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Residual pollution and vegetation distribution in amended soils 20 years after a pyrite mine tailings spill (Aznalcóllar, Spain)

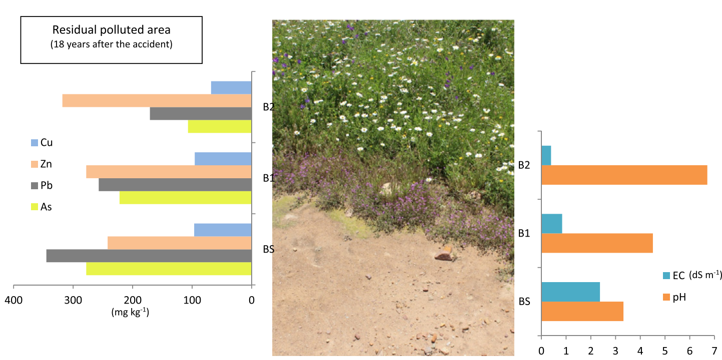
M. García-Carmona [a], H. García-Robles [b], C. Turpín Torrano [a], E. Fernández Ondoño [a], J. Lorite Moreno [b], M. Sierra Aragón [a,*], F.J. Martín Peinado [b]

[a] Departamento de Edafología y Química Agrícola, Universidad de Granada, Campus Fuentenueva s/n, 18001 Granada, Spain
[b] Departamento de Botánica, Universidad of Granada, Campus Fuentenueva s/n, 18001 Granada, Spain

HIGHLIGHTS

- Areas with residual polluted soils persist in the Guadiamar Green Corridor 18 years after the recovery.
- High concentrations of potentially bioavailable metal(loid)s were found in some soils in the area.
- Plant richness and vegetation cover shows small-scale differences in spatial distribution.
- Transfer of trace elements to plants poses a risk of pollution for the ecosystem and livestock.

GRAPHICAL ABSTRACT



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ABSTRACT

The present work assesses the residual pollution in the Guadiamar Green Corridor (SW, Spain) after a long-term aging process (18 years) since the accident of the Aznalcóllar pyrite mine. We have focused on the study of trace elements (Cu, Zn, Cd, As and Pb) in soils, their fractionation and the transference to the surrounding vegetation. The residual polluted areas are characterized by scattered plots with absence of vegetation, presenting high concentrations of trace elements, acidic pH and low organic carbon content. Surrounding these polluted plots, two vegetation gradient belts are clearly identified by changes in plant cover and richness. The inhibition of plant growth in the bare soils is related to the highest mobility of soluble and exchangeable Cu, Zn and Cd forms, which significantly decrease with the distance to the polluted plots.

Plant richness and cover show differences between belts; bioaccumulation of trace elements in plants also differs, with a preferential accumulation in roots. Despite the low bioavailability of As and Pb in soils, bioaccumulation factors in plants for these elements are significantly higher in belt 1 in relation to belt 2. High Cu and Cd potential toxic concentrations in aerial parts of vegetation are found, posing a risk for livestock and a potential entrance to the food-chain. On the other hand, *Lamarckia aurea* (L.) Moench (in belt1) and *Trifolium campestre* Schreb. (in belt2) were the most dominant species in severely polluted soils. Elevated concentrations of trace elements in the vegetation growing in the area indicate plant adaptation mechanisms to live in these severely polluted soils, which can be used as a good bioindicator of pollution in similar polluted areas.

* Corresponding author.
E-mail address: msierra@ugr.es (M. Sierra Aragón).

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1. Introduction

The potential of trace elements (TE) to degrade ecosystems and their significant toxicological risk for human health by passing through the food-chain are widely known. Otherwise, soil is an essential component of ecosystems, with a great capacity to cope with pollution, and so to protect the other components, both abiotic (air and water) and biotic ones (living organisms). There are many processes that influence toxicity in soils over time (Lock and Jannsen, 2003); soil properties largely control the mobility, bioavailability and consequently, the potential toxicity of trace elements in the environment. Since total concentration of TE provides limited information in relation to their toxic effects, selective extractions are a more suitable approach to assess the TE fractionation in soils and thus their bioavailability for living organisms (Margui et al., 2007; Quevauviller et al., 1998). Plants absorb TE from the soil in their bioavailable forms or solubilize them with their root exudates. Some TE are essentials for plant growth and development, such as Cu and Zn, but over a certain threshold of concentration, they become toxic for plants, as it happens with non-essential elements. Hence, the potential TE toxicity for plants primarily depends on phytoavailability. This strongly relies on the uptake capacity of each plant species, and on TE concentration and bioavailability in soils (Liu et al., 2013), where soil physicochemical properties like pH, Eh, water regime, clay content, SOM, CEC and nutrient balance, are key factors in the control of TE mobility (Kabata-Pendias, 2011).

Plants can act as bioindicators of soils contaminated by TE due to their capacity of interacting with them. To cope with TE toxicity, plants adopt different strategies, including exclusion mechanisms and prevention of transfer to aerial parts, or accumulation in the above-ground tissues (Raskin et al., 1994; Sarwar et al., 2017). Plants that concentrate TE above normal values are called hyperaccumulators (Chaney, 1983). From a restoration point of view, the ability to grow in highly polluted soils is interesting for phytomanaging soil pollution (Moreno-Jiménez et al., 2009), since it is a cost-effective and environmentally friendly technique to decrease environmental risk in soils affected by mine tailings (Parraga-Aguado et al., 2014). The restoration of vegetation cover can stabilize and control the dispersion of pollutants throughout the ecosystem, which can also avoid soil erosion (P. Madejón et al., 2006). However, accumulation of TE in excess on plant tissues can create an exposure pathway into the food-chain (Christou et al., 2017; McLaughlin et al., 1999).

A remarkable example of the damage that this type of pollution can produce, was the mine spill occurred in 1998 in Aznalcóllar (SW Spain), where 45×10^5 m^3 of acidic waters and toxic tailings were spilled into the Agrio and Guadiamar rivers (Simón et al., 2001), finally affecting 45 km^2 of soils, mainly with agricultural use. However, the environmental impact was highly minimized thanks to the high buffer capacity of soils (Aguilar et al., 2007; Simón et al., 2008) and the rapid restoration of the affected area. During the following three years after the accident, an important restoration program was implemented to recover the affected area, which involved the removal of tailings and heavily polluted soils, the extensive application of organic and inorganic amendments, and the general phytostabilization of the area (Aguilar et al., 2004; Simón et al., 2008). The restoration concluded with the affected area reconverted in a natural protected area, the Guadiamar Green Corridor (CMA, 2003). Multiple monitoring studies have been conducted on the affected area since then, deepening the knowledge about long-term TE pollution and the evolution of the remediation (Madejón et al., 2018). Eighteen years after the accident, despite the aging process decreasing the pollutant concentration and bioavailability in soils, residual pollution is still found in the area (Martín et al., 2015), posing a toxic risk to living organisms (García-Carmona et al., 2017; Romero-Freire et al., 2016a). The residual pollution is easily identified in soils by the total absence of vegetation; those areas are surrounded by soils with a gradual change in plant cover and richness, which indicates an interaction between pollutants and vegetation that should be monitored over time.

Bare soils in Aznalcóllar are a synonym of severe pollution, posing a risk for the safety of the surrounding ecosystem (García-Carmona et al., 2017). More information is needed on the TE mobility in long-term contaminated soils and their influence in the soil-plant system. In this study, we aimed at investigating the fractionation of the main trace elements in highly polluted soils and the influence of soil properties, in order to determine the phytoavailability and its effect on the vegetation distribution. Therefore, physicochemical soil properties and constituents, mobility and bioavailability of pollutants (Cu, Zn, Cd, As and Pb) using selective extraction methods and species richness and cover were measured in the area. In addition, TE bioaccumulation in plants was analyzed in order to study their response against highly polluted soils and so, to assess the potential environmental risk to the ecosystem.

2. Material and methods

2.1. Soil and vegetation samples

Soils with residual pollution were detected in previous works (Martín et al., 2015) using satellite images from Google Earth for the identification and quantification of the bare soil surface. Soils without vegetation were concentrated in the first 18 km downstream from tailing pond, representing about 7% of the total area affected by the spill. The residual polluted areas were randomly distributed, and had a highly variable size, with bare soil spots from <1 to >200 m^2.

Four residual polluted plots were selected and divided into three subareas according to a soil recovery gradient in terms of presence of vegetation. Soils were sampled from the bare soil parts (BS) right in the centre, to the two surrounding belt subareas characterized by a progressive vegetation appearance, the intermediate moderately revegetated belt (B1) and the outer and more recovered belt (B2) (Fig. 1). Soil samples were taken from the upper soil layer (0–10 cm), with three replicates composed of 5 soil sub-samples (center and four corners from a square meter) and intensively homogenized to provide a single sample for each plot.

Vegetation samples representing the whole community were collected in the two vegetated belts for the four selected plots. A 50 $\times$ 50 cm^2 grid divided into 100 cells was used for the study of plant cover and species richness.

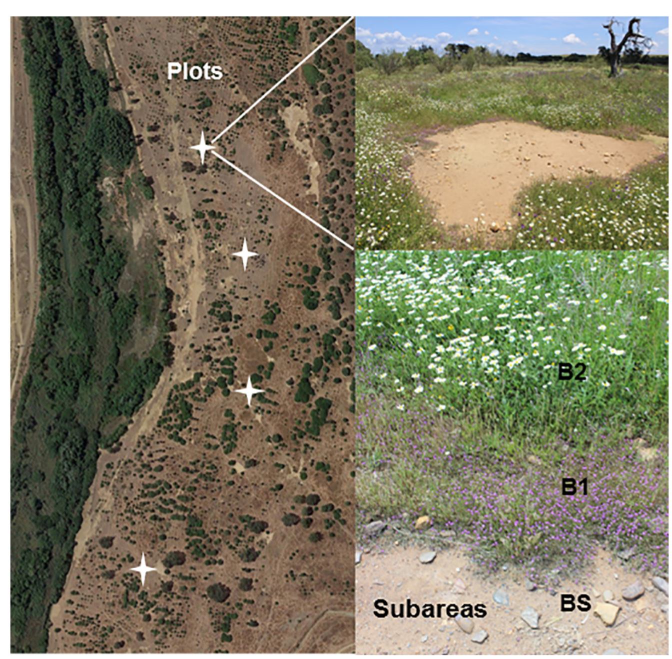

Fig. 1. Plots location in the Guadiamar Green Corridor, and a detail of the different subareas divided by different vegetation composition (BS: bare soils; B1: belt 1; B2: belt 2).

2.2. Analytical methods

2.2.1. Soil analysis

Soil samples were air dried at room temperature and sieved at 2-mm. Soil texture was determined by the Robinson pipette method (Soil Conservation Service, 1972); calcium carbonate content by volumetric method (Barahona, 1984); soil pH was measured in water and 0.1 M KCl in a ratio 1:2.5 with a 914 pH/Conductometer Metrohm; total organic carbon (OC) was analyzed by a LECO® TruSpec CN (St. Joseph, MI, USA) after soil samples were acid-washed (HCl 1 mol/l for 24 h) to remove carbonates, following Ussiri and Lal (2008); soil: water extract (1:5) was prepared to determine the electrical conductivity (EC) using a Eutech CON700 conductivity-meter; cation exchange capacity (CEC) was determined according to the methodology of the Soil Conservation Service (1972). Amorphous iron and manganese oxides (Fe_o, Mn_o) were extracted according to Schwertmann and Taylor (1977) procedure and measured by atomic absorption spectroscopy (SpectrAA 220FS Varian).

Total concentrations (Cu, Zn, Cd, As and Pb) were determined in soils by X-ray fluorescence in a NITON XLt 792 analyzer, with a 40 kV X-ray tube with Ag anode target excitation source, and a Silicon PIN-diode with a Peltier cooled detector. The procedure followed the manufacturer's instructions and the recommendations of the Method 6200 (US EPA, 1998). The accuracy of the method, the analytical precision and the detection limits were evaluated according to US EPA (2006) in Martín Peinado et al. (2010).

2.2.2. Trace elements fractionation

Selective extractions were performed in order to assess the potential mobility and bioavailability of trace elements in soils, determining from mobile or available fractions to unavailable fractions. Soluble fraction (S) was extracted by distilled water from soil:water extract 1:5 according to Sposito et al. (1982). Exchangeable fraction (E) was obtained by 0.01 M $CaCl_2$ extraction according to Novozamsky et al. (1993). Bioavailable fraction (B) for plants was extracted using 0.05 M EDTA (pH 7) as described by Quevauviller et al. (1998). Fraction bounded to amorphous Fe/Mn oxides (O) was extracted by 0.1 M oxalic acid-ammonium oxalate extract (pH 3) as described by Schwertmann and Taylor (1977). Total concentrations (T) of main pollutants (Cu, Zn, Cd, As and Pb) were additionally obtained by strong acid digestion (HNO_3:HF, 3:1) in a microwave oven XP1500Plus (Mars®). Trace element content in all extracted fractions was analyzed by inductively coupled plasma-mass spectrometry (ICP-MS) in a PE SCIEX ELAN-5000A spectrometer. The accuracy of the method, the analytical precision and the detection limits are detailed in Romero-Freire et al. (2016b).

Residual fraction (R) was calculated as the difference between the total fraction (T) and the fraction with the most effective extractant for each element. The oxalic acid- ammonium oxalate reagent was generally the most effective for all the elements. According to Ure (1995), this reagent can extract the fraction of the elements bound to oxides and secondary clay minerals, the organically bound, exchangeable and water-soluble forms.

2.2.3. Plant analysis

Collected plants were divided into aerial and root parts to analyse them separately. The different parts were washed with distilled water, dried (70 °C for 48 h), ground and digested in a microwave XP1500Plus (Mars®) in HNO_3:H_2O_2 (1:1) (Sah and Miller, 1992). Concentration of micro and macronutrient elements in plants (Fe, Mn, Ca, Mg, K, Na) were measured by atomic absorption spectrometry (SpectrAA 220FS Varian) and potential pollutants (Cu, Zn, Cd, As and Pb) by ICP-MS in a PE SCIEX ELAN-5000A spectrometer.

Bioaccumulation factor (BAF) for the uptake of trace elements by vegetation was calculated by dividing concentrations in plants (mg kg^{-1} dry weight) by TE bioavailable concentrations (extracted by EDTA) in the tested soils (mg kg^{-1} dry soil) (Kidd et al., 2007; Wang et al., 2006). Concentrations extracted by EDTA were selected for BAF calculation as this fraction represents the bioavailable forms of TE for plants (Margui et al., 2007; Parra et al., 2014).

Richness and plant cover were also estimated. Richness was calculated as the number of species found per grid, and species cover as the percentage of the total surface covered by a given specie. The 50 × 50 cm^2 grid was randomly placed and replicated three times per sampling site.

2.3. Data analysis

Non-parametric Kruskal-Wallis test for the analysis of mean comparison was chosen due to sample size (Theodorsson-Norheim, 1986). Significant differences were determined by Kruskal-Wallis post hoc test ($P < 0.05$). In order to analyse the influence of soil properties on the selective extractions of metals, Spearman's correlations were performed. PCA analysis was made to assess relationships among trace elements, and a non-metric multidimensional scaling (NMDS) ordination diagrams were performed to determine the effects of trace elements concentration in the vegetation response. All these analyses were performed with a confidence level of 95% by using SPSS v.20.0 (SPSS Inc., Chicago, USA), and the non-metric multidimensional scaling (NMDS) with Rstudio (2015) (Rstudio Team, Boston, USA).

3. Results and discussion

3.1. Soil properties

Soil properties analyzed in bare soils (BS) and in the surrounding vegetated areas (B1 and B2) showed significant differences in most parameters (Table 1). Bare soils were extremely acidic (pH < 4), showed high EC (>2.3 dS m^{-1} in a 1:5 soil:water extract) and an elevated content in total iron (Fe_T). These conditions, along with the high presence of trace elements (TE), are a consequence of the continued contamination process, characterized by the oxidation of the remaining tailings in soils, as a result of the rapid and deficient first extensive cleanup operation that mixed the tailings with the soil in depth (Simón et al., 2008). Strong acidity, high EC, and low OC have a severe impact in the mobility of trace elements (Ivezić et al., 2012; Rodríguez-Vila et al., 2017), being these

Table 1
Main soil properties of the subareas: bare soils (BS), belt1 (B1) and belt2 (B2). (EC: electrical conductivity; OC: organic carbon content; $CaCO_3$: calcium carbonate content; CEC: cation exchange capacity; V: base saturation percentage; Fe_t/Mn_t: total concentration of Fe and Mn; Fe_o/Mn_o: amorphous concentration of Fe and Mn; sd: Standard deviation).

	BS		B1		B2	
	Mean	*sd*	Mean	*sd*	Mean	*sd*
Clay (%)	24.23	*4.98*	19.6	*6.66*	22.89	*5.03*
Coarse silt (%)	13.37b	*2.00*	13.00b	*0.32*	11.19a	*0.78*
Fine silt (%)	17.47	*1.28*	16.13	*3.25*	18.95	*2.82*
Sand (%)	44.94	*6.46*	51.28	*9.63*	46.97	*7.74*
Gravel (%)	3.97	*1.77*	3.41	*1.21*	4.67	*2.21*
pH (H_2O)	3.32a	*0.28*	4.51b	*0.40*	6.71c	*1.16*
pH (KCl)	3.40a	*0.27*	4.19b	*0.35*	6.33c	*0.87*
EC (dS m^{-1})	2.37b	*0.41*	0.84a	*0.83*	0.39a	*0.13*
$CaCO_3$ (%)	0.68a	*0.05*	0.67a	*0.09*	1.39b	*0.67*
OC (%)	0.79a	*0.15*	2.01b	*0.70*	2.42b	*0.28*
Ca^{2+} ($cmol_+$ kg^{-1})	6.33a	*1.6*	7.53a	*1.61*	11.01b	*0.60*
Mg^{2+} ($cmol_+$ kg^{-1})	2.08b	*0.61*	0.84a	*0.68*	0.62a	*0.07*
K^+ ($cmol_+$ kg^{-1})	0.03	*0.01*	0.04	*0.01*	0.04	*0.01*
Na^+ ($cmol_+$ kg^{-1})	0.07a	*0.02*	0.11ab	*0.02*	0.12b	*0.02*
CEC ($cmol_+$ kg^{-1})	8.99a	*0.46*	10.55b	*0.94*	11.79b	*0.62*
V (%)	94.45ab	*11.10*	80.75a	*13.32*	100.00b	*0.00*
Fe_T (mg kg^{-1})	40802b	*1935*	33242a	*4801*	30818a	*2814*
Fe_O (mg kg^{-1})	5641b	*747*	3812a	*954*	4393ab	*653*
Mn_T (mg kg^{-1})	387.3a	*34.6*	365.3a	*33.8*	740.9b	*53.9*
Mn_O (mg kg^{-1})	181.1a	*53.7*	190.8a	*46.4*	574.9b	*90.9*

Lowercase letters represent significant differences among areas (Kruskal Wallis test P < 0.05).

parameters directly related to the seed emergence inhibition in BS. In contrast, this situation was different in the subareas surrounding the unvegetated soils (B1 and B2), where pH rose from 4.5 in B1 to 6.7 in B2, and $CaCO_3$ progressively increased. Calcium carbonate was intensely applied in the affected soils during the restoration actions, what enhanced the pH and enabled the establishment of vegetation (Aguilar et al., 2004). The EC significantly decreased in belt areas (B1 and B2) in relation to bare soils (BS), also promoting favorable conditions for the establishment of vegetation. The presence of vegetation over time produced a significant increase of CEC and OC in both belt areas (B1 and B2) in relation to the bare soils (BS).

3.2. Trace elements in soils

Total concentrations measured in these residual polluted plots were compared with the background values reported by Simón et al. (1999) for the soils in the area which remained unaffected by the mine spill. In all cases, concentrations of trace elements studied exceeded the background concentrations, with maximum values exceeding 19 and 10-fold in the case of As and Pb, respectively, and 3, 2 and 2.5-fold in the case of Cu, Zn and Cd, respectively. Furthermore, As and Pb concentrations exceed the established levels (36 and 275 mg kg^{-1}, respectively) used for declaring a soil as contaminated by the Regional Government in Andalusia (Spain) (Decreto18/2015).

Changes in soil TE solubility over time within the affected area by the influence of soil properties were previously reported (Martín et al., 2015; Romero-Freire et al., 2016a). The application of different amendments, including organic matter, iron-rich clayey materials, and sugar-refinery scum (rich in calcium carbonate), modified the properties in the treated soils, promoting changes in the TE mobility. To assess the current mobility and bioavailability of trace elements in the affected soils and, hence, the influence on the vegetation distribution, soil TE fractionations were carried out. Trace elements fractions were analyzed from mobile or available forms, what involves soluble in water, exchangeable and bioavailable forms; to immobile or unavailable fractions, what involves those bounded to amorphous Fe/Mn oxides and the residual fraction.

Statistically significant differences ($P < 0.05$) were found among subareas for the different extracted fractions depending on the specific TE mobility (Table 2). Previous studies in the area (Kraus and Wiegand, 2006; Simón et al., 2008) determined high mobility with strong leaching in depth under acidic conditions for Zn, Cd, and to a lesser extent, for Cu; while low mobility was detected for Pb and As, with significant accumulation in the uppermost part of the soil. In our results, the highest values measured were generally concentrated in BS subareas, especially for soluble and exchangeable forms in the case of Cu, Zn and Cd, and bounded to Fe/Mn oxides forms for Cu and As, while B2 subareas showed the lowest values for TE mobile forms except for Pb extracted with EDTA and bounded to Fe/Mn oxides. According to the soil properties, the mobility of Cu, Zn and Cd was mainly related negatively to pH and OC content and positively to EC for soluble and exchangeable fractions, meanwhile for As and Pb mobile forms, no significant correlation with any soil properties was found (Spearman analysis, Table A. 1).

Soluble and exchangeable Cu fractions, decreased significantly in B2 from BS subareas, 74 and 54-fold, respectively. According to our findings, the reduction of Cu mobility was related to the presence of OC and to the rise in pH. Both properties have been reported as highly significant in Cu retention (Kabata-Pendias, 2011); while acidic conditions with pH below 5.5 enhance Cu mobility in soils (Martínez and Motto, 2000). Copper bounded to Fe/Mn oxides reached the highest percentage extracted in relation to total concentrations in all areas (ranged between 68.04 and 61.65%), followed by Cu extracted by EDTA (33.18–47.13%), although without statistical differences among subareas. Copper associated with organic matter could be extracted by EDTA (Kidd et al., 2007), even from organic complexes characterized by their low bioavailability (Violante et al., 2010). According to our

Table 2
Trace elements concentrations (mg kg^{-1} dry soil) for the different soil fractions among subareas: bare soils (BS), belt1 (B1) and belt2 (B2) (R: residual fraction; O: bounded to amorphous Fe/Mn oxides fraction; B: bioavailable fraction; E: exchangeable fraction; S: soluble fraction; T: total concentration; sd: Standard deviation).

		BS		B1		B2	
		Mean	*sd*	Mean	*sd*	Mean	*sd*
Cu	Cu_T	96.60b	*12.71*	95.58b	*20.17*	68.20a	*7.70*
	Cu_S	0.74b	*0.79*	0.03a	*0.02*	0.01a	*0.00*
	Cu_E	9.41c	*9.92*	0.93b	*0.64*	0.18a	*0.05*
	Cu_B	37.01	*12.74*	31.72	*7.53*	32.14	*2.72*
	Cu_O	65.73b	*18.69*	58.92ab	*13.99*	42.12a	*5.44*
	Cu_R	30.86ab	*6.25*	36.64b	*7.88*	26.09a	*4.54*
Zn	Zn_T	242.33	*57.06*	277.73	*37.66*	317.80	*76.03*
	Zn_S	6.77b	*4.19*	1.47a	*1.63*	0.07a	*0.04*
	Zn_E	70.42c	*52.41*	23.66b	*7.65*	1.52a	*2.79*
	Zn_B	89.10	*48.37*	56.22	*8.41*	43.09	*15.78*
	Zn_O	97.78	*54.29*	59.52	*16.46*	75.49	*24.52*
	Zn_R	142.75a	*26.05*	193.48b	*22.80*	242.31b	*52.15*
Cd	Cd_T	0.86	*0.34*	0.80	*0.09*	0.87	*0.32*
	Cd_S	0.03	*0.02*	0.01	*0.01*	bdl	–
	Cd_E	0.34b	*0.21*	0.15b	*0.04*	0.02a	*0.03*
	Cd_B	0.54	*0.25*	0.45	*0.04*	0.57	*0.22*
	Cd_O	0.81	*1.28*	0.24	*0.06*	0.47	*0.58*
	Cd_R	bdl	–	0.37	*0.06*	0.06	*0.56*
As	As_T	278.08b	*58.03*	222.05b	*50.55*	106.98a	*30.90*
	As_S	bdl	–	bdl	–	0.01	*0.00*
	As_E	0.08	*0.10*	0.02	*0.02*	0.03	*0.02*
	As_B	2.15	*1.99*	0.87	*0.40*	1.37	*0.95*
	As_O	88.20b	*27.11*	59.52ab	*16.46*	36.56a	*13.41*
	As_R	189.89b	*36.65*	162.54b	*38.07*	70.45a	*17.86*
Pb	Pb_T	345.13b	*80.68*	256.58ab	*64.27*	171.23a	*26.07*
	Pb_S	bdl	–	0.01	*0.01*	0.01	*0.01*
	Pb_E	0.02	*0.03*	0.01	*0.01*	0.01	*0.00*
	Pb_B	0.61a	*0.09*	2.48b	*1.99*	18.91c	*6.97*
	Pb_O	5.54a	*1.44*	6.89ab	*4.18*	16.07b	*5.72*
	Pb_R	339.57b	*339.57*	249.67b	*65.86*	154.11a	*31.86*

Lowercase letters represent significant differences among areas (Kruskal Wallis test $P < 0.05$). bdl: below detection limit.

results, the main soil components related to Cu retention are iron oxides and organic matter, being even more important than clay minerals (McLaren et al., 1981).

A similar behavior was found in Zn fractionation. Soluble and exchangeable Zn concentrations were significantly higher in BS and decreased in the adjacent belts (100 and 46-fold lower, B1 and B2, respectively). The residual fraction was the most abundant form in revegetated areas (69.67% in B1 and 76.25% in B2) and even in bare soils (58.91% of the total concentration). pH is the most important parameter for determining Zn mobility, which is very relevant at acidic conditions (Lock and Jannsen, 2003; Romero-Freire et al., 2016b). In addition, organic matter is a natural sink of Zn in soils where it is easily absorbed (Kumpiene et al., 2008). The correlation between Zn and these two parameters (Table A. 1) was shown for both revegetated areas (B1 and B2), where the increase in pH and OC content promoted Zn retention in soils.

Similar to Cu and Zn, soluble and exchangeable Cd fractions showed a strong reduction from BS to B2 (76-fold for soluble Cd and 18-fold for exchangeable). Cd extracted by EDTA was high in all subareas (61.91% in BS, 55.67% in B1 and 64.79% in B2), being the predominating fraction in both vegetated belts in relation to the total concentration, whereas Cd bounded to Fe/Mn oxides was the most abundant fraction in BS, reaching 93.16%, indicating that Cd in bare soils could be retained in different forms from those that appear in soils with vegetation. Hence, Cd showed higher bioavailability in the vegetated soils (B1 and B2) than in bare soils compared to the other elements, with a very low percentage of residual fraction, especially in B2. Soluble and exchangeable forms were controlled by pH, confirming that Cd is very mobile under acidic conditions even after a long-term aging process; similar results were also showed by Clemente et al. (2008) for soils related to metallurgical activity. Moreover, Kirkham (2006) indicated that pH was the most important factor controlling Cd bioavailability for plants. Along with pH, OC is highly related to the reduction of Cd mobility in soils (Bur et al., 2010; Kabata-Pendias et al., 2011); in our study area, OC was significantly correlated with the retention of soluble and exchangeable Cd in the subareas with significant increase in organic carbon (B1 and B2) in relation to bare soils (Table A. 1).

Arsenic presented a very low mobility in relation to the total concentration in all cases (<2% for mobile fractions, <0.03% for soluble As); meanwhile the residual fraction predominated in all areas (68.29% in BS, 73.20% in B1, and 65.85% in B2), followed by the fraction bounded to Fe/Mn oxides (ranging between 26.80 and 34.17% of the total As concentration). Arsenic bounded to Fe/Mn oxide was mainly related to amorphous oxides forms (Table A. 1); the retention of As by soil oxides, corroborated by many authors (Acosta et al., 2015; Manaka, 2006), is due to the anionic behavior of As, what promotes the bound to soil Fe/Mn oxides. The presence of poorly crystalline forms of iron oxides was previously reported in these soils (Aguilar et al., 2007), and provides the main sorption sites for As, along with aging can co-precipitate and finally immobilize this pollutant into the solid phase (Suda and Makino, 2016).

The residual fraction of Pb predominated in all subareas (ranging from 98.39% in BS to 90.01% in B2); whereas percentage of soluble and exchangeable forms were negligible (<0.01% of the total concentration in all cases), indicating low bioavailability for plants. Lead is a very immobile element in soils, being clay minerals, pH and organic matter the most important factors determining Pb fixation (Kabata-Pendias, 2011; Romero-Freire et al., 2015). Generally, Pb reaches high accumulation in the uppermost part of the soils, mainly due the sorption by OM, forming stable complexes and so reducing phytotoxicity at low soil pH (Udom et al., 2004). However, in our study Pb extracted by EDTA and bounded to Fe/Mn amorphous oxides showed a significant increase in B2, from 0.18% and 1.16% of the total concentration in BS to 11.04% and 9.38% in B2, respectively. This could be related to the increase of $CaCO_3$ content in B2, where Pb could be forming co-precipitates with Fe easily extracted by EDTA and oxalic acid-ammonium oxalate reagents. Simón et al. (2005) reported that Pb co-precipitate with Fe coating the surface of carbonates that were used as an amendment in these soils; moreover, their findings showed that the quantity precipitated decreased at pH > 6.5, increasing bioaccessibility. Therefore, the addition of calcium carbonate is a suitable remediation technique to raise pH in contaminated soils, but monitoring studies are strongly recommended after every remediation program to prevent future mobilizations.

3.3. Relationship between TE and vegetation

3.3.1. Vegetation distribution

A total of 27 species were identified within the studied area. Species richness showed significant differences among subareas, nearly twice higher in B2 than in B1, both values far from those in BS (Fig. 2). Total cover showed a similar pattern, reaching the highest values in B1 and B2 (95.75% - 100%, respectively) and the lowest in BS (4%). Therefore, cover and richness were significantly higher in all cases for the surrounding belts and drastically low in BS.

To assess the distribution of the different species among the three subareas, an NMDS analysis was performed ($R^2 = 0.98$) (Fig. 3). The graphic, in which line length represents the abundance of species, showed an inverse relationship between pollutants and richness. Whereas in BS rarely appeared a species (only *Lamarckia aurea* (L.) Moench and *Spergularia rubra* (L.) J. Presl & C. Presl, both with 4% of cover), the two vegetated belts concentrated most of the species, although there were significant differences in terms of species distribution between them.

Important differences were found among subareas regarding the percentage of cover per specie. In BS, only *L. aurea* and *S. rubra* were found with a low cover value. In B1 *L. aurea* was dominant (80%) and appeared associated with other species such as *Anthemis arvensis* (L.), *Hypochaeris glabra* (L.), *S. rubra* and *Vulpia membranácea* (L.) Dumort. In contrast, in B2 the dominant species was *Trifolium campestre* Schreb., with >70% of cover, together with *Leontodon longirostris* (Finch & P.D. Sell) Talavera, *Avena sterilis* (L.), *Chrysanthemum coronarium* (L.) or *Anagallis arvensis* (L.). Therefore, the dominant species were different in each belt and so their distribution. Consequently, *L. aurea* (dominant species in B1), rarely appeared in B2 and was replaced by *T. campestre* as dominant species.

Lamarckia aurea showed the highest tolerance to TE toxicity, dominating above other species also tolerant to this kind of pollution (B1). According to E. Madejón et al. (2006), *L. aurea* can accumulate high quantities of Cu, Pb and Zn (the latter one with concentrations even over phytotoxic levels) without affecting their nutrient absorption capacity. *S. rubra*, the other species that accompanies *L. aurea*, is also able to cope with high levels of trace elements beyond normal values (Hernández and Pastor, 2008). On the other hand, *T. campestre* also showed a good ability to cope with elevated levels of this kind of pollution (B2), results that fit with those of Bidar et al. (2007), who reported that this species could grow in soils highly polluted by Cd, Pb and Zn, by accumulating them in its roots. Both *L. aurea* and *T. campestre*, were the species best adapted to this kind of soil pollution; moreover, the high TE concentration bioaccumulated in vegetation indicates that these plants may have developed adaptation mechanisms that allow them to live under these stress conditions. Hence, and in-depth study about the bioaccumulation capacity of the highlighted species within the residual polluted areas, could identify the potential species to be used in the phytoremediation of both the study area and other similar polluted areas.

3.3.2. Trace element bioaccumulation

Differences in TE accumulation were found for roots and aerial parts of the plant and between belts (Table A. 2). In B1, significant Cu and Zn accumulation in roots were shown, whereas in B2 roots significantly accumulated Cu and As (no significant TE accumulation was detected in

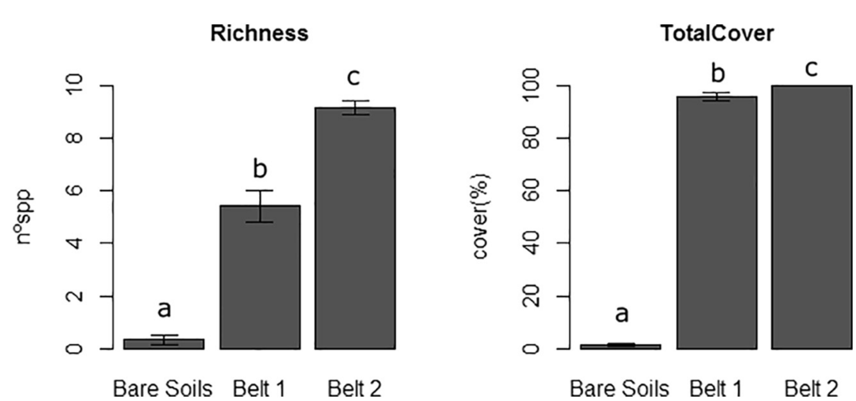

Fig. 2. Vegetation richness and total cover per subarea. Lowercase letters represent significant differences among subareas (Kruskal Wallis test P < 0.05).

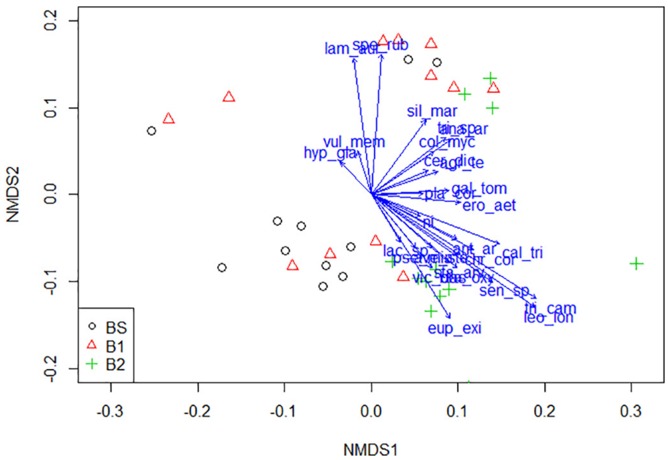

Fig. 3. NMDS analysis representing plant species distribution among subareas. Bare soils (BS): black circles, belt1 (B1): red triangles, and belt2 (B2): green cross. Species (in blue): agr_te = *Agrostis tenerrima*; ana_ar = *Anagallis arvensis*; ant_ar = *Anthemis arvensis*; ave_ste = *Avena sterilis*; bra_oxy = *Brassica oxyrrhina*; cal_tri = *Calendula tripterocarpa*; cer_dic = *Cerastium dichotomum*; chr_cor = *Chrysanthemum coronarium*; col_myc = *Coleostephus myconis*; ero_aet = *Erodium aethiopicum*; eup_exi = *Euphorbia exigua*; gal_tom = *Galactites tomentosa*; hyp_gla = *Hypochaeris glabra*; sta_arv = *Stachys arvensis*; lac_sp = *Lactuca* sp.; lam_aur = *Lamarckia aurea*; leo_lon = *Leontodon longirostris*; ni = No identified; pla_cor = *Plantago coronopus*; sen_sp = *Senecio lividus*; sil_mar = *Silybum marianum*; spe_rub = *Spergularia rubra*; tri_cam = *Trifolium campestre*; tri_sp = *Trifolium* sp.; pse_min = *Pseudorlaya minuscula*; vic_das = *Vicia dasycarpa*; vul_mem = *Vulpia membranacea*. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

aerial parts). Comparing between belts, vegetation in B1 accumulated significantly more As and Pb than vegetation in B2; on one hand, the bioavailable As (extracted with EDTA) did not show significant differences between belts, but on the other hand, the bioavailable Pb fraction did increase in B2 soils. Antagonistic influence was identified between Ca-Mg and As-Pb (PCA analysis, Table A. 3); a higher presence in B2 of Ca could be related to the decrease in As and Pb bioaccumulation in roots. The protective action of calcium in vegetation against the presence of As has been previously reported in these soils by E. Madejón et al. (2006). Other studies have reported the reduction in Pb toxicity due to the presence of Ca, blocking Pb transport into the root and therefore the toxicity (Kim et al., 2002).

Bioaccumulation factor (BAF) using EDTA extraction of soils was calculated to evaluate the transfer of TE into plants (Table 3). Since total concentrations have been commonly used to predict the TE transfer from soil to plant without considering the bioavailability of TE in soils, several authors highly recommend the EDTA extraction to predict this potential for bioaccumulation (Wang et al., 2006). In most cases BAF > 1 indicates accumulation in plants, and despite the high variability of the values, accumulation was preferentially observed in roots.

In B1, Cu BAF was significantly higher in roots than in aerial parts, while in B2 significant As, Cu and Cd BAF were shown in roots, indicating high transfer of these elements from soil to plants. Cu, Zn and Cd are elements easily absorbed by plants roots due to their high solubility in soils (Kabata-Pendias, 2011). Although no differences were shown for Zn, high values of BAF indicated high transference of Zn into the plant. In plants, Cu usually shows low mobility in contrast to other elements, easily accumulated in roots where is strongly retained (Ali et al., 2002), similar results were found in our study in both belts. Cadmium in soils, which also showed significant transfer from soil to roots, can be tolerated by plants in a relatively high content (Chan and Hale, 2004). Arsenic and Lead belong to the group of trace elements that, even absorbed by plants, hardly translocate to aerial parts. According to that, our results showed As transfer and accumulation into roots; in addition Del Río et al. (2002) showed high accumulation in shoots for diverse plant species in the area in relation to the unaffected soils, and this accumulation seems to be maintained over time. On the other

Table 3
Bioaccumulation factor (BAF) calculated for trace elements dividing concentrations in plants (mg kg^{-1} dry weight) by concentration extracted by EDTA (mg kg^{-1} dry soil), for the different belts (B1 and B2) and different part of the plants (root and aerial) (sd: Standard deviation).

	B1				B2			
	Root		Aerial		Root		Aerial	
	Mean	*sd*	Mean	*sd*	Mean	*sd*	Mean	*sd*
CuBAF	2.34b	*0.71*	0.71a	*0.22*	1.85b	*0.16*	0.54a	*0.10*
ZnBAF	4.45	*0.85*	3.32	*0.55*	4.93	*1.61*	2.60	*0.26*
AsBAF	31.49B	*15.57*	10.93	*11.37*	8.20Ab	*4.82*	1.12a	*0.67*
CdBAF	4.79	*1.97*	2.86	*1.24*	3.40b	*1.72*	1.50a	*0.97*
PbBAF	33.25B	*30.55*	17.467B	*17.37*	1.43A	*1.17*	0.65A	*0.22*

Lowercase letters represent significant differences between part of the plants (Kruskal Wallis test P < 0.05). Capital letters represent significant differences between belts (Kruskal Wallis test P < 0.05).

hand, the increase of Pb extracted by EDTA in B2 soils resulted in a higher transfer into plants, with translocation from roots to aerial parts. Furthermore, Kabas et al. (2012) reported the influence of the presence of organic matter in the increase of Pb accumulation in vegetation, due to the soluble organic ligands formation for the root exudations that increase the uptake of the trace elements.

Comparing between belts, only As and Pb BAF showed significant differences; higher BAF was observed in roots from B1 for As and Pb, also for aerial parts in B1 for Pb. Despite their usual low mobility in soils, corroborated by the limited percentage of As and Pb extracted by EDTA, both elements reached the highest bioaccumulation factor of all the trace elements, with significant higher transfer from soil to plant in B1 than in B2. Therefore, both elements could have a greater influence on the differences in vegetation distribution between B1 and B2 soils.

Bioaccumulation values in aerial parts of the vegetation were higher than the considered normal for some TE according to the values reported by Chaney (1983); therefore, plants grown in this polluted soil would have certain degree of tolerance. Concentrations of Cu, Zn and As in the aerial parts were in the phytotoxic range defined in Kabata-Pendias (2011), also for Fe with concentrations in plants far exceeding the maximum value reported (500 mg kg^{-1}), maybe with toxicity implication for the vegetation. Chaney (1983) established the maximum levels of dietary for trace elements for domestic livestock (cattle, sheep, swine and chicken) in comparison with levels in forages. According to these results, Cu concentration in our plants exceeded the maximum levels chronically tolerate for cattle (25 mg kg^{-1}), and in all type of livestock for Cd (limit value 0.5 mg kg^{-1}). That let us conclude that exists a potential risk of pollutant transference through food-chain, hence is necessary to take action and control the polluted soils in the area to safeguard the ecosystem and the human health.

4. Conclusions

Twenty years after the remediation program involved after the Aznalcóllar mine spill, residual pollution is still found in the area. As a consequence of the high content in trace elements, patches of soils continue without vegetation, surrounded by soils with two different vegetation gradients depending on the levels of soil pollution. These soils pose a high risk of pollution dispersion to the rest of the ecosystem.

The lack of vegetation was influenced by the most mobile elements, Cu, Zn and Cd, which reached the highest mobility in the bare soils. The surrounding vegetated belts registered a significant decrease in TE concentration, an immobilization of potentially pollutant elements due to the change in soil conditions, mainly an increase in pH and organic matter content. Regarding bioaccumulation in vegetation, trace elements accumulated preferentially in vegetation roots, highlighting the As and Pb bioaccumulation factor significantly higher in roots of Belt 1 in relation to Belt 2 despite their general low bioavailability in soils. Hence, As and Pb are possibly key factors affecting the differences in vegetation distribution through belts.

Vegetation is a good indicator of soil pollution. In our study, richness and plant cover changed according to soil pollution patterns. Furthermore, many species can tolerate high concentrations of trace metals in soils through different strategies, like exclusion or accumulation. *Lamarckia aurea* and *Trifolium campestre* have demonstrated the highest capacity to cope with severe soil pollution. However, trace elements bioaccumulated in plants can result in a risk for the ecosystem and the living organisms, by their introduction into the food-chain. Despite low translocation to aerial parts was shown, high concentrations of Cu and Cd measured in aerial parts poses a risk to livestock. A continuous monitoring of trace elements in soils and the transfer to wild vegetation is necessary to ensure the ecosystem safety in the area; moreover, an assisted remediation process is required for bare soils to control bioavailability and toxicity of pollutants.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2018.09.092.

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