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Assessment of native shrubs for stabilisation of a trace elements-polluted soil as the final phase of a restoration process

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ABSTRACT

Re-vegetation is the main aim of ecological restoration projects, where the use of native plants is recommended over exogenous species, which may result in an undesirable modification of the ecosystem. A 10-year phytoremediation programme was carried out in a site affected by the toxic spill of pyritic (iron sulphide) residue at Aznalcóllar (Spain) in 1998, contaminated with heavy metals (Cd, Cu, Pb and Zn) and arsenic. The success of the re-vegetation of the area with native species after a large (6 years) active phytoremediation intervention was evaluated during 4 years as the final step of the ecological restoration process. Mediterranean native shrubs (*Retama sphaerocarpa*, *Tamarix gallica*, *Rosmarinus officinalis* and *Myrtus communis*) were selected and their potential for restoration of the soils affected by the pyritic residue was assessed. Plant survival was negatively affected by soil acidity, which was the main factor controlling trace elements (TEs) solubility and soil microbial biomass, and therefore, soil quality. Nevertheless, the surviving plants were well developed and reached a large size at the end of the experiment (except *M. communis*). Trace element transfer from soil to harvestable parts was low for all species, and some species have been able to decrease TEs availability in the soil. The results suggest that *R. sphaerocarpa* was the most adequate plant species for the restoration of these soils, as it showed the highest survival rate, elevated tolerance to strong soil acidity and low TEs transfer factors.

1. Introduction

Mining activities generally involve the contamination of large areas with potentially toxic elements, such as trace elements (TEs), whose dispersion may suppose a serious threat to nearby communities and ecosystems (Alloway, 2013). In mine affected sites, unvegetated areas and tailings piles exposed to wind and water erosion and lixiviation are the main sources of contamination (Clemente et al., 2007; Zornoza et al., 2012). In addition, catastrophic tailing dam failures such as those that occurred in China, Romania, Sweden, USA, Spain, and Hungary are another possible contamination source associated to mining activities (Macklin et al., 2003). These accidents frequently result in huge amounts of highly polluted sludge being rapidly discharged into the ecosystem, covering land surface, destroying plant and wildlife, and the soil matrix becoming saturated with contaminant particles (Alloway, 2013).

In the last decades, phytoremediation has moved forward from a conceptual methodology to a practical and commercially-viable technology for environmental clean-up for both organic and some inorganic contaminants, such TEs (Dickinson et al., 2009). The primary aim of phytostabilisation is the reduction of contaminants' mobility and of the effects of pollutants on humans and ecosystems (Domínguez et al., 2008). Therefore, re-vegetation is the main goal of ecological restoration projects as the soil surface must be stabilised so that wind and water erosion are minimised and there is a reduced risk for humans and animals (Vangronsveld et al., 2009). The success of phytoremediation of TEs-contaminated sites requires a thorough understanding of the physico-chemical and biological constraints upon plant growth, as well as the combination of suitable soil amendments and well-chosen plant species that tolerate particular local conditions, including the elevated concentrations of TEs (Mench et al., 2009).

The Aznalcóllar mining district (Seville, SW Spain) includes several deposits of sulphide ore, from which Ag, Cu, Pb and Zn sulphides used to be separated by flotation from unwanted sulphides such as pyrite (López-Pamo et al., 1999). On April 1998, an accident at the Aznalcóllar mine provoked a toxic spill consisting of approximately 4 million m^3 of acid water and 2

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million m^3 of mining sludge, which were released through a breach that opened in the dam of the mine's tailings pond (Grimalt et al., 1999). Since then, different phytoremediation experiments have been carried out at the Aznalcóllar site (Clemente et al., 2006; Domínguez et al., 2008; Moreno-Jiménez et al., 2011), where elevated concentrations of toxic TEs remained in the soil after the removal of the pyritic sludge (Taggart et al., 2004; Vázquez et al., 2011). Long term reclamation of these soils requires the establishment of stable nutrient cycles for plant growth and microbial processes (Kavamura and Esposito, 2010; Lone et al., 2008; Singh et al., 2002). However, the success of remediation projects frequently remains unevaluated.

Restoration of the vegetation cover can fulfil the objectives of stabilisation, pollution control, visual improvement and removal of threats to human beings (Singh et al., 2002; Wong, 2003). The re-vegetation must be carried out with plant species selected on the basis of their ability to survive and regenerate in the local environment. In this sense, the use of native Mediterranean shrubs in phytoremediation is of special interest (Lepp and Dickinson, 1998), as they are well adapted to this specific environment, and avoids introduction of non-native and potentially invasive species, stimulating at the same time natural ecological succession (Mendez and Maier, 2008; Pardo et al., 2014a). The extensive canopy of this type of plants and their deep root network could effectively reduce leaching and wind erosion of contaminant particles, minimising the dissemination of the contamination while improving soil ecosystem functions (Pardo et al., 2014b).

In this study we sought to assess the success of re-vegetation with selected native shrubs (*Retama sphaerocarpa*, *Tamarix gallica*, *Rosmarinus officinalis* and *Myrtus communis*) as the final step of the restoration process of soils affected by residual contamination from the Aznalcóllar spill that had undergone an active phytoremediation intervention. The evolution of the main soil physico-chemical properties including TEs availability, plant survival, TEs accumulation by plants, and transfer factors were assessed during three years, once plants were well stabilised in the soil (one year after transplanting). At the end of the experiment, a soil quality index based on soil microbial biomass and soil physico-chemical properties was determined to assess the efficacy of the recovery process and to identify the most-interesting species for phytostabilisation.

2. Materials and methods

2.1. Experimental site

The experiment was performed in the experimental site "El Vicario", which is located on the right bank of the Guadiamar river (37°26′21″N; 06°13′00″W), 10 km downstream from the Aznalcóllar mine, and was affected by pyritic sludge contamination from the mine accident that occurred in 1998. This site was previously subjected to two different intervention steps: (i) a two year (2000–2001) active phytoremediation experiment, with the addition of organic amendments (compost and cow manure) and lime to the soil, and the growth of two successive crops of *Brassica juncea* (L.) Czern. as a metal-accumulator species; and (ii) a natural attenuation phase, without external intervention (2002–2005) (Clemente et al., 2003, 2006). The present experiment is framed in a third phase that sought the restoration of the site using native plant species (2006–2010). For this final phase, any possible remaining effects of the addition of amendments in the initial phase were disregarded, because of both the long period of time that had passed since the application of the amendments and the fact that the top 20 cm of the soil were tilled before the establishment of the new experiment. This soil presented a wide range of pH (from 2 to 7), low contents of organic matter (12–16 mg of TOC kg^{-1}) and carbonates (5%), and a loam texture. Total concentrations of TEs in these soils varied widely due to the patches of sludge that remained in the area: Zn (457–1617 mg kg^{-1}), Cu (126–305 mg kg^{-1}), Pb (172–839 mg kg^{-1}), As (135–634 mg kg^{-1}) and Cd (0.6–5.5 mg kg^{-1}), which also provoked high levels of DTPA-extractable Zn (up to 965 mg kg^{-1}), Cu (up to 109 mg kg^{-1}), Pb (up to 11 mg kg^{-1}) and Cd (up to 3.3 mg kg^{-1}) in the soil (Clemente et al., 2003, 2005, 2006).

2.2. Experimental design

The experimental area (18 × 24 m) was divided into 12 plots (8 × 4 m, distributed in two rows separated by a corridor of 1 m) for the development of the two previous intervention phases. Each plot was further divided into 6 subplots of approximately 2.6 × 2 m for the present experiment, resulting in a total of 72 subplots (Fig. 1).

Four native species were selected amongst those used for the Green Corridor of the Guadiamar river restoration project (Domínguez et al., 2008): *R. sphaerocarpa* L., *T. gallica* L., *R. officinalis* L. and *M. communis* L. These species had been previously studied to understand their interaction with TEs (Moreno-Jiménez et al., 2011, 2012). Seedlings of similar size (around 20 cm height), provided by the Red de Viveros (native plants nursery) of the Junta de Andalucía, were transplanted to the soil in December 2005. In each subplot, one plant of two of the selected species was grown combining large-sized and low-sized species in a random distribution, i.e. *R. sphaerocarpa* or *T. gallica* with *M. communis* or *R. officinalis* (Fig. 1). Three plants of each species were planted per plot; thus, a total of 36 plants of each species was grown in the experimental area. Plants were grown under natural conditions (average annual rainfall and temperature around 500 mm and 18–20 °C, respectively) without any agricultural practice or irrigation system.

Soil and plants were sampled at three different dates once plants were stabilised in soil (one year after transplanting): June 2007, June 2008 and May 2010. Soil sampling consisted of extracting three subsamples from the surface soil (<20 cm depth) in each subplot that were mixed to form a composite sample. Soil pH, electrical conductivity (EC), total (in the first sampling) and soluble and exchangeable ($CaCl_2$-extractable) concentrations of heavy metals (Cd, Cu, Fe, Mn and Zn) and As ($NaHCO_3$-extractable) were measured. Soil microbial biomass C and N were determined only in the last sampling as soil quality indicators, as these parameters are sensitive to trace element pollution (McGrath, 1994). Aerial plant material, consisting in young and fully-grown leaves from the middle part of the plants, was also collected at the three sampling times. Survival percentage of each plant species was monitored every 6 months after transplanting during the first year, and then annually until the end of the experiment.

2.3. Analytical methods

The soil samples were air-dried and sieved to <2 mm prior to analysis. Soil pH was measured for saturated pastes and EC was determined in 1:5 (soil:water) extracts. Soil pseudo-total heavy metals were determined after nitric–perchloric acid (2:1) digestion. Soluble and exchangeable metals were extracted with 0.1 M $CaCl_2$ (1:10 w/v) for 12 h. Available As concentrations in soil samples were measured after extraction (1:10 w/v) with 0.5 M $NaHCO_3$ (Clemente et al., 2006). Heavy metals were measured by flame atomic absorption spectrometry (FAAS, UNICAM 969, Thermo Elemental), and As concentrations were determined using electro-thermal atomic absorption spectrometry (ETAAS, PerkinElmer Analyst 800) or atomic fluorescence spectrometry (AFS, PS Analytical Millennium Excalibur System).

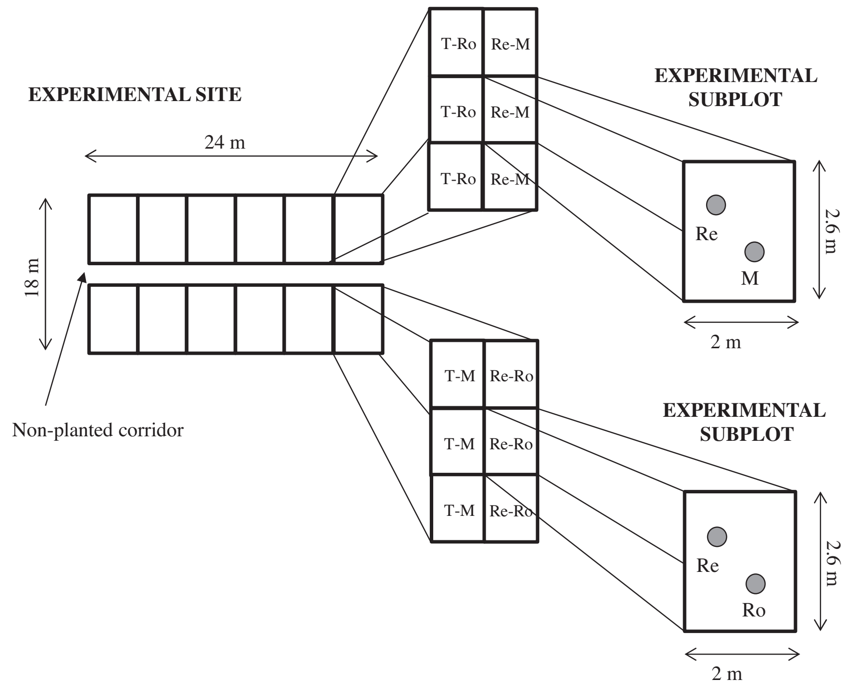


Fig. 1. Experimental set-up in the "El Vicario" site. M: *M. communis*, T: *T. gallica*, Re: *R. sphaerocarpa* and Ro: *R. officinalis*.

Soil microbial biomass carbon (B_C) and nitrogen (B_N) were determined after a fumigation-extraction method (Vance et al., 1987) in a total organic carbon analyser for liquid samples (TOC-V CSN + TNM-1 Analyzer, Shimadzu), and calculated according to Wu et al. (1990). All values were referred to oven-dried soil weight (105 °C for 24 h).

Plant samples were washed thoroughly with distilled water, dried at 70 °C for at least 48 h and ground. Plant material was subjected to a nitric–perchloric acid (2:1) digestion for determination of heavy metals (FAAS) and As (AFS) concentrations.

2.4. Data processing and statistical analysis

Due to the highly dispersed pattern of the data as a consequence of the heterogeneity of the contamination in the study site and of the elevated size of the dataset (n = 72), non-parametric statistics (box-plots) was used to assess the evolution with time of the different parameters determined. Pearson's coefficients were used to determine correlations between variables. The spatial distribution of the variables was depicted using diagrams resembling level curves, in which relative values are plotted to facilitate comparison between the different variables in the consecutive samplings. Simple linear regression, Student's T-test and correlations were also performed using the IBM SPSS 19 software package.

For the plants of the 2007 sampling, the transfer factor (TF) of the TEs was calculated as the ratio between the element concentration in the plant and its total concentration in soil ($[TE]_{shoot}/[TE]_{soil}$). The survival percentage of each plant species was determined by referencing the number of individuals found at each sampling time to those initially planted.

3. Results and discussion

3.1. Monitoring of soil physico-chemical properties

In all the samplings carried out during the restoration experiment, soil pH in the subplots ranged from 2.3 to 7.8 and up to 85% of the soil samples showed an acid character (Fig. 2). As already reported in the previous experimental phases, such acidity was a consequence of the oxidation of residual pyrite in the soil (Clemente et al., 2003, 2006). However, soil pH only slightly changed with time throughout the present experiment, mean values scarcely decreasing from 2007 to 2010 (Fig. 2), suggesting that pyrite oxidation was almost completed during the previous years after the pyritic spill. The electrical conductivity (EC) remained quite constant and did not show a clear trend with time (Fig. 2), and correlated negatively with soil pH ($P < 0.001$) when considering all the samplings. This correlation indicates that the main contribution to EC was the presence of high concentrations of soluble sulphates coming from the oxidation of the sludge, accumulated in the soil surface especially during dry seasons (Vázquez et al., 2011). Soils with pH values close to 7 had the lowest EC values, suggesting precipitation of soluble sulphates as $CaSO_4$ and the co-precipitation of metal (hydro) oxides (Clemente et al., 2003).

Soil pseudo-total concentrations (mg kg^{-1}) of metals and arsenic measured at the beginning of this phase of the experiment (June 2007) followed the order (mean ± standard deviation): Fe (42,113 ± 7654) > Mn (701 ± 299) ≈ Zn (531 ± 291) ≈ Pb (411 ± 150) > As (225 ± 69) ≈ Cu (199 ± 46) > Cd (9.6 ± 2.9). Most of the subplots showed As, Cd, Pb and Zn concentrations above the intervention levels established by the regional government of Andalucía in agricultural soils (As: >50, Cd: >7, Pb: >350, Zn: >600 mg kg^{-1}, for soils with a pH < 7; BOJA, 1999), and for As, even higher than the intervention levels established for natural parks and forest areas (>100 mg kg^{-1}; BOJA, 1999).

There was a large spatial heterogeneity in the concentrations of easily-available trace elements ($CaCl_2$-extractable fraction) in the experimental plots, associated with the uneven presence of the sludge that remained in the soils, as occurred with soil pH and EC. However, the spatial distribution observed for pH, EC and $CaCl_2$-extractable Cd, Cu, Mn and Zn remained almost the same throughout the experiment (from 2008 to 2010; Fig. 3). The values of $CaCl_2$-extractable metals concentrations found were within the same range as those reported by Clemente et al. (2006) for DTPA-extractable Cd (0.4–3.3 mg kg^{-1}), Cu (6–109 mg kg^{-1}) and

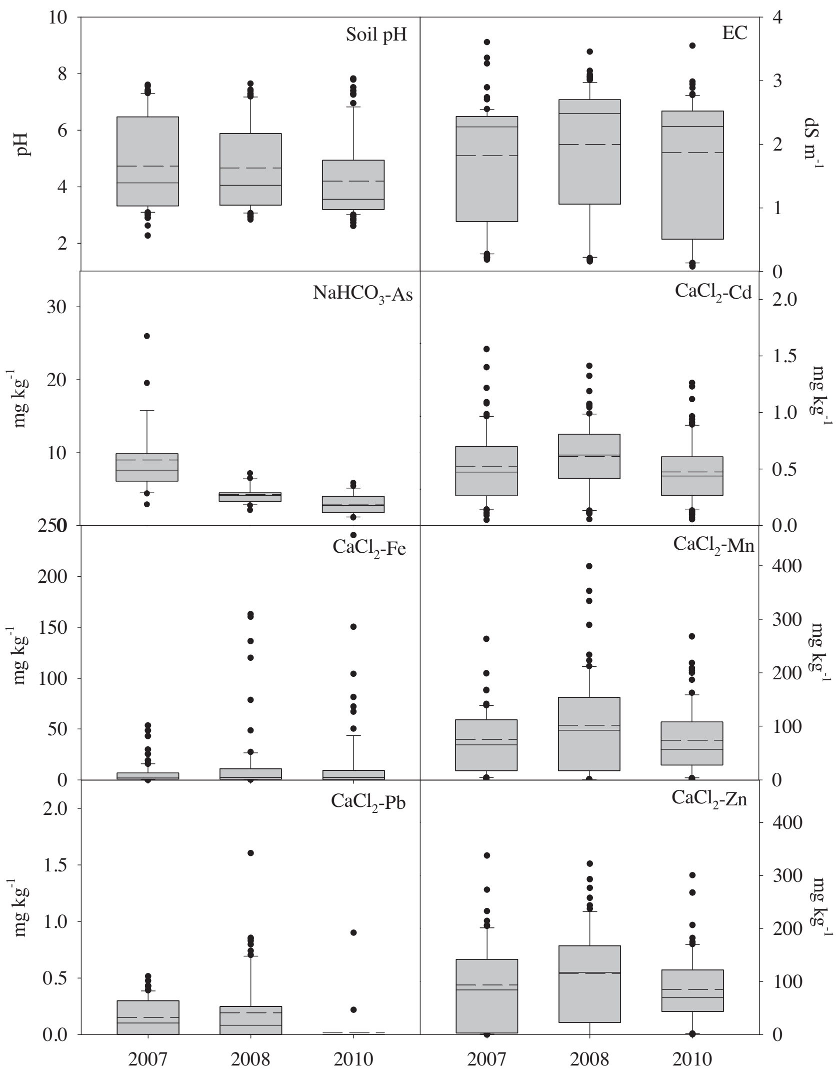


Fig. 2. Distribution of the pH values of the soil, electrical conductivity (EC) and concentrations of $CaCl_2$-extractable metals and $NaHCO_3$-extractable As in the different samplings. The box indicates the upper and lower quartiles, the dotted line within the box indicates the mean and the thin line indicates the median; the vertical lines indicate the 90–10 percentiles and the dots are outliers ($n = 72$).

Zn (31–965 mg kg^{-1}), but with the maximum concentrations found in the present experiment being clearly lower (Cd: 0.7 mg kg^{-1}, Cu: 19.4 mg kg^{-1} and Zn: 191 mg kg^{-1}), indicating certain reduction of the available pool of these elements in the soil (Clemente et al., 2003, 2006). Metal availability in the soil increased slightly from 2007 to 2008, but then showed a general decrease in 2010 (Fig. 2). A total of 71, 47, 65, 64 and 58% of the sampled soils showed significantly lower $CaCl_2$-extractable concentrations in 2010 for Cd, Cu, Fe, Mn and Zn, respectively, than those obtained in 2008.

As expected, Pb and Fe showed very low availability (<0.5% of the pseudo-total concentrations were found to be $CaCl_2$-extractable). Contrastingly, the percentages of extractable Mn, Cu and Zn were elevated, especially in soils with pH < 5 (4–35% for Mn, 0.7–13% for Cu and 7–53% for Zn) compared to those observed in soils with pH > 5 (from 0 to a maximum of 2.9, 0.2 and 1.6% for Mn, Cu and Zn, respectively). This fact agrees well with the strong negative correlation found for soil pH ($P < 0.001$) with the soluble concentrations of Mn, Cu and Zn in each sampling time throughout the restoration phase. Surprisingly, Cd did not show a similar behaviour: low percentages of pseudo-total Cd were found in $CaCl_2$-extractable form, even in soils with pH < 5 (values ranged from 1.3 to 14%), and no significant correlation with pH was found in 2010. Seven years after the mine accident, Álvarez-Ayuso et al. (2008) pointed out an important increase of Cd with the soil depth due to this metal being mostly leached through the surface acid soil layers. Therefore, the decrease in $CaCl_2$-extractable Cd at the end of the monitoring period (71% of the sampled soils showed lower concentrations of Cd in 2010 compared to the same soils in 2008) could be due partly to metal leaching during wet periods. Moreover, despite the high influence of soil pH on Cu, Fe, Mn and Zn availability found, the decrease of these elements extractability observed from 2008 to 2010 was not accompanied by a decrease in soil acidity, suggesting the influence of other soil factors. For instance, Clemente et al. (2006) suggested the possible adsorption of metals on soil minerals, as amorphous Fe and Mn oxides may have been formed in the soil as a consequence of the slow oxidation of pyrite coming from the sludge in the soil (Sun et al., 2012), and these compounds could retain soluble metals reducing their availability.

In 2007, available-As concentrations ($NaHCO_3$-extractable) in soils could be considered rather high (from 1.2 to 8.8% of pseudo-total As concentration in soil), although the availability of this element significantly decreased throughout the experiment (Fig. 2). Vázquez et al. (2011) also found a decrease of easily-

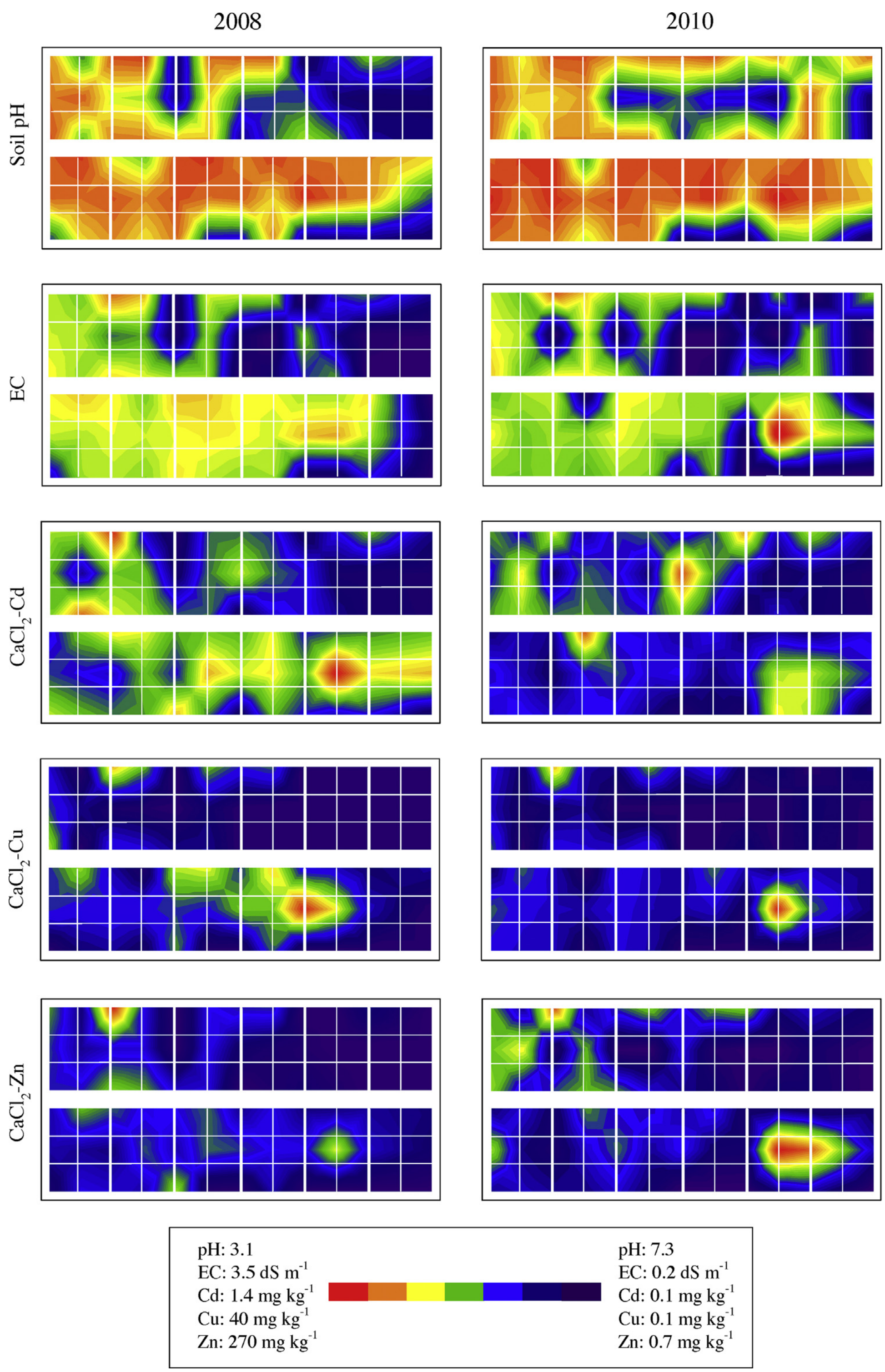


Fig. 3. Evolution of the spatial distribution of soil pH, electrical conductivity (EC) and concentrations of $CaCl_2$-extractable metals (Cd, Cu and Zn) in the experimental plots (see Fig. 1 for details).

available As in soils from a close area over time, suggesting a certain capacity of these soils for natural attenuation. In general, the highest extractable As concentrations occurred in soils with pH > 6 in all the samplings although significant and positive correlations were only found for $NaHCO_3$-extractable As concentrations and soil pH in June 2007 ($r = 0.532$; $P < 0.01$). This fact can be associated to the competition between OH-groups, present at alkaline pH values, and As oxyanions for the retention sites of the soil (Fitz and Wenzel, 2002). Nevertheless, the decrease in As availability observed with time could be due to its adsorption on iron oxides and hydroxides formed in the soil after sulphide oxidation (Sun et al., 2012), as they are known to be the principal retention agents for As in aerobic soils (Kabata-Pendias, 2001).

Even though significant differences between subplots regarding the presence of the different native plant species were not found (due to the spatial heterogeneity), the overall influence of the plant

Table 1
Metals and As concentrations ($mg\,kg^{-1}$) in shoots of plants growing in the experimental plots in 2007 and 2010, and their corresponding transfer factors (TF) in 2007.

Plant species	Cd		Cu		Zn		Fe		Mn		Pb		As	
	Jun-07	May-10	Jun-07	May-10	Jun-07	May-10	Jun-07	May-10	Jun-07	May-10	Jun-07	May-10	Jun-07	May-10
R. sphaerocarpa														
Mean	0.32	<0.01	8.64	10.3	74.6	105	157	55.5	131	53.5	0.38	<0.01	1.60	<0.01
sd	0.37		10.6	2.03	107	71.5	57.4	9.89	238	26.0	0.76		1.43	
TF[a]	0.03		0.04		0.19		0.00		0.26		0.00		0.01	
R. officinalis														
Mean	0.33	<0.01	7.54	6.97	59.6	62.8	385	86.5	36.6	19.5	1.97	<0.01	5.19	0.10
sd	0.10		2.33	1.02	26.2	21.1	314	35.7	13.5	7.56	3.71		8.51	0.19
TF[a]	0.04		0.04		0.12		0.01		0.05		0.00		0.02	
T. gallica														
Mean	0.59	<0.01	8.59	6.01	64.1	111	215	80.3	43.5	43.9	0.21	<0.01	0.60	<0.01
sd	0.31		3.44	1.34	27.3	49.4	226	7.56	42.3	23.6	0.51		0.47	
TF[a]	0.06		0.04		0.11		0.01		0.07		0.00		0.00	
M. communis														
Mean	0.65	<0.01	12.5	7.05	351	248	378	274	319	116	0.69	1.51	2.12	1.15
sd	0.70		4.40	0.79	374	30.8	152	53.1	331	58.5	3.08	0.54	1.90	0.44
TF[a]	0.07		0.07		1.10		0.01		0.71		0.00		0.01	

[a] Mean value (*R. sphaerocarpa* $n = 13$; *R. officinalis* $n = 8$; *T. gallica* $n = 6$; *M. communis* $n = 4$). sd: standard deviation.

establishment was assessed by observing the changes with time of the soil physico-chemical properties of the subplots where plants were able to grow. In these subplots, significant differences were only observed for the concentrations of $CaCl_2$-extractable Mn and Zn, which decreased ($P < 0.05$) from 2007 to 2008 ($\Delta Mn = -26.5$ $mg\,kg^{-1}$ and $\Delta Zn = -21.9\,mg\,kg^{-1}$), and then increased in the last sampling ($\Delta Mn = 28.2\,mg\,kg^{-1}$ and $\Delta Zn = 30.5\,mg\,kg^{-1}$, $P < 0.01$), opposite to the general trend observed in the plots. Contrastingly, in the soils with plants the easily available fraction of As showed significant decreases ($P < 0.001$) throughout the experiment, reaching an $\Delta As = -6.0\,mg\,kg^{-1}$ between 2007 and 2010, similar to the general trend observed in the soil. Our results agree with those of Moreno-Jiménez et al. (2011) who found a significant depletion of As extractability when *R. sphaerocarpa* and *M. communis* were grown in soils with $pH < 5$. As commented later in the text (Section 3.3) the amount of TEs retained in plant tissues (Table 1) in our experiment was very low in comparison with the available pool in the soil. So, reductions in the available fraction of TEs cannot be attributed to their absorption and accumulation by the plants, but to rhizosphere processes, such as chelation by plant exudates and immobilisation by root-associated microorganisms that indeed reduced TEs uptake and translocation to the aerial parts of the plants (Martínez-Alcalá et al., 2009).

3.2. Soil microbial biomass C and N concentrations as indicators of soil quality

Soil microorganisms play an essential role in the ecosystem, as they are involved in different critical soil functions such as in biogeochemical cycles of the main nutrients and in OM degradation (Gil-Sotres et al., 2005). Therefore, due to their relation with the soil functionality and their high sensitivity to TEs, microbial properties have been widely used as indicators of soil quality in TEs contaminated soils (Hinojosa et al., 2004; Zornoza et al., 2012). In this sense, soil microbial biomass C (B_C) and N (B_N), as an estimation of the microbial community size in the soil, have been frequently used in order to monitor the success of the reclamation process of contaminated land (Clemente et al., 2007; Pardo et al., 2014a; Zornoza et al., 2012), and to evaluate the efficiency of phytostabilisation processes (Pardo et al., 2014b).

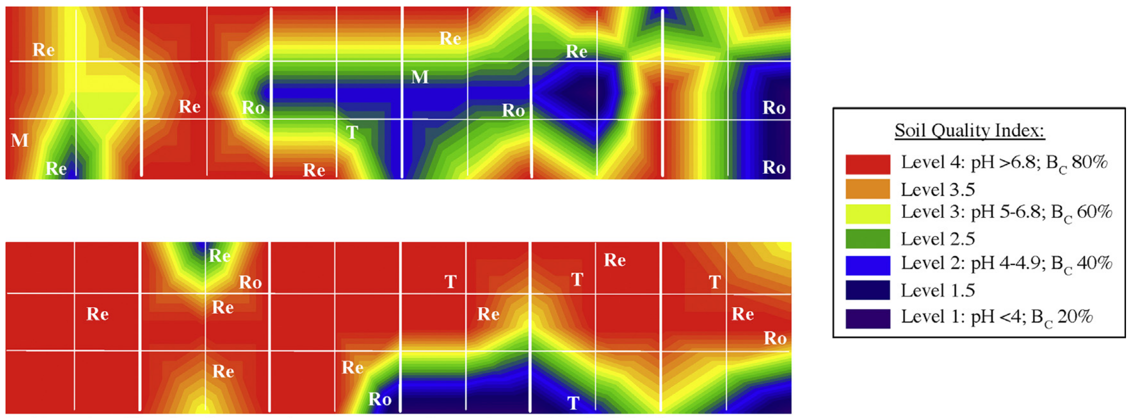
At the end of the experiment, these parameters (B_C and B_N) showed high variability among soils, reaching values from 18.7 to 667 for B_C and from 1.2 to 82.7 for B_N ($mg\,kg^{-1}$). The lower values were within the same range as those in untreated soils surrounding the treated plots (Burgos et al., 2010; Perez de Mora et al., 2006) and in other TEs-contaminated soils (Clemente et al., 2007; de la Fuente et al., 2010; Zornoza et al., 2012), reflecting the poor microbiological status of this type of soils. However, it is worth mentioning that the largest concentrations observed were within the same range as that observed in soils remediated with organic amendments (Pardo et al., 2014b; Perez de Mora et al., 2006), reflecting the success of the restoration programme applied here.

In the present experiment, concentrations of B_C positively correlated with soil pH ($r = 0.912$, $P < 0.001$) and negatively with $CaCl_2$-extractable Mn ($r = -0.540$, $P < 0.01$), Cu ($r = -0.797$, $P < 0.001$) and EC ($r = -0.811$, $P < 0.001$), showing the influence of these soil properties in microbial growth and in their spatial distribution in the soil. TEs are known to be toxic for soil microorganisms, as they may provoke functional alterations, protein denaturation and cell membranes modifications, influencing their growth and metabolism (Leita et al., 1995). Several studies have reported that high TEs-availability in polluted soils negatively affects the size of the soil microbial communities, resulting in low B_C and B_N concentrations (de la Fuente et al., 2010; Perez de Mora et al., 2006; Zornoza et al., 2012).

When linear regression analyses were performed (both forward and backward), the following equation was the most-significant:

$$B_C(mg\,kg^{-1}) = -414 + 139pH; P < 0.001; F = 111$$

This equation indicates that soil pH was the main factor controlling microbiological status of soil, and therefore, soil biological quality, in agreement with the results obtained by Clemente et al. (2003) in the same experimental plots in the first experimental remediation phase. At that stage plant survival (*Brassica juncea* was used in that experiment), biomass production and heavy metal bioavailability were also mostly conditioned by soil acidity. Given the representativeness of the B_C-pH relation observed in the present experiment, a soil quality index based on the regression equation was calculated. Then, four intervals of soil pH were selected to determine the different quality levels: level 1, $pH \leq 3.9$; level 2, pH between 4 and 4.9; level 3, pH between 5 and 6.8; and level 4, $pH > 6.8$ (corresponding to 20%, 40%, 60% and 80% of the maximum B_C concentrations found in the experimental site,



T: *Tamarix gallica*; **Re:** *Retama sphaerocarpa*; **Ro:** *Rosmarinus officinalis*; **M:** *Mirtus communis*.

Fig. 4. Spatial distribution of the soil quality index based on soil microbial biomass-C (B_C) and soil pH, and plants survival (white labels) in 2010 in the experimental plots (see Fig. 1 for details).

respectively). These levels were used to depict the spatial distribution of soil microorganisms in the experimental site (Fig. 4).

3.3. Viability of plant species: survival and TEs accumulation

The first year of the present experimental phase was crucial for plant species survival (Fig. 5). Six months after transplanting (May 2006), plant survival was around 50% for all species and decreased a few months later (after the first summer), except for *R. sphaerocarpa*. But since then plant survival remained constant (Fig. 5). At the end of the experiment, *R. sphaerocarpa* showed the highest success regarding plant survival (44%), while those of *R. officinalis*, *T. gallica* and especially *M. communis* were rather low (<20%). A similar behaviour was found by Moreno-Jiménez et al. (2011) in a field experiment using the same species in soils from the same area. These authors reported the highest survival rate for *R. sphaerocarpa* (34% and 24% in soils with pH > 5 and in soils with pH < 5, respectively, after 2 years of field experiment). Despite the low survival values reported in the present experiment, the surviving specimens were fully and healthy developed, and reached a large size (average height of adult plants was around 160–190 cm, except for *M. communis* that did not exceed 25 cm height; Supplementary Information (SI), Fig. S1).

The vegetation and the soil microflora of an ecosystem are closely interrelated. Plants influence soil microbial processes by delivering organic compounds and improving soil conditions (Moynahan et al., 2002), whereas soil microorganisms have a positive impact on plant growth by the decomposition and mineralisation of plant material (Gil-Sotres et al., 2005). Numerous studies have reported the relation between stimulation of microbiological parameters and plant presence in TEs contaminated soils, this showing their relevance in the recovery of soil health (Pardo et al., 2014a, 2014b; Zornoza et al., 2012). However, in the present study plant survival and B_C were not clearly related (probably due to the spatial heterogeneity; Fig. 4), and plant survival was not always related with the highest quality levels proposed (based on soil pH and B_C). In fact, many of the developed plants at the end of the experiment had grown in soils with the lowest quality. Nevertheless, the relation between the proposed quality index and the presence of each plant species allowed observing trends in their preferences for different soil conditions. *R. sphaerocarpa* was able to grow even in soils with the poorest quality and tolerated acidic soil conditions as it was able to grow at soil pH as low as 3.1 (Figs. 3 and 4). This xerophytic shrub, the only leguminous species used in this experiment, has been frequently used in the restoration of semi-arid land, and has caused significant improvements in the physico-chemical and biological properties of the rhizosphere soil associated in part with its nitrogen fixing capacity (Valladares et al., 2002). *R. sphaerocarpa* creates favourable conditions in the rhizosphere for the development of microorganisms, which can accelerate nutrient cycles in soil and may in turn facilitate the survival of this plant species (Moreno-Jiménez et al., 2011). Contaminant elements in soil inhibit enzymatic activities, but rhizosphere habitats protect microorganisms and partly alleviate the toxic effects (Martínez-Iñigo et al., 2009). This species also has a deep root system (Haase et al., 1996), which provides access to deep water sources during dry seasons and may help to avoid toxicity of metals retained in the surface soils at the Aznalcóllar site (30–60 cm depth; Álvarez-Ayuso et al., 2008).

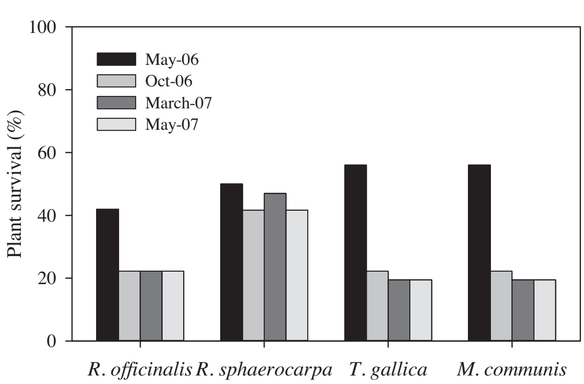


Fig. 5. Plant species survival (2006–2007) in the experimental plots.

Both *R. officinalis* and *T. gallica* grew only in subplots with intermediate quality levels (Fig. 4). Although the survival of *R. officinalis* and *T. gallica* was very low, the plants showed good appearance and development; these two species are considered as drought- and salt-tolerant plants, which could explain their ability to become established in these soils with a high electrical conductivity (Fig. 3). Contrastingly, *M. communis* was only found in sub-plots with the highest quality and the survival of this species was extremely low, and still alive plants were rather small showing impaired development.

A high variability was found in heavy metal concentrations in plant tissues in the present experiment, which could explain the absence of correlations between heavy metal concentrations in plants and in soils. In general, the concentrations of heavy metals and arsenic in plant tissues (Table 1) were within normal ranges (Kabata-Pendias, 2001). Only Cd concentrations in plants sampled in 2007 were above normal levels for all the species, but none of them can be considered toxic (5–30 mg kg^{-1}; Kabata-Pendias, 2001). *M. communis* showed the highest concentrations of most heavy metals in 2007 and 2010, reaching toxic concentrations of Zn (>100–400 mg kg^{-1}; Kabata-Pendias, 2001), which could partially explain the low survival of this species. Therefore, the mechanism of this plant species for limiting transport of Zn may have been altered. In fact, the transfer factor for Zn was >1 (Table 1), indicating transport from soil to the plants aboveground parts.

Trace element concentrations in plant tissues remained almost constant throughout the experiment and no significant differences were found between species (Table 1). The *t*-test for related samples showed only significant decreases in Fe (ΔFe = −103 mg kg^{-1}; $P < 0.001$) and As (ΔAs = −1.6 mg kg^{-1}; $P < 0.01$) concentrations in *R. sphaerocarpa* and in Mn concentration in *R. officinalis* (ΔMn = -16.2 mg kg^{-1}; $P < 0.05$) from 2007 to 2010. The transfer factor (Table 1) was generally bellow 1, indicating a low and controlled transport of contaminants from soil to shoots and therefore plants TE exclusion mechanisms. This is a common mechanism for plant resistance (Clemens et al., 2002; Dahmani-Muller et al., 2000) and this excluder behaviour was reported before for these Mediterranean shrubs (Moreno-Jiménez et al., 2011). Thus, restoration with these species is a good option for the remediation of the area, with a low risk for food chain transfer (Domínguez et al., 2008) and development of a permanent plant cover.

4. Conclusions

Soil pH was the main factor controlling soil microbial biomass, TE solubility and EC, which condition soil quality. After 10 years of the pyritic spill the soil properties were stabilised from a chemical point of view. The selected plant species were fully developed and reached a large size four years after their transplanting, except *M. communis*. *R. sphaerocarpa* showed the highest survival rate and high resistance to strong soil acidity, and seems the most promising species regarding successful soil remediation potential. This species was able to grow even in the poorest soils (low pH, high EC and high $CaCl_2$-extractable metals), maybe due to different adaptations responses that allowed this plant to survive in this area. Low transfer of contaminants from soils to plants was found, linked to a certain capacity of these soils for natural attenuation, which point out phytostabilisation as the best alternative for soil reclamation.

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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.agee.2014.06.030.

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