil, plant fruits, plant cover);
- April 2010: Sampling 3 (soil, plant leaves, biomass, soil pore water);
- December 2010: Sampling 4 (soil, plant leaves and fruits, soil pore water).

2.3. Analytical methods

The physico-chemical characteristics of the soils and the organic amendments were determined according to the methods described in Pardo et al. [22]. Soil extractable TE concentrations (0.1 M CaCl₂, 1:10 w/v) were determined by AAS. Soil available-As was determined in a 0.5 M NaHCO₃ extract (1:10 w/v) by hydride generation atomic fluorescence spectrometry (PSAnalytical Millennium Excalibur, UK). The concentration of available-K was extracted with 10 mM NaNO₃ (1:5 w/v) and measured by ICP-OES. All the analyses were done at least in duplicate and adjusted to values for oven-dried soil (105 °C for 24 h). Analytical errors (standard error) were below 2%.

The concentrations of the TEs (As, Cd, Cu, Fe, Mn, Pb and Zn) and nutrients (Ca, K, Mg, Na and P) in the acid-digested leaves and fruit of *A. halimus* and in the reference plant material were determined by ICP-OES (model Varian Vista-MPX; Varian Ltd., Murgate, Australia). For the reference plant material, the recoveries were 80–105%. The leaf N concentration was determined by an automatic microanalyser (Euro EA; EuroVector, Milan, Italy).

Trace element concentrations in the soil pore water were determined by ICP-OES (Thermo Scientific), and dissolved organic-C (DOC) and N were determined in the TOC-V analyzer (Shimadzu).

2.4. Statistical analyses

Using SPSS Version 19.0 software (SPSS Inc.), simple correlations between the different variables were performed. Data were also subjected to ANOVA and differences between means were determined using Tukey's test. The normality of the data and the variance homogeneity were tested by the Kolmogorov–Smirnov and Levene tests, respectively, before the ANOVA: values were logarithm- or arcsin-transformed when necessary, to satisfy these tests.

3. Results

3.1. Soils

Certain heterogeneity of pH values in the soil was initially observed, even between sub-plots receiving the same treatment, and some plots acidified throughout the experiment. The most acidic (<6) subplots for each treatment were statistically classified as outliers (extreme values) and those data were not considered for further calculations. Soil pH remained around neutral throughout

the experiment, with slightly lower values in CM treated plots (statistically significant in the second sampling; Fig. 1), and no significant effects of the presence of plants or time. Soil salinity was very high in all samplings (EC > 2.5 dS m⁻¹ in 1:5 (w/v) water extracts, equivalent to 17.8 dS m⁻¹ in saturated paste [29]) with no significant effects of the treatments (data not shown).

The CaCl₂ extractable metal concentrations remained relatively low throughout the experiment; Zn and Cd concentrations did not show significant differences between treatments, whereas Mn (in the 1st and 2nd sampling) and Pb (in the 2nd and 4th samplings) extractable concentrations in CM were significantly higher than in HL or PS treated plots (Fig. 1). However, the concentrations of those elements (Cd, Mn, Pb and Zn) correlated negatively with soil pH in all the samplings (data not shown), which may explain the elevated extractable metal concentrations found in CM plots. No significant effects were found for plants or time, and treatment × plant (T × P) significant effects were only found for Zn and Mn in the first sampling and for Pb in the last (SI Table A1).

Soil OM and TOC concentrations were significantly higher in plots with compost than in HL or PS amended plots in all the samplings (Fig. 2). No significant effects on soil TOC of the presence of the plants or time were observed, although significant differences were found for the interaction T × P in every sampling, which indicate a different plant effect for each treatment. The organic-C turnover, affected by the high OM-mineralisation rate under warm climates and by the leaf loss during hot summers, may be responsible for such changes. For the amended soils TOC and TN decreased from samplings 1 to 3 before increasing (Fig. 2); also, the available K was increased in the presence of plants in the last sampling ($p < 0.05$). This suggests that the fading of the amendment effect was compensated by the incorporation of plant matter (leaf litter and roots) as the plants grew.

Soil microbial biomass-C (B_C) and -N (B_N) concentrations were highest in CM treated soils and lowest in HL plots (Fig. 2), with significant T × P effects in almost every sampling; the presence of *A. halimus* augmented the biomass-C and -N from the second sampling onwards. Available P (NaHCO₃-extractable) was only measured at the last sampling: it increased in the order HL (0.21 ± 0.04) ≈ PS (0.86 ± 0.12) < CM (1.49 ± 0.39) (mg kg⁻¹, mean ± standard error; $p < 0.001$) and was significantly correlated with leaf P ($r = 0.688$, $p < 0.05$). Available As concentrations increased slightly in CM plots (samplings 1 and 3), although they were still quite low (0.8–1.9 mg kg⁻¹) and did not change significantly with time (Fig. 2). In samplings 1–3, significantly higher NaNO₃-extractable K concentrations were shown in soils receiving CM (up to 5–10 fold those in HL treatment), with significantly higher concentrations in planted subplots ($p < 0.01$) and significant T × P ($p < 0.01$) effects in the last sampling.

3.2. Pore water

Soil pore water pH varied between 5.8 and 7.3 (considering all treatments) in the 1st sampling and decreased significantly in the 2nd one, then remaining almost constant until the last sampling (Table 2). It was significantly higher in PS treated plots in the 1st and 3rd samplings ($p < 0.01$ and 0.05, respectively); significant T × P effects ($p < 0.05$) were found in the 1st sampling too, but no further significant effects (plants, time, time × treatment × plant (t × T × P)) were found.

Dissolved organic C (DOC) in pore water was significantly higher in non-planted sub-plots compared to their corresponding planted ones in the first sampling, and significant T × P effects were shown in the 1st and 2nd samplings (Table 2). The presence of plants had also a significant effect regarding total N (TN) in pore water in the first sampling, with higher concentrations in samples from non-planted than from planted plots in all treatments ($p < 0.01$),

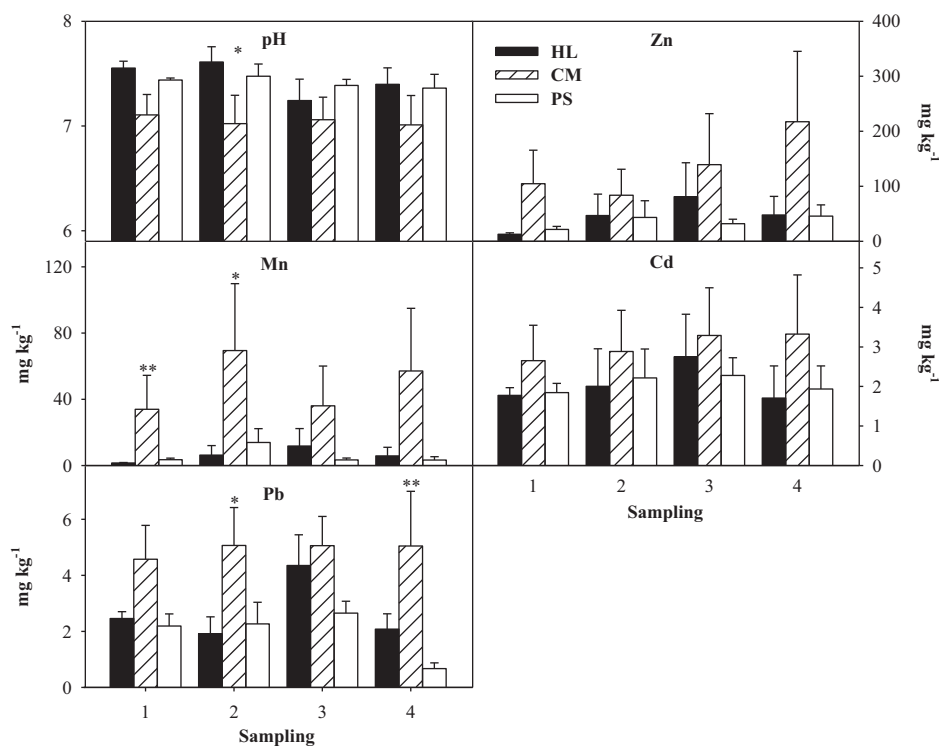


Fig. 1. Distribution of pH values and CaCl₂-extractable metal (Zn, Mn, Cd, and Pb) concentrations in the soils from the different treated plots in the different samplings ($n = 7$ or 8). * and ** indicate $p < 0.05$ and 0.01 , respectively. Bars indicate standard errors. Samplings 1–4 were performed 4, 12, 16 and 24 months, respectively, after transplanting.

indicating plant N uptake. Significantly higher ($p < 0.01$) TN concentrations were found in HL than in CM and PS soil pore water in the 4th sampling ($p < 0.01$), together with significant $T \times P$ ($p < 0.05$) effects (Table 2).

Copper concentrations in pore water were mostly below the detection limit of ICP-OES (0.01 mg l^{-1}). The concentrations of Cd and Pb in pore water were generally low ($< 2 \text{ mg l}^{-1}$; Table 2) and did not show any significant effect of the treatments. Zinc and Mn

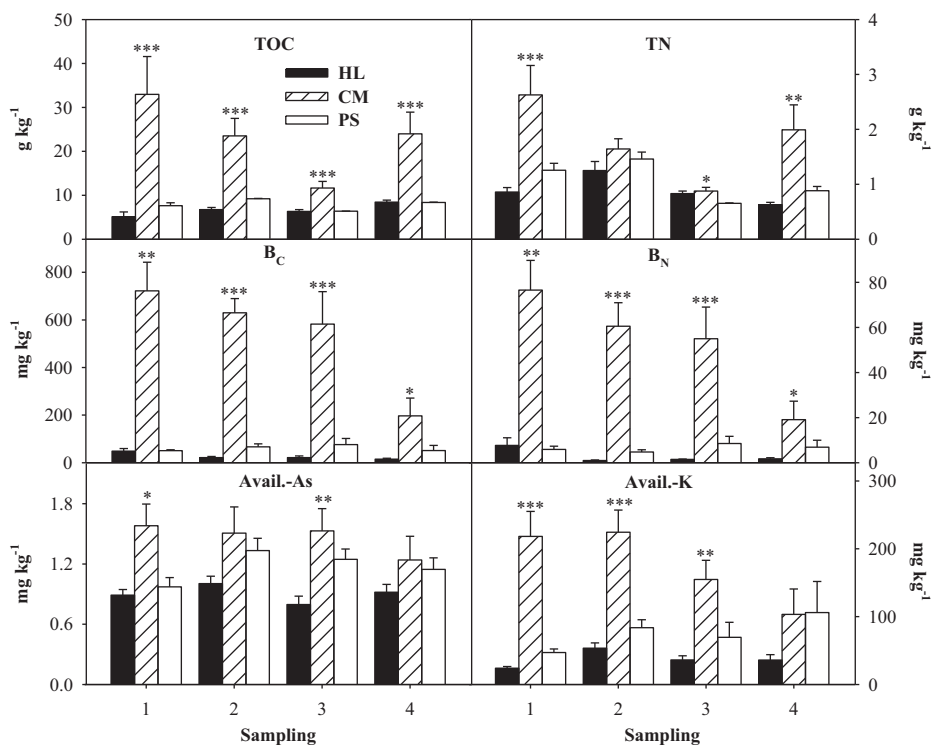


Fig. 2. Total organic-C (TOC), total-N (TN), microbial biomass-C (B_C) and -N (B_N), and available As and K concentrations in the soils with the different treatments in the 4 samplings (see legend of Fig. 1) ($n = 7$ or 8, except B_{C,N}, $n = 4$). *, ** and *** indicate $p < 0.05$, 0.01 and 0.001 , respectively. Bars indicate standard errors.

Table 2

The pH, dissolved organic C (DOC) and trace element concentrations (mg l^{-1}) in soil pore water ($n=4$). In the ANOVA, T= treatment; P= presence of plants (*A. halimus*) and t = time.

		pH	DOC	TN	Pb	Zn	Mn	Cd
Sampling 1	HL no plant	6.6±0.3ab	7.7±0.3abc	6.2±2.6a	0.55±0.00	26.3±17.0ab	6.09±4.03ab	0.06±0.00
	HL plant	6.0±0.5b	3.8±0.6bc	2.5±0.5ab	0.39±0.15	117.9±62.4a	16.48±10.19a	0.56±0.17
	CM no plant	6.9±0.2ab	14.9±9.5ab	2.1±1.1ab	0.01±0.00	22.1±14.2ab	0.11±0.04ab	0.05±0.02
	CM plant	6.7±0.3ab	3.4±0.4c	1.4±0.3ab	0.20±0.08	40.9±21.2ab	3.04±2.11a	0.07±0.03
	PS no plant	7.1±0.1ab	16.0±9.0a	3.4±1.0ab	<0.01	1.3±0.6b	0.07±0.02ab	<0.01
	PS plant	7.3±0.0a	8.4±1.4abc	1.2±0.2b	<0.01	0.7±0.4b	0.02±0.01b	<0.01
ANOVA	Treatment	**	NS	NS	NS	**	**	NS
	Plant	NS	**	**	NS	NS	NS	NS
	T × P	*	*	*	NS	**	**	NS
Sampling 2	HL no plant	6.1±0.6	11.2±1.3bc	8.2±3.5	0.36±0.32	56.6±36.2	8.40±4.58a	0.17±0.12
	HL plant	6.0±0.6	8.1±0.8c	3.6±1.2	0.53±0.49	57.0±31.2	3.53±2.50a	0.25±0.15
	CM no plant	7.0±0.1	54.4±13.5a	2.9±0.5	<0.01	2.0±0.1	0.13±0.03ab	<0.01
	CM plant	6.1±0.5	14.2±3.5abc	3.6±0.4	0.53±0.36	116.1±80.6	14.91±11.24a	0.33±0.23
	PS no plant	6.9±0.1	7.9±1.0c	4.5±1.2	0.01±0.00	1.9±0.4	0.04±0.01b	0.01±0.01
	PS plant	6.5±0.6	60.4±40.4ab	2.7±0.7	<0.01	4.0±2.3	0.26±0.21b	<0.01
ANOVA	Treatment	NS	NS	NS	NS	*	*	NS
	Plant	NS	NS	NS	NS	NS	NS	NS
	T × P	NS	**	NS	NS	NS	*	NS
Sampling 3	HL no plant	6.2±0.5	12.0±2.9	3.5±1.4	0.2±0.2	37.5±26.9	3.7±2.6	0.1±0.1b
	HL plant	6.0±0.4	9.3±0.7	3.5±1.9	0.4±0.3	129.3±56.0	16.6±9.1	0.5±0.2ab
	CM no plant	6.4±0.5	16.4±0.0	0.7±0.0	0.1±0.1	64.6±41.8	7.3±3.1	0.2±0.1ab
	CM plant	6.3±0.5	11.0±0.6	1.6±0.6	0.3±0.2	101.7±62.3	11.5±8.8	0.4±0.2a
	PS no plant	7.1±0.0	15.8±0.5	10.4±3.1	<0.01	0.9±0.1	<0.01	<0.01
	PS plant	7.2±0.1	13.0±0.4	1.7±0.6	<0.01	2.3±1.1	0.1±0.0	<0.01
ANOVA	Treatment	*	NS	NS	NS	NS	NS	NS
	Plant	NS	NS	NS	NS	NS	NS	*
	T × P	NS	NS	NS	NS	NS	NS	*
Sampling 4	HL no plant	6.7±0.4	10.4±0.5	8.1±3.9a	0.11±0.11	18.8±16.6	0.97±0.86	0.1±0.1
	HL plant	5.8±0.5	13.4±2.2	4.0±0.7ab	0.43±0.29	138.4±97.4	15.38±15.30	0.4±0.3
	CM no plant	6.5±0.3	17.2±3.2	1.4±0.8b	0.23±0.16	75.0±52.9	3.55±3.22	0.3±0.2
	CM plant	6.3±0.5	15.0±2.3	1.7±0.9ab	0.22±0.13	70.8±47.2	4.12±3.12	0.2±0.2
	PS no plant	6.5±0.2	16.5±0.1	1.2±0.1b	0.03±0.02	1.1±0.4	0.01±0.00	<0.01
	PS plant	7.2±0.0	10.2±0.1	1.2±0.1b	<0.01	0.5±0.4	<0.01	<0.01
ANOVA	Treatment	NS	NS	**	NS	*	NS	NS
	Plant	NS	NS	NS	NS	NS	NS	NS
	T × P	NS	NS	*	NS	NS	NS	NS
ANOVA	Time	NS	NS	NS	NS	NS	NS	NS
	$t \times T \times P$	NS	***	**	NS	NS	NS	**

***, **, *: $p < 0.001$, 0.01, 0.05, respectively. NS: not significant. Values denoted by the same letter in a column for each sampling do not differ significantly according to Tukey's test at $p < 0.05$.

concentrations were significantly ($p < 0.01$) higher in HL and CM treated plots than in PS treated ones in the 1st sampling, and both showed also significant ($p < 0.05$) $T \times P$ effects. These two elements presented some instances of elevated concentrations in pore water (up to 400 mg l^{-1} Zn and 50 mg l^{-1} Mn) (Table 2) and, as formerly reported for 'in situ' field collected pore water, rather high variability [27]. Pore water metal (Zn, Mn, Cd) concentrations negatively correlated soil pH and positively correlated their corresponding CaCl_2 -extractable concentrations (data not shown).

3.3. Plants

Survival of *A. halimus* was improved by the organic amendments, the mean values being 59, 79 and 89%, respectively, in the HL, CM and PS plots one year after transplanting. The stimulation of growth by the organic amendments is reflected in the data for ground cover (1, 13 and 17% ($p < 0.001$), and 11, 79 and 90% ($p < 0.01$) for HL, CM and PS plots in the first and second sampling, respectively) and in the above-ground dry biomass production (taking into account the number of plants, it was 165, 811 and 1580 g per sub-plot for the HL, CM and PS treatments, respectively) 16 months after transplanting.

The addition of the amendments did not cause significant increases in the concentrations of K or Na in plant leaves in the different samplings (Fig. 3). Only N concentrations in PS plots in the 1st sampling and P concentrations in CM plots in all the samplings were significantly higher than for HL (Fig. 3). Both N and P concentrations significantly ($p < 0.001$) decreased with time and showed

significant ($p < 0.001$) time \times treatment ($t \times T$) effects. The leaf concentrations of Cd, Mn and Zn significantly ($p < 0.001$) increased with time, whereas those of Cu, Fe, As and Pb ($p < 0.001$) decreased throughout the experiment (Fig. 4). Concentrations of As and Cu in plant leaves positively correlated TOC in soils in the first sampling ($p < 0.05$), whilst those of Zn and Cd negatively correlated soil pH ($p < 0.01$). Arsenic and Fe concentrations in the fruits harvested 12

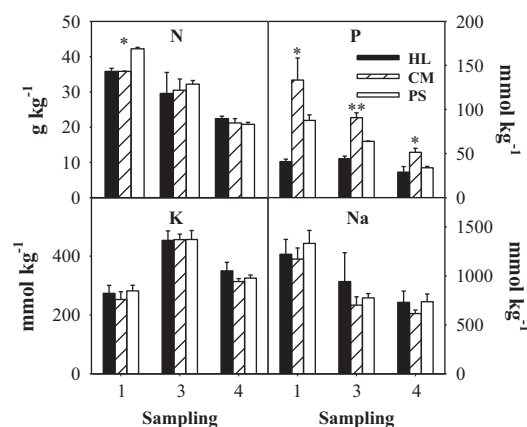


Fig. 3. Nutrient concentrations in plant leaves ($n=3$) from the different treated plots in the three samplings (see legend of Fig. 1). * and ** indicate $p < 0.05$ and 0.01, respectively. Bars indicate standard errors.

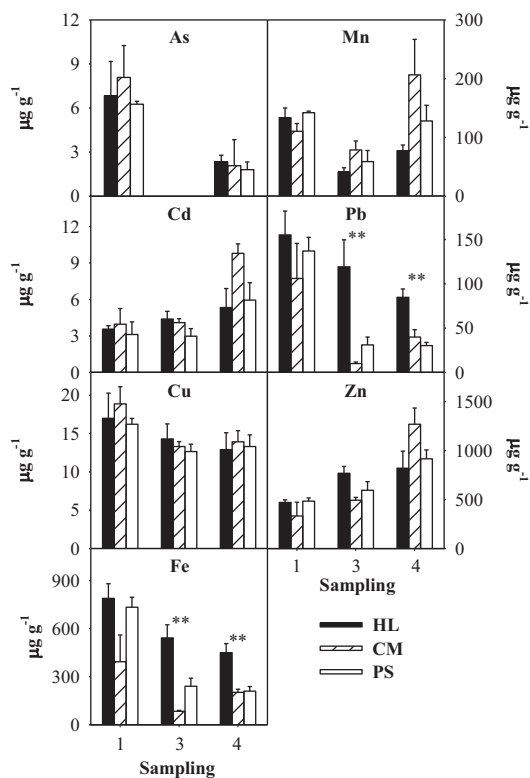


Fig. 4. Trace element concentrations in plant leaves ($n=3$) from the different treated plots in the three samplings (see legend of Fig. 1). ** indicates $p < 0.01$. At sampling 3, the As concentrations were below the ICP detection limit (equivalent to approximately $0.5 \mu\text{g g}^{-1}$ in leaves). Bars indicate standard errors.

months after transplanting (the 2nd sampling) were significantly decreased by the addition of the organic amendments compared to HL treatment. The concentrations of As and Pb decreased in plant fruits with time, whilst those of Cd, Mn and Zn significantly increased from the 2nd to the 4th sampling ($p < 0.001$; Fig. 5). No significant correlations were found between plant trace element concentrations and the corresponding pore water ones.

4. Discussion

4.1. Effects of the amendments in soils

The solubility of metals (Cd, Pb, Zn) remained almost unaltered after the addition of the amendments, in agreement with the results reported previously by Pardo et al. [22] and Martínez-Fernández and Walker [16] from incubation and pot experiments, respectively, that used the same soils and amendments. In the latter, the lack of a pH effect of the amendments was thought to have contributed to the limited impact of the amendments on the concentrations of plant available HMs, which could be also the case in the present experiment as HL was added to compensate the pH effect of the organic amendments. In another pot experiment, Pérez-Esteban et al. [17] found a metal (Zn) immobilising effect of compost only in acid mine soils and at the highest dose (60 t ha^{-1}). Farrell et al. [30] also reported no effect of compost on heavy metals solubility in surface soil in a column experiment after the addition of the amendments, although an initial pulse of soluble metals was found through the column, reportedly as a result of leached salts from the compost. Similarly, in the present experiment mean soluble concentrations of the metals (Cd, Mn, Pb and Zn) were initially higher in CM plots, although due to the heterogeneity of the data between plots significant differences were only found for

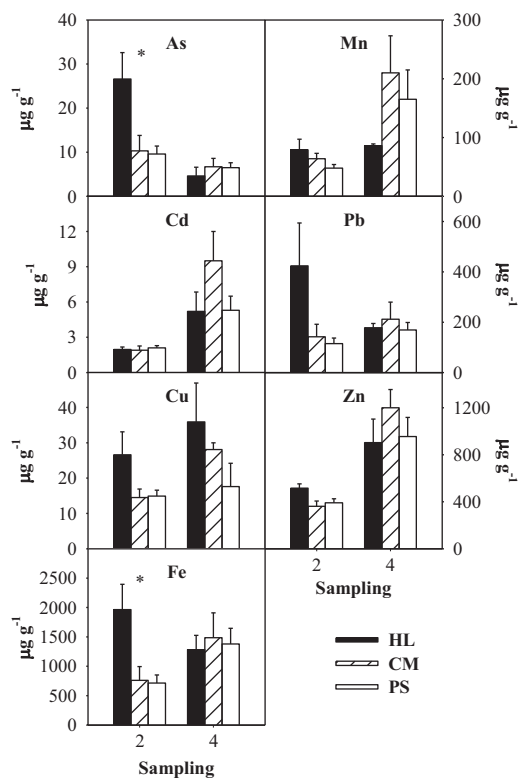


Fig. 5. Trace element concentrations in plant fruits ($n=3$) from the different treated plots in the two samplings (see legend of Fig. 1). * indicates $p < 0.05$. Bars indicate standard errors.

Mn ($p < 0.01$). Metal concentrations in both CaCl_2 extracts and 'in situ'-collected pore water were rather high in some of the samples, reflecting the heterogeneity of soil pH in the experimental site, this being especially relevant in CM-treated plots: lower pH values coincided with higher metal concentrations. The slight mobilisation of As observed in CM soils (increased NaHCO_3 -extractable concentrations) was also previously observed in incubation and pot experiments [16,22]. The presence of phosphates and soluble organic compounds in the compost that may compete for adsorption sites in the soil rather than an effect on pH may be responsible for this [31]. The positive correlation between available As and P supports this idea, although available As also positively correlated soil pH and pore water pH and DOC concentrations in the last sampling ($p < 0.05$), so As mobilisation may be a consequence of the sum of both effects.

The addition of nutrients and a certain amount of degradable OM with the CM significantly increased the soil microbial biomass-C and N throughout the experiment. This effect was also observed in the incubation experiment mentioned above [22], as was an increase in soil microbial biomass activity (soil respiration). This increase may be, at least in part, responsible for the observed decrease in soluble N in pore water samples from organically-amended plots compared to the limed ones, due to microbial immobilisation of inorganic-N. Farrell et al. [30] found that initial rises in pore water N levels following compost addition had disappeared after three months. The concentrations of B_C and B_N correlated positively with available P concentrations, suggesting restriction of the microbial biomass in the control soil due to P deficiency [32].

A number of experiments where different composted materials have been added to TE-contaminated soils have also reported increased microbial biomass content [33] and increased bacterial and fungal diversity and activity [34,35]. However, Rosario et al.

[10] found that it was the growth of *Atriplex canescens* (Pursh) Nutt., not compost addition, that determined the development of the bacterial community in a mine tailings soil. Barness et al. [36] found that underneath the canopy of *A. halimus* there was increased TOC, CO₂ evolution and microbial biomass at soil depths down to 50 cm. We found a significant effect of both amendments on B_C and B_N as the experiment progressed, which was enhanced by the presence of *A. halimus*; although the values declined with time, they were well above those of the limed plots after two years. This, together with the effects on TOC, TN and available K and P, indicates a sustainable improvement in soil quality due to the halophyte-amendment combination.

4.2. Plant growth and macronutrient concentrations

The promotion of growth by the addition of organic amendments to contaminated soils results from improvements in the nutrient supply and physical conditions, together with decreased bioavailability of heavy metals and/or metalloids [20,21]. Here, compost (in the long term) and slurry (immediately after its addition to soil) stimulated *A. halimus* growth, whereas Rosario et al. [10] found that compost addition to a mine tailings soil did not increase *A. canescens* plant volume significantly.

The small increases in the leaf concentrations of N due to PS 4 months after transplanting, which may have contributed to the promotion of growth, were followed by a decline to levels that may have been growth-limiting [16]. This limited effect of the amendments mirrors the results of Castillejo and Castelló [37] for *A. halimus* following addition of different doses of municipal solid waste compost to gypsum spoil. Leaf P concentrations $\geq 70 \text{ mmol kg}^{-1}$ are needed for maximal growth of *A. halimus* [16]. Thus, in the first 16 months after transplanting, the growth enhancement provoked by both amendments was probably due, at least in part, to raised P plant accumulation. Two years after transplanting, the leaf P was still higher for the compost treatment, reflecting the higher P availability. Castillejo and Castelló [37] also found a decrease in P availability with time after compost application and that the shoot P concentration in *A. halimus* increased with increasing P availability in the soil. Growth was increased by the amendments without an increase in leaf K concentration, which at four and 24 months after transplanting may have been below the level required for maximal growth, around 400 mmol kg^{-1} [16]. Anyway, under saline conditions (as in this study) *A. halimus* can withstand lower tissue K concentrations whilst tissue Na rises, and growth is unaffected (or even stimulated) [38].

4.3. Trace elements accumulation in plant tissues.

The leaf As concentrations four months after transplanting were probably phytotoxic [39], although accumulation of As by *A. halimus* has not been studied before, but after 16 months had declined to below $0.5 \mu\text{g g}^{-1}$ and at 24 months were non-phytotoxic (Fig. 4). Vromman et al. [40] proposed a role for polyamines in the intracellular sequestration of As by *Atriplex atacamensis* Phil. A decrease in As concentration with time was observed also in fruits. These declines were not related to soil As availability, that remained almost constant throughout the experiment, although a positive correlation was found between As concentration in plant leaves and available As concentrations in soils in the last sampling ($r=0.693$, $p<0.05$). Such decreases in tissue TE concentrations with time can occur if the rate of uptake falls below the rate of biomass production [41]. Pérez-Esteban et al. [17] found that *A. halimus* accumulated up to $100 \mu\text{g g}^{-1}$ of Cu in its shoot dry matter when grown in a soil contaminated strongly by Cu; here, the leaf Cu concentrations were probably not phytotoxic [6,39]. The leaf concentrations of Fe and Mn required by *A. halimus* for maximal growth are ≤ 150

and $\leq 23 \mu\text{g g}^{-1}$, respectively [14]. If leaf Fe concentrations above $500 \mu\text{g g}^{-1}$ are phytotoxic [42], then the decreases in Fe concentrations in plant leaves brought about by both amendments (Fig. 4) could have contributed to the observed growth stimulation. Leaf Mn concentrations in excess of $400 \mu\text{g g}^{-1}$ are, in general, phytotoxic [39]; so, the increase in leaf Mn provoked by compost at 24 months (Fig. 4) probably did not restrict growth.

Since shoot Pb concentrations $>100 \mu\text{g g}^{-1}$ represent toxicity [16,39], the reductions effected by both organic amendments probably had a role in their promotion of growth. Although the compost used in the current study was rich in P and Fe, which can immobilise Pb in the soil [20], and there was a negative correlation between leaf Pb and soil available P ($r=-0.594$, $p<0.05$), there was no significant effect of compost on soil Pb availability. However, Pb uptake by *A. halimus* may have been reduced by its precipitation as $\text{Pb}_{10}(\text{PO}_4)_6\text{Cl}_2$ on the root surface [43]. Considering the extremely-high Pb level of the soil, *A. halimus* seems efficient at excluding Pb from its leaves, confirming the results of Manousaki and Kalogerakis [15].

Leaf and fruit Cd concentrations were highest at the last harvest (24 months) (Fig. 5), possibly reflecting the slight acidification observed in the soil throughout the experiment. Generally, phytotoxicity appears at values of leaf Cd concentrations of $5\text{--}30 \mu\text{g g}^{-1}$ [39], so it is possible that at 24 months Cd phytotoxicity was occurring, although Lutts et al. [14] found that values as high as $618 \mu\text{g g}^{-1}$ after a 3-week exposure did not reduce *A. halimus* shoot growth significantly. Precipitation of Cd with oxalate [14] and/or its excretion into trichomes and increased synthesis of glycinebetaine [19] may contribute to the tolerance of *A. halimus*. The high soil salinity initially may have restricted the uptake of Cd [19], whilst subsequent root penetration into less-saline soil layers may have contributed to the increase in tissue Cd with time. Leaf Zn concentrations exceeding $100\text{--}300 \mu\text{g g}^{-1}$ are, in general, phytotoxic [39,42]. Although Lutts et al. [14] found that *A. halimus* has a high tolerance of elevated external and internal Zn concentrations, possibly due to co-precipitation of Zn with oxalate [14], the leaf levels measured here (up to $1200 \mu\text{g g}^{-1}$) could be a concern regarding dissemination of Zn. The highest Zn concentrations were found in the last sampling (after two years of growth), possibly because the root system reached deeper soil layers, especially for plants in CM treatment with a great development, where the effect of soil amendments was less. High levels of foliar Cd and Zn were found for other species native to the Sierra Minera by Conesa et al. [44] and, in the case of Cd, for *A. canescens* by Rosario et al. [10].

The relatively-high TE availability in this soil is reflected in the leaf concentrations of Cd, Pb and Zn, which were higher than for *A. halimus* grown in pots of other contaminated soils for comparable lengths of time [15,17]. Although the ingestion of its leaves and stems by herbivorous mammals is limited by their high salt content, *A. halimus* is colonised by arthropods (phytophages and their predators [45]), a route by which contaminants could move through the food chain. Therefore, if the aim is the phytostabilisation of the soil, PS could be considered a better candidate as soil amendment due to lower plant metal accumulation compared to CM, although these differences were not statistically significant (due to variability of the data) and CM improved soil quality more, in terms of soil microbial biomass and long-term nutrients supply.

5. Conclusions

The current findings demonstrate the potential of *A. halimus* for the phytostabilisation of contaminated soils, with respect to achieving a stable vegetation cover and improved soil OM and nutrient contents and microbial population, but also highlight the difficulties of trying to implement phytoremediation in a

pluri-contaminated, heterogeneous soil. The use of the amendments positively influenced plant establishment in the plots and activated the soil microbial biomass. Changes in TE concentrations in plant leaves with time show a need for long-term monitoring and for an ecotoxicological evaluation of the stabilised soils, and strengthen the interest in studying soil pore water collected 'in situ' in the remediated site. Overall, the halophyte plus organic amendment approach to phytostabilisation seems to be appropriate under (semi-)arid conditions, although its suitability for a particular site will depend on the specific contaminant(s) present. The ability of *A. halimus* to accumulate TEs in its leaves and fruits to levels which could accelerate their dissemination, but which are not high enough for phytoextraction, may limit its use to moderately-contaminated sites.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jhazmat.2012.04.048>.

References

- [1] H.M. Conesa, R. Schulin, The Cartagena-La Unión mining district (SE Spain): a review of environmental problems and emerging phytoremediation solutions after fifteen years research, *J. Environ. Monit.* 12 (2010) 1225–1233.
- [2] O. Gonzalez-Fernandez, I. Queralt, J. Manteca, G. Garcia, M. Carvalho, Distribution of metals in soils and plants around mineralized zones at Cartagena-La Unión mining district (SE, Spain), *Environ. Earth Sci.* 63 (2011) 1227–1237.
- [3] M.C. Navarro, C. Perez-Sirvent, M.J. Martínez-Sánchez, J. Vidal, P.J. Tovar, J. Bech, Abandoned mine sites as a source of contamination by heavy metals: a case study in a semi-arid zone, *J. Geochem. Explor.* 96 (2008) 183–193.
- [4] M.J. Marqués, L. Jiménez, R. Pérez-Rodríguez, S. García-Ormaechea, R. Bienes, Reducing water erosion in a gypsic soil by combined use of organic amendment and shrub revegetation, *Land Degrad. Dev.* 16 (2005) 339–350.
- [5] M.O. Mendez, E.P. Glenn, R. Maier, Phytostabilization potential of quailbush for mine tailings: growth, metal accumulation, and microbial community changes, *J. Environ. Qual.* 36 (2007) 245–253.
- [6] M.O. Mendez, R. Maier, Phytostabilization of mine tailings in arid and semiarid environments – an emerging remediation technology, *Environ. Health Persp.* 116 (2008) 278–283.
- [7] C.J. French, N.M. Dickinson, P.D. Putwain, Woody biomass phytoremediation of contaminated brownfield land, *Environ. Pollut.* 141 (2006) 387–395.
- [8] M. Mench, S. Bussièrre, J. Boisson, E. Castaing, J. Vangronsveld, A. Ruttens, T. De Koe, P. Bleeker, A. Assunção, A. Mancaeu, Progress in remediation and revegetation of the barren Jales gold mine spoil after in situ treatments, *Plant Soil* 249 (2003) 187–202.
- [9] C. Ortiz-Calderón, O. Alcaide, J. Li Kao, Copper distribution in leaves and roots of plants growing on a copper mine-tailing storage facility in Northern Chile, *Rev. Chil. Hist. Nat.* 81 (2008) 489–499.
- [10] K. Rosario, S.L. Iverson, D.A. Henderson, S. Chartrand, C. McKeon, E.P. Glenn, R. Maier, Bacterial community changes during plant establishment at the San Pedro River mine tailings site, *J. Environ. Qual.* 36 (2007) 1249–1259.
- [11] S.S. Kachout, A.B. Mansoura, R. Mechergui, J.C. Leclerc, M.N. Rejeb, Z. Ouerghi, Accumulation of Cu, Pb, Ni and Zn in the halophyte plant *Atriplex* grown on polluted soil, *J. Sci. Food Agric.* 92 (2012) 336–342.
- [12] H.N. Le Houérou, The role of saltbushes (*Atriplex* spp.) in arid land rehabilitation in the Mediterranean Basin: a review, *Agroforest Syst.* 18 (1992) 107–148.
- [13] H. Conesa, G. García, A. Faz, R. Arnaldos, Dynamics of metal tolerant plant communities development in mine tailings from the Cartagena-La Unión Mining District (SE Spain) and their interest for further revegetation purposes, *Chemosphere* 68 (2007) 1180–1185.
- [14] S. Lutts, I. Lefèvre, C. Delpèrre, S. Kivits, C. Deschamps, A. Robledo, E. Correal, Heavy metal accumulation by the halophyte species Mediterranean saltbush, *J. Environ. Qual.* 33 (2004) 1271–1279.
- [15] E. Manousaki, N. Kalogerakis, Phytoextraction of Pb and Cd by the Mediterranean saltbush (*Atriplex halimus* L.): metal uptake in relation to salinity, *Environ. Sci. Pollut. Res.* 16 (2009) 844–854.
- [16] D. Martínez-Fernández, D.J. Walker, The effects of soil amendments on the growth of *Atriplex halimus* and *Bituminaria bituminosa* in heavy metal-contaminated soils, *Water Air Soil Pollut.* 223 (2012) 63–72.
- [17] J. Pérez-Esteban, C. Escolástico, J. Ruiz-Fernández, A. Masaguer, A. Moliner, Bioavailability and extraction of heavy metals from contaminated soil by *Atriplex halimus*, *Environ. Exp. Bot.*, <http://dx.doi.org/10.1016/j.envexpbot.2011.12.003>.
- [18] Y. Tapia, V. Cala, E. Eymar, I. Frutos, A. Gárate, A. Masaguer, Phytoextraction of cadmium by four Mediterranean shrub species, *Int. J. Phytoremediat.* 13 (2011) 567–579.
- [19] I. Lefèvre, G. Marchal, P. Meerts, E. Correal, S. Lutts, Chloride salinity reduces cadmium accumulation by the Mediterranean halophyte species *Atriplex halimus* L., *Environ. Exp. Bot.* 65 (2009) 142–152.
- [20] M.P. Bernal, R. Clemente, D.J. Walker, The role of organic amendments in the bioremediation of heavy metal-polluted soils, in: R.B. Gore (Ed.), *Environmental Research at the Leading Edge*, Nova Publishing, New York, 2007, pp. 1–57.
- [21] M.P. Bernal, R. Clemente, D.J. Walker, Interactions of heavy metals with soil organic matter in relation to phytoremediation, in: J.P. Navarro-Aviño (Ed.), *Phytoremediation: The Green Salvation of the World*, Research Signpost, Trivandrum, 2009, pp. 109–129.
- [22] T. Pardo, R. Clemente, M.P. Bernal, Effects of compost, pig slurry and lime on trace element solubility and toxicity in two soils differently affected by mining activities, *Chemosphere* 84 (2011) 642–650.
- [23] M.J. Martínez, C. Pérez-Sirvent, Niveles de fondo y niveles genéricos de referencia de metales pesados en suelos de la Región de Murcia, University of Murcia, Murcia, 2007.
- [24] BOE. Real Decreto 9/2005, de 14 de enero, por el que se establece la relación de actividades potencialmente contaminantes del suelo y los criterios y estándares para la declaración de suelos contaminados. BOE 15, de 18 de enero de 2005.
- [25] M.P. Bernal, A.F. Navarro, M.A. Sánchez-Monedero, A. Roig, J. Cegarra, Influence of sewage sludge compost stability and maturity on carbon and nitrogen mineralization in soil, *Soil Biol. Biochem.* 30 (1998) 305–313.
- [26] R. Clemente, N.M. Dickinson, N.W. Lepp, Mobility of metals and metalloids in a multi-element contaminated soil 20 years after cessation of the pollution source activity, *Environ. Pollut.* 155 (2008) 254–261.
- [27] E. Moreno-Jiménez, L. Beesley, N.W. Lepp, N.M. Dickinson, W. Hartley, R. Clemente, In situ sampling of pore water under field conditions as a methodology to monitor trace elements in soils: data compilation and approaches, *Environ. Pollut.* 159 (2011) 3078–3085.
- [28] International Union of Pure and Applied Chemistry, Reference material for trace analysis by radio analytical methods: Bowen's Kale, *Pure Appl. Chem.* 51 (1979) 1183–1193.
- [29] D.J. Walker, M.P. Bernal, The effects of olive mill waste compost and poultry manure on the availability and plant uptake of nutrients in a highly saline soil, *Bioresour. Technol.* 99 (2008) 396–403.
- [30] M. Farrell, W.T. Perkins, P.J. Hobbs, G.W. Griffith, D.L. Jones, Migration of heavy metals in soil as influenced by compost amendments, *Environ. Pollut.* 158 (2010) 55–64.
- [31] W.J. Fitz, W.W. Wenzel, Arsenic transformations in the soil–rhizosphere–plant system: fundamentals and potential application to phytoremediation, *J. Biotechnol.* 99 (2002) 259–278.
- [32] L. Liu, P. Gundersen, T. Zhang, J. Mo, Effects of phosphorus addition on soil microbial biomass and community composition in three forest types in tropical China, *Soil Biol. Biochem.* 44 (2012) 31–38.
- [33] C. de la Fuente, R. Clemente, I. Martínez-Alcalá, G. Tortosa, M.P. Bernal, Impact of fresh and composted solid olive husk and their water-soluble fractions on soil heavy metal fractionation, microbial biomass and plant uptake, *J. Hazard. Mater.* 186 (2011) 1283–1289.
- [34] A. de Varennes, M.M. Abreu, G. Qu, C. Cunha-Queda, Enzymatic activity of a mine soil varies according to vegetation cover and level of compost applied, *Int. J. Phytorem.* 12 (2010) 371–383.
- [35] M. Farrell, G.W. Griffith, P.J. Hobbs, W.T. Perkins, D.L. Jones, Microbial diversity and activity are increased by compost amendment of metal-contaminated soil, *FEMS Microbiol. Ecol.* 71 (2010) 94–105.
- [36] G. Barness, S. Rodríguez Zaragoza, I. Shmueli, Y. Steinberger, Vertical distribution of a soil microbial community as affected by plant ecophysiological adaptation in a desert system, *Microb. Ecol.* 57 (2009) 36–49.
- [37] J.M. Castillejo, R. Castelló, Influence of the application rate of an organic amendment (municipal solid waste [MSW] compost) on gypsum quarry rehabilitation in semiarid environments, *Arid Land Res. Manag.* 24 (2010) 344–364.
- [38] M. Bajji, J.-M. Kinet, S. Lutts, Salt stress effects on roots and leaves of *Atriplex halimus* L. and their corresponding callus cultures, *Plant Sci.* 137 (1998) 131–142.
- [39] A. Kabata-Pendias, *Trace Elements in Soils and Plants*, third ed., CRC Press LLC, Boca Raton, 2001.
- [40] D. Vromman, A. Flores-Bavestrello, Z. Šlejko, S. Lapaille, C. Teixeira-Cardoso, M. Briceño, M. Kumar, J.-P. Martínez, S. Lutts, Arsenic accumulation and distribution in relation to young seedling growth in *Atriplex atacamensis* Phil., *Sci. Total Environ.* 412–413 (2011) 286–295.

- [41] B.H. Robinson, M. Leblanc, D. Petit, R.R. Brooks, J.H. Kirkman, P.E.H. Gregg, The potential of *Thlaspi caerulescens* for phytoremediation of contaminated soils, *Plant Soil* 203 (1998) 47–56.
- [42] H. Marschner, *Mineral Nutrition of Higher Plants*, second ed., Academic Press, London, 1995.
- [43] X. Cao, L.Q. Ma, M. Chen, S.P. Singh, W.G. Harris, Impacts of phosphate amendments on lead biogeochemistry at a contaminated site, *Environ. Sci. Technol.* 36 (2002) 5296–5304.
- [44] H. Conesa, A.B. Moradi, B.H. Robinson, G. Kühne, E. Lehmann, R. Schulin, Responses of native grasses and *Cicer arietinum* to soil polluted with mining wastes: implications for the management of land adjacent to mine sites, *Environ. Exp. Bot.* 65 (2009) 198–204.
- [45] E.M. Hegazi, J.K. Wangberg, J.R. Goodin, D.K. Northington, Field observations on arthropods associated with *Atriplex halimus* in Egypt, *J. Arid Environ.* 3 (1980) 305–308.