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Using Mediterranean shrubs for the phytoremediation of a soil impacted by pyritic wastes in Southern Spain: A field experiment

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ABSTRACT

Re-vegetation is the main aim of ecological restoration projects, and in Mediterranean environments native plants are desirable to achieve successful restoration. In 1998, the burst of a tailings dam flooded the Guadiamar river valley downstream from Aznalcóllar (Southern Spain) with sludges that contained elevated concentrations of metals and metalloids, polluting soils and waters. A phytoremediation experiment to assess the potential use of native shrub species for the restoration of soils affected by the spillage was performed from 2005 to 2007, with soils divided into two groups: pH < 5 and pH > 5. Four native shrubs (*Myrtus communis*, *Retama sphaerocarpa*, *Rosmarinus officinalis* and *Tamarix gallica*) were planted and left to grow without intervention. Trace element concentrations in soils and plants, their extractability in soils, transfer factors and plant survival were used to identify the most-interesting species for phytoremediation. Total As was higher in soils with pH < 5. Ammonium sulphate-extractable zinc, copper, cadmium and aluminium concentrations were higher in very-acid soils, but arsenic was extracted more efficiently when soil pH was >5. Unlike As, which was either fixed by Fe oxides or retained as sulphide, the extractable metals showed significant relationships with the corresponding total soil metal concentration and inverse relationships with soil pH. *T. gallica*, *R. officinalis* and *R. sphaerocarpa* survived better in soils with pH > 5, while *M. communis* had better survival at pH < 5. *R. sphaerocarpa* showed the highest survival (30%) in all soils. Trace element transfer from soil to harvestable parts was low for all species and elements, and some species may have been able to decrease trace element availability in the soil. Our results suggest that *R. sphaerocarpa* is an adequate plant species for phytostabilising these soils, although more research is needed to address the self-sustainability of this remediation technique and the associated environmental changes.

1. Introduction

Trace element levels in soils have increased in many soils since the beginning of human industrial activity. Trace element-contaminated soils can pose an environmental risk for humans and other organisms: thus, reclamation activities are frequently recommended (Mench et al., 2010). Pyrite mines are a major source of trace elements and soils impacted by this activity can show high levels of pollutants (Adriano, 2001). Phytoremediation is a promising technique for large areas of soil where the emphasis lies on the environmental rather than the economic value. When contamination exists in the top layer of the soil, plants can root in this zone and play a role in pollutant immobilisation (Vangronsveld et al., 2009). In 1998, the burst of a tailing dam flooded the Guadiamar river valley downstream from Aznalcóllar (Southern Spain) with sludges that contained elevated concentrations of metals and metalloids, polluting top soils and waters over an area of ~40 km^2 (Simón et al., 1999). After the initial treatment of the spill, a restoration project, the Green Corridor of the Guadiamar River, was initiated with the aim of re-vegetating the affected area with an open forest ecosystem (Domínguez et al., 2008). However, the concentrations of trace elements in soils remained above the background levels in some sites and pyrite oxidation also promoted soil acidification. Re-vegetation is the main aim of ecological restoration projects, and in Mediterranean environments native plants are desirable to achieve successful restoration (Moreno-Jiménez et al., 2008). Trace metals in soil can be transferred to the vegetation, but the uptake, translocation and phytoaccumulation will be intimately linked to plant species. Phytoremediation techniques try to optimise the interactions between soil and plants, in order to decontaminate soils and improve the environmental quality of the site (Mench et al., 2009).

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The main aspect to be restored at the site that is the subject of the work described here is the ecological value of the soil and, therefore, phytoremediation is a better alternative than other, more-destructive techniques such as physical and chemical methods (Rodríguez et al., 2009; Moreno-Jiménez et al., 2010a). Some phytoremediation experiments have been carried out at the study site; however, most of them used crop species, and none of the species that would be planted in an ecological restoration project have been evaluated with regard to phytoremediation. Our field experiment explores the potential of native shrub species for phytoremediation under real conditions.

2. Materials & methods

2.1. Field experiment

The experiment was performed in plot B2 (1000 m^2) in the experimental site “El Vicario” (Aznalcóllar, Spain), 7 km downstream of the toxic spill. The experimental plot has been described by Vázquez et al. (2006), and has a wide range of pH (2–8), low contents of organic matter (<2%), nitrogen (<0.1) and carbonates (<0.1%) and a loamy-sandy texture.

This site has been the subject of different research experiments: for instance, Clemente et al. (2005), Madejón et al. (2006) and Dary et al. (2010). For this experiment, plants were selected from among those used for the Green Corridor of Guadiamar River restoration project (Domínguez et al., 2008). Four species were selected: *Myrtus communis* L., *Retama sphaerocarpa* L., *Rosmarinus officinalis* L. and *Tamarix gallica* L., which have been studied previously under hydroponic conditions in order to understand their interaction with trace elements (Moreno-Jiménez et al., 2008; 2009a). The plants used were similar in size and aspect to those used in the revegetation activities carried out in the area.

Because of the high pH variability in the study plot (1000 m^2), it was divided into subplots of 25 m^2, grouping them into soils with pH < 5 and soils with pH > 5. Eight subplots from each group were used for the experiment. Each subplot was divided into 4 units of 1.5×1.5 m^2, leaving a corridor of 50 cm between units and subplots as a barrier to avoid interactions between them. In each unit, 16 plants of one species were transplanted, so that in each subplot all the species were grown. Unplanted subplots were maintained as control soils. A scheme of the experimental set-up is detailed in Fig. 1.

The experiment lasted for two years, from December 2005 to December 2007. Plants were grown under natural conditions; neither agricultural practices nor irrigation were carried out. The survival of the transplanted plants was monitored annually. Surviving plants were those still present in the soil and with clear visual symptoms of biological activity, such as green colour, fresh tissues and turgid leaves or stems.

2.2. Soil and plant sampling, processing and analysis

In planted subplots, the plants were rooted out and the soil in contact with the roots was collected. In control subplots, soils were sampled from the top 5–30 cm. Once in the laboratory, roots were initially cleaned by hand, removing all the adhering soil particles. Afterwards, all plant material was rinsed for 5 min with tap water, which was shaken off by hand. Finally, plant material was submerged in distilled water for 2 min, dried at 60 °C for 3 days and milled to a fine powder in a grinder. Soils were dried in a glasshouse for 7 days and sieved to <2 mm.

Plant material (0.5 g) was digested in an autoclave (Autoester-G, Selecta) with 3 mL HNO_3 (65%), 2 mL H_2O_2 (33%) and 10 mL mili-Q water, at 125 °C and 1.25 kPa for 30 min, filtered and diluted to 25 mL with mili-Q water (Lozano-Rodríguez et al., 1995).

Soil pH was measured in a 1:2.5 (soil:water) suspension (MAPA, 1994). Total element concentrations in soil samples were processed after autoclave digestion (0.5 g) with 6 mL HNO_3, 4 mL H_2O_2 and 6 mL of mili-Q water, filtration and dilution to 50 mL with mili-Q water (Moreno-Jiménez et al., 2010a). Weakly-retained metals and As in soil samples were determined after extraction with 0.1 M $(NH_4)_2SO_4$ (1:10 w:v) for 4 h (Vázquez et al., 2008).

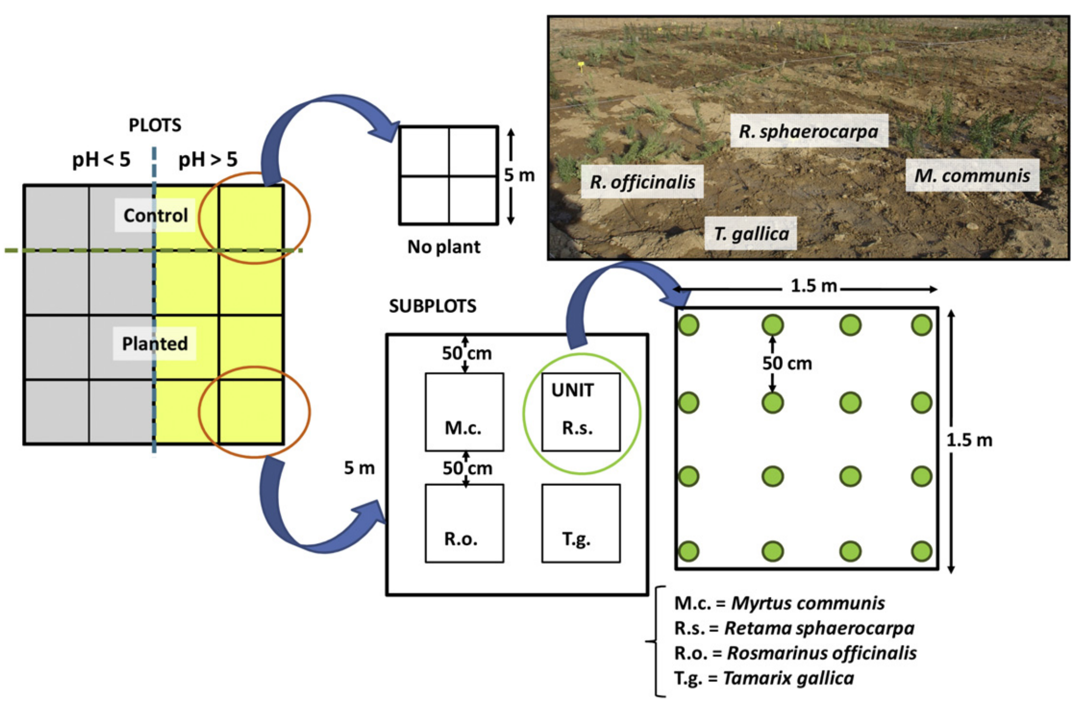

Fig. 1. Experimental set-up in the plot at El Vicario (Aznalcóllar, Southern Spain). Top right, plants at the beginning of the experiment.

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Metal concentrations were determined by AAS (Perkin Elmer Analyst 800) for soil and plant extracts and arsenic by atomic fluorescence (P S Analytical 10.055, Millenium Excalibur system).

Certified reference materials (CTA-VL2, tobacco leaves, 0.97 μg As g^{-1}; CMR048-050, soil, 150 mg kg^{-1}) were also digested and analysed. The recoveries in both materials were >88%, >84%, >87% and >84% for As, Cd, Cu and Zn, respectively, with low coefficients of variance (<6%).

Two soil samples, one from subplots with pH < 5 and another from subplots with pH > 5, were selected for sequential extraction of As. Arsenic fractionation in soil samples was assessed following the procedure of Wenzel et al. (2001), with five steps: (1) 0.1 M $(NH_4)_2SO_4$-extractable fraction, (2) 0.05 M $NH_4H_2PO_4$-extractable fraction, (3) 0.2 M NH_4-oxalateextractable, (4) 0.2 M NH_4-oxalate and 0.2 M ascorbic acid-extractable at 96 °C, and (5) residual phase (digested with HNO_3/H_2O_2 under 125 °C and 1.5 kPa).

2.3. Data processing and statistical analysis

Data were processed with Excel and SPSS. ANOVA and Duncan's test were used to study the influence of plants on the availability of the elements in the soil. Two-way ANOVA was used for mean comparison, with soil pH and plant species as factors. The transfer factor (TF) was calculated as the ratio $[TE]_{shoot}$:$[TE]_{soil}$. The translocation rate was evaluated by the $[TE]_{shoot}$:$[TE]_{root}$ ratio, where TE = trace element.

3. Results

3.1. Plot soils

Both pH and total trace element concentrations are shown in Fig. 2. Subplots were grouped in pH < 5 and pH > 5, with the former group ranging from pH 2.9 to 4.8 and the latter from 5.2 to 8.3. Total metal concentrations in the different soils were within the same range, whilst As concentrations were higher in the most-acidic soils. The average concentrations of Al, Cd and Cu in soil were 2% and 1.2 and 22 mg kg^{-1}, respectively. The total Zn and As concentrations averaged 252 and 312 mg kg^{-1} and 87 and 46 mg kg^{-1}, for pH < 5 and pH > 5, respectively.

The ammonium sulphate-extractable concentrations of Zn and Al were significantly higher than those of the other elements (Fig. 3). Extractable metal concentrations were higher at pH < 5 than at pH > 5, while concentrations of extractable As were similar or even higher in soils with pH > 5.

The percentages of extractable elements were calculated on the basis of the total element in every single sample (Fig. Supplementary Material). Arsenic, the only element presumably present in anionic forms, showed the lowest extractability and a slight tendency to be more mobile at pH > 5. Zinc and Cd were the most-soluble metals, mainly in acidic soils. Aluminium was present in soluble forms only at low pH.

In order to establish the relationships between the extractable concentrations of the different elements in the soils ($[TE]_{Ext}$) and soil physicochemical properties, a linear regression analysis was performed by both forwards and backwards methods and the most-significant of the equations was selected for each element. Significant regression equations were found for all the elements except As:

$$[Al]_{Ext} = 136 - 18 \cdot pH;\ r = 0.57;\ P < 0.001.$$

$$[Cd]_{Ext} = 0.27 - 0.05 \cdot pH + 0.07 \cdot [Cd]_T;\ r = 0.52;\ P < 0.01.$$

$$[Cu]_{Ext} = 4.73 - 0.63 \cdot pH;\ r = 0.46;\ P < 0.01.$$

$$[Zn]_{Ext} = 92 - 26 \cdot pH + 0.23 \cdot [Zn]_T;\ r = 0.84;\ P < 0.001.$$

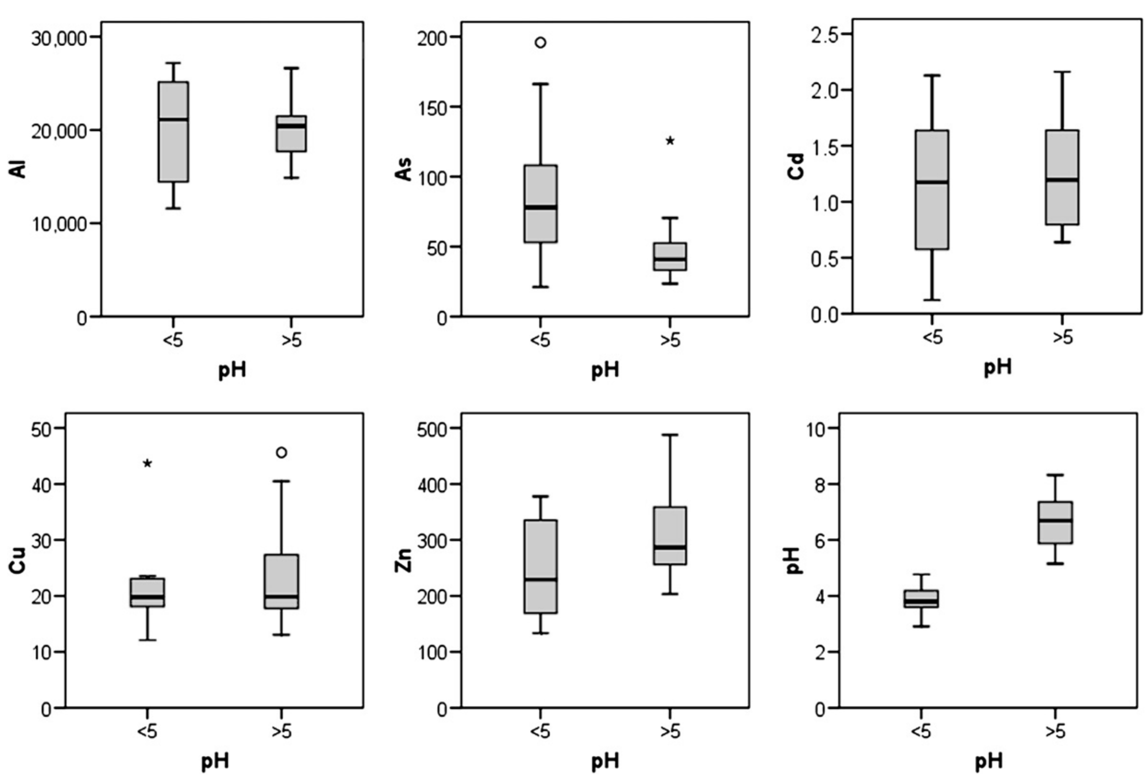

Fig. 2. The total concentrations of trace elements (mg kg^{-1}) and pH in soils in the subplots of the experimental plot.

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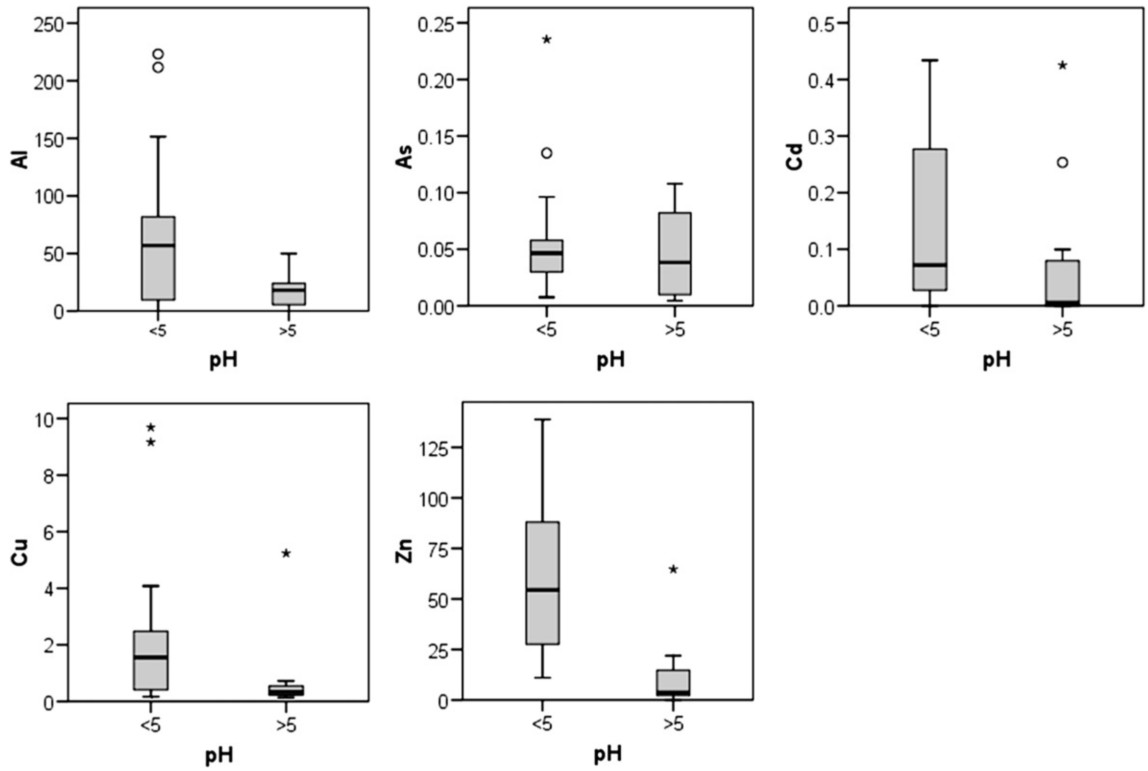

Fig. 3. The $(NH_4)_2SO_4$-extractable concentrations (mg kg^{-1}) of Al, As, Cd, Cu and Zn in the subplots.

Since extractable As was not explained by the regression analysis, As fractionation in soil was assessed: the results are shown in Table 1. Weakly-retained As (F1 and F2) in soils with pH $<$ 5 was lower than at pH $>$ 5. Up to 60% of As in soils with pH $>$ 5 was extracted in F3 and 4, mainly associated with Fe oxides (Wenzel et al., 2001). The residual fraction (F5) was higher for the soils with low pH.

Table 1
Percentage (%) of As retained in the different fractions of the sequential extraction and total concentration. Mean, $n = 2$.

	F1 (%)	F2 (%)	F3 (%)	F4 (%)	F5 (%)	Total (mg As kg^{-1})
pH = 3,3	0.11	8.8	14.3	31.2	45.6	148
pH = 6,4	0.50	10.2	15.8	44.1	29.8	55

As an example of the influence of plant establishment on trace element extractability, the ammonium sulphate-extractable As and Zn concentrations are shown in Fig. 4, for both planted and control soils. Significant differences ($p < 0.10$) were observed only in soils with pH $<$ 5, where both *R. sphaerocarpa* and *M. communis* decreased the extractable fraction of As. In the case of Zn, some plants showed a tendency to decrease its extractability, but the high variability of the results meant that significant differences did not exist.

3.2. Plants grown in the experimental plot

Plant survival decreased during the experiment (Table 2). At the end, survival was low, as plants had been exposed to pollution,

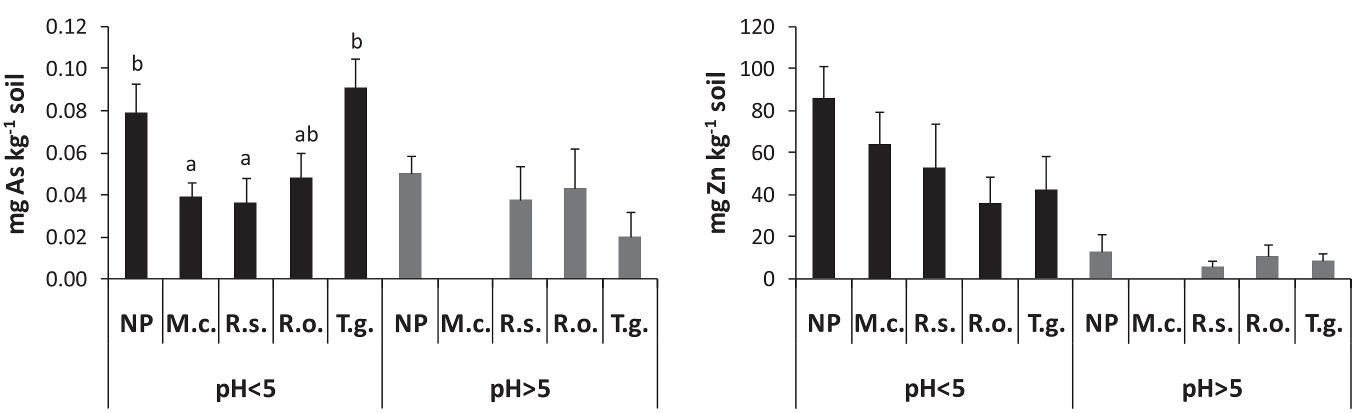

Fig. 4. The extractable arsenic and zinc concentrations in soils with and without plants. NP: non-planted; M.c.: *Myrtus communis*; R.s.: *Retama sphaerocarpa*; R.o.: *Rosmarinus officinalis*; T.g.: *Tamarix gallica*. Mean ± SE, $n = 3$–8. Significant differences between means ($p < 0.1$) are indicated by different letters. No data were obtained for *M. communis* at pH $>$ 5.

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Table 2
Survival rate (%) of plants in the different subplots after 2 years of field experiment. Mean ± SE, $n = 8$; n.s. = not significant, * = $p < 0.05$, ** = $p < 0.01$.

Species (Sp)		Plant survival (%)	
		pH < 5	pH > 5
Myrtus communis		13.8 ± 8.9	0.8 ± 0.9
Retama sphaerocarpa		23.8 ± 8.7	34.4 ± 9.0
Rosmarinus officinalis		10.2 ± 4.9	27.8 ± 6.2
Tamarix gallica		3.1 ± 1.4	16.7 ± 6.8
ANOVA	pH n.s.	Sp **	pH*Sp *

Table 4
Shoot:root ratio ($[TE]_{shoot}/[TE]_{root}$) and transfer factor ($TF = [TE]_{PA}/[TE]_{soil}$) for metals and As in the plants growing in the experimental. Mean, $n = 3–8$. M.c.: *Myrtus communis*; R.o.: *Rosmarinus officinalis*; R.s.: *Retama sphaerocarpa*; T.g.: *Tamarix gallica*. n.a.: no analysed.

	$[TE]_{shoot}/[TE]_{root}$					TF				
	Al	As	Cd	Cu	Zn	Al	As	Cd	Cu	Zn
pH < 5										
M.c.	2.35	1.53	0.51	0.37	1.96	0.009	0.026	0.19	0.29	0.73
R.s.	0.74	0.31	0.33	0.71	0.87	0.006	0.018	0.10	0.45	0.46
R.o.	1.50	0.50	0.95	1.02	1.68	0.007	0.016	0.10	0.53	0.32
T.g.	2.48	0.43	8.80	1.46	2.04	0.010	0.008	0.58	0.46	0.57
pH > 5										
M.c.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
R.s.	5.43	0.98	0.20	0.76	0.66	0.007	0.027	0.05	0.37	0.17
R.o.	2.69	0.98	0.89	0.85	2.10	0.007	0.017	0.18	0.40	0.25
T.g.	3.98	0.63	1.96	1.01	0.86	0.007	0.021	0.24	0.47	0.15

drought, occasional flooding by the river and animal disturbance. *R. sphaerocarpa* showed the highest success in both soils (pH < 5 and > 5). *R. officinalis* showed good survival at pH > 4, while the survival of *T. gallica* and *M. communis* was generally low.

The Al, As, Cd, Cu and Zn concentrations in plant organs are detailed in Table 3. The levels of metals were slightly higher in subplots with pH < 5. Arsenic levels in *M. communis* shoots were higher than in the other plants. As a general rule, the concentrations of trace elements in both roots and shoots were of the same order of magnitude.

The transfer factor was generally below 1, indicating a low transfer of contaminants to shoots. The shoot:root ratio ranged between 0.1 and 10, being in many cases >1 (Table 4).

4. Discussion

4.1. *Trace element contamination in soils*

The total As and Zn concentrations in soils exceeded the toxic thresholds that could be hazardous for the biota (Ross, 1994); As exceeded the permitted level of 100 mg kg^{-1} for soils in natural areas of Andalucía (Aguilar et al., 2004). In fact, 30% of the soils with pH < 5 and 5% of the soils with pH > 5 were above this latter limit. Although the levels of Zn were not alarming, they can be considered of environmental concern. The total concentrations of trace elements are in the range reported by Aguilar et al. (2004) for the same area; they seem to have decreased over time in the experimental plot if our data are compared to previous studies just after the mine spill, in very-close plots at the same site (Clemente et al., 2006; Madejón et al., 2006). The high heterogeneity of the soils of this area (Vanderlinden et al., 2004) may contribute to this, as may either natural attenuation or pollutant dispersion to adjacent soils/waters. Further studies are desirable to ensure that deeper layers of soils or adjacent soils or waters are not being impacted by the mobility of the contaminants.

If we calculate the fraction of extractable trace elements in the soil in relation to the total in the same sample, these values are consistent with other reports (Fellet et al., 2007; Beesley et al., 2010; Moreno-Jiménez et al., 2009a,2010a), following generally this order: Cd ~ Zn > Cu >> Al > As. In the case of metals, the percentage of extractable element was higher when soil pH decreased, while As extractability was slightly higher in soils with pH > 5. Total element concentration, pH, oxides, organic matter and texture are main variables affecting metal (loid) extractability (Adriano, 2001). In the studied soils, a narrow range of organic matter content (1–2%), Fe content (2–5%), Al content (1.2–2.7%) and texture (loamy-sand) was found, but total concentration and pH varied widely. This variability of the two latter factors makes them useful for explanation of differences in extractability in soils, using linear regression analysis. Both pH and total element concentration explained to a high degree the extractable metal concentrations in soils. All the metals (Al, Cd, Cu and Zn) showed lower extractability as soil pH increased, which corresponds with previous findings (Adriano, 2001; Kabata-Pendias, 2004; Moreno-Jiménez et al., 2009b). Arsenic was analysed in the same way as the metals, but no relationship existed between the extractable As concentration and the pH and total As concentration in the linear

Table 3
Metals and As concentrations (μg g^{-1}) in shoots (S) and roots (R) of plants growing in the experimental plot. Mean (range), $n = 3–8$. M.c.: *Myrtus communis*; R.o.: *Rosmarinus officinalis*; R.s.: *Retama sphaerocarpa*; T.g.: *Tamarix gallica*. n.d.: non detected; n.a.: non analysed.

		Al	As	Cd	Cu	Zn
pH<5						
M.c.	S	226 (169–333)	2.56 (1.60–4.57)	0.29 (0.23–0.41)	6.3 (5.4–8.3)	196 (84–290)
	R	96 (35–205)	1.67 (0.64–3.82)	0.57 (n.d.-1.22)	17.0 (6.8–35.5)	100 (61–141)
R.s.	S	137 (81–182)	1.53 (0.46–2.12)	0.17 (0.05–0.38)	8.4 (6.4–11.7)	149 (90–249)
	R	186 (47–398)	4.99 (3.98–5.76)	0.53 (0.24–0.75)	11.8 (10.2–14.3)	172 (133–196)
R.o.	S	177 (139–202)	0.78 (0.74–0.83)	0.15 (0.12–0.19)	13.1 (11.3–15.6)	90 (82–100)
	R	118 (93–205)	1.57 (1.23–1.83)	0.16 (n.d.-0.29)	12.8 (9.1–19.2)	54 (46–68)
T.g.	S	147 (118–183)	0.79 (0.50–0.93)	0.32 (0.18–0.59)	9.2 (8.9–9.4)	114 (71–196)
	R	15 (6.8–44)	1.84 (0.73–3.82)	0.04 (n.d.–0.11)	6.3 (5.1–7.1)	56 (33–85)
pH>5						
M.c.	S	n.a.	n.a.	n.a.	n.a.	n.a.
	R	n.a.	n.a.	n.a.	n.a.	n.a.
R.s.	S	153 (123–180)	0.82 (0.66–0.95)	0.07 (0.04–0.10)	6.4 (4.7–9.0)	61 (44–73)
	R	28 (4.1–76)	0.84 (0.48–1.56)	0.33 (0.02–0.67)	8.4 (5.8–9.6)	92 (32–133)
R.o.	S	148 (83–196)	0.67 (0.43–0.83)	0.19 (0.07–0.34)	11.9 (10.4–14.4)	66 (42–88)
	R	55 (6–136)	1.16 (0.37–2.97)	0.12 (0.06–0.34)	14.1 (8.6–20.8)	32 (20–50)
T.g.	S	134 (103–170)	0.71 (0.43–0.83)	0.31 (0.20–0.41)	10.3 (8.2–12.3)	43 (33–52)
	R	54 (23–83)	1.13 (0.70–1.84)	0.16 (n.d.–0.42)	10.2 (9.0–14.4)	50 (29–83)

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regression analysis. In fact, the highest extractable As concentrations were observed with pH < 3 and pH > 6. For the former case, this can be attributed to the protonation of As(V) in soils to arsenic acid, which is poorly retained in soils as it is uncharged (Zhang and Selim, 2008). For the latter, OH^- can compete with As anions for the retention sites of the soil, releasing As from the exchangeable anion fraction (Smith et al., 1999; Fitz and Wenzel, 2002). The sequential extraction of As from the soils demonstrates the low availability (<11% in the first two fractions) of this metalloid in these mine-impacted soils. It was retained primarily in the most-insoluble soil fractions: bound by oxides, as precipitated salts and as sulphur salts. The role of oxides controlling the solubility of As in these Fe-enriched soils may be predominant (La Force et al., 2000). The results are similar to those reported for soils in other pyritic mine sites (Conesa et al., 2008; Moreno-Jiménez et al., 2010a).

4.2. *Plant–soil interactions and phytoaccumulation of trace elements*

Plant survival after two years was low (<40%), which was attributed to the harsh climatic conditions and the lack of agronomic practices or protection for the plants. *R. sphaerocarpa* showed the highest survival rate, standing out as the most-suitable species for the re-vegetation of these soils under the experimental conditions.

Plant establishment had effects on contaminant availability. When the ammonium sulphate-extractable concentrations were compared for soils from planted and unplanted plots (Fig. 4), As extractability was significantly depleted only when *R. sphaerocarpa* and *M. communis* were growing in soils with pH < 5. Similar depletions in available metal concentrations were also observed in some other planted plots, but these differences were not significant, likely due to the variability of the results. For instance, plants decreased the average extractable Zn concentrations, but not significantly so. This depletion of the available fraction is one of the main goals of phytoremediation, since it decreases the environmental risk associated with the contamination. Plant accumulation may partly explain this effect (Mench et al., 2009), but the amount of contaminant retained in plant tissues in our experiment was low in comparison with the available pool in the soil. So, reductions in the available fraction can be attributed to rhizosphere processes, such as chelation by plant exudates and immobilisation by root-associated microorganisms (Mench et al., 2009; Kidd et al., 2009).

The transfer factors showed this order: Zn $>$ Cd $\sim$ Cu $>$ As $>$ Al, which, to some extent, reflects the extractability of these elements in the soils. This agrees with previous reports (Domínguez et al., 2008). Trace element transfer from soil to the harvestable parts was low for all species and elements (always lower or much lower than 1). Therefore, none of the studied species are useful for phytoextracting metal(loid)s from these soils. However, as the main aim of phytoremediating these soils would be the preservation of the ecological quality of the environment, phytostabilisation may provide a good alternative: these plants do not accumulate large amounts of trace elements in their edible parts, therefore posing a low risk for food chain transfer. These results agree with those of Domínguez et al. (2008), who described phytomanagement as a good option for this site.

The trace element concentrations in plants exceeded the normal levels reported by Kabata-Pendias and Pendias (1992), but they were below the toxicity threshold concentrations described by other authors (Álvarez et al., 2003; Gardea-Torresdey et al., 2005). Only As slightly exceeded the limit of 3 mg kg^{-1} reported by Chaney (1989). Domínguez et al. (2008) reported similar ranges of trace element concentrations in native re-vegetated plants within the Green Corridor of the Guadiamar River. The levels of trace elements in plants reported in the current work correspond to plants growing in contaminated soils for two years; Domínguez et al. (2008) found higher concentrations in mature plants than in young ones. Therefore, monitoring plants in the long-term is desirable, to ensure low concentrations of contaminants in plants. Alexandre (2003) carried out a field trial in a nearby plot, reporting higher concentrations of Cd, Cu and Zn in the aerial parts of *Lupinus angustifolius*, *Helianthus annuus*, and *Zea mays* than in our work with Mediterranean shrubs. Clemente et al. (2005) also found, in the same site, concentrations of Zn, Cu, Cd and As in above-ground tissues of *Brassica juncea* that were higher or much higher than in our species. Although Domínguez et al. (2008) recently measured trace elements in the shoots of some of the species studied in the current experiment, as far as we know there are no data reported about trace element concentrations in the roots of plants grown under field conditions in these soils.

Trace element exclusion is a widespread mechanism for plant resistance (Dahmani-Muller et al., 2000; Clemens et al., 2002), and this excluder behaviour was reported before for the studied Mediterranean shrubs (Moreno-Jiménez et al., 2008; 2009a). Focussing on As, the studied plant species showed lower shoot:root ratios of As concentration under hydroponic conditions than in the field. This could be explained partly by a previous observation: the higher the As dose in the nutrient solution, the lower the shoot:root ratio of its concentration since more As is retained by the roots (Moreno-Jiménez et al., 2010b). Studies with lupin showed the same pattern, with higher ratios under field conditions for As and Cd (Vázquez, 2004). These differences between water- and soil-based cultures can be due to the high availability of trace elements in hydroponic cultures compared to soil (Moreno-Jiménez et al., 2010b). The sampling procedure might also explain these differences: only old, coarse roots can be sampled in the field, while in hydroponics both fine and coarse roots are easily accessible. Vamerali et al. (2009) found the highest concentrations and accumulation of trace elements in fine roots, so field sampling can underestimate contaminant concentrations in roots if the whole root system is not obtained.

4.3. *Concluding remarks and future research*

The ability to survive under the prevailing environmental conditions, depletion of contaminant availability in soils, native character and a low soil-to-shoot transfer of the contaminant(s) are the most-important plants traits for the application of phytostabilisation (Mench et al., 2009; Kidd et al., 2009). We highlight the native species *R. sphaerocarpa* as a promising candidate for the successful re-vegetation and stabilisation of the studied site. A previous experiment to evaluate the feasibility of phytostabilisation with Mediterranean shrubs demonstrated that As-loaded roots did not increase the risk of As remobilisation during their mineralisation in these soils (Moreno-Jiménez et al., 2009c). This finding supports our recommendation. Future work should determine the environmental sustainability of phytostabilisation, by assessing the soil health and the primary ecological succession in the reclaimed soil over time, and monitoring the long-term availability of trace elements in the soil and their transfer to biota.

5. Conclusions

Arsenic solubility in the soils of Aznalcóllar was low, but metal extractability was higher in those soils with low pH. Although very-acid pH was toxic for plants, some species were resistant to trace element toxicity and soil acidity in the experimental plot, with a low transfer of contaminants from soils to plants: this makes

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phytostabilisation the best alternative. Among all the tested species, *R. sphaerocapra* showed the highest survival rate and may help to decrease As availability in these soils.

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Appendix. Supplementary material

Supplementary data related to this article can be found online at doi:10.1016/j.jenvman.2011.01.022.

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