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RESEARCH ARTICLE

Gypsum mining spoil improves plant emergence and growth in soils polluted with potentially harmful elements

Helena García-Robles · Eliane G. P. Melloni · Francisco B. Navarro · Francisco José Martín-Peinado · Juan Lorite

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Abstract

Purpose Soil pollution is a major problem worldwide. Some anthropogenic activities, such as mining, may exceed soil capacity, causing relevant health and ecosystem hazards. The use of mineral amendments can help reduce soil pollution. Gypsum mining spoil (GS) is a waste material highly produced in gypsum mining industry, which has never been used in soil remediation despite its high potential as amendment of polluted soils. In this study, we carried out an *ex-situ* experiment to assess for the first time the capacity of GS to both reduce the availability of Potentially Harmful Elements (PHEs) in soils and promote seed emergence.

Methods Soils affected by residual pollution after the Aznalcóllar mine spill were collected, treated with GS in three different proportions, and sown with seeds of two non-genetically related species. Seed emergence and biomass production were monitored, and PHE content in soils and plants were analysed.

Results We have observed a direct and very positive relation between GS and both the reduction of PHE availability and PHE uptake by plants, and the increase of plant emergence and growth, especially with the addition of the highest doses of the amendment.

Conclusion This study highlights the promising results of GS as a novel soil amendment to be used in the remediation of polluted soils and vegetation recovery. Moreover, using GS as soil amendment will bring the opportunity to sustainably manage this waste material and reduce its social and environmental impact parallelly to the mitigation of PHE hazards.

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H. García-Robles (✉) · J. Lorite
Department of Botany, University of Granada, Campus Fuentenueva s/n, 18001 Granada, Spain
e-mail: helenagr@ugr.es

J. Lorite
e-mail: jlorite@ugr.es

E. G. P. Melloni
Natural Resources Institute, Federal University of Itajubá, Av. BPS, Itajubá 1303 - CEP 37500-903, Brazil
e-mail: elianegp@unifei.edu.br

F. B. Navarro
Andalusian Institute of Agricultural Research and Training (IFAPA), Camino de Purchil s/n, 18004 Granada, Spain
e-mail: fbruno.navarro@juntadeandalucia.es

F. J. Martín-Peinado
Department of Soil Science and Agricultural Chemistry, University of Granada, Campus Fuentenueva s/n, 18001 Granada, Spain
e-mail: fjmartin@ugr.es

J. Lorite
Interuniversity Institute for Earth System Research, University of Granada, 18006 Granada, Spain

Keywords Heavy metal immobilization · Inorganic amendment · Mining waste reuse · *Cynodon dactylon* · *Medicago sativa*

Introduction

Soils provide crucial environmental functions and services, being their productivity the most essential service for human survival and development (Kabata-Pendias and Pendias, 2000). Centuries of anthropogenic activities have resulted in the accumulation of pollutants in soils (Rodríguez-Eugenio et al. 2018). Hence, pollution is one of the main concerns affecting soils globally (FAO and ITPS 2015; Payá-Pérez et al. 2018; and Rodríguez-Eugenio 2018; Rodríguez-Eugenio et al. 2018). Among all chemical pollutants, Potentially Harmful Elements (PHEs), which include heavy metals and metalloids, are of major concern since they can persist in soils for a long period of time (Pilon-Smits 2005) and produce negative cumulative effects on organisms. Consequently, they represent the main source of global environmental pollution with noxious implications for human health (Muyessar and Linsheng, 2016). PHEs are naturally present in soils, and some of them are essential micronutrients for plants, however, when concentrations exceed a specific threshold, they may cause toxicity (DalCorso et al. 2014; Higueras et al. 2016). For instance, high concentrations of PHEs can affect plant nutrition and fitness by displacing other essential nutrients, what may cause deficiencies limiting plant performance (DalCorso et al. 2014; Kabata-Pendias 2011). Otherwise, some plants can stabilize PHEs in soils (phytostabilizers) or accumulate high concentrations of PHEs in their tissues (hyperaccumulators). However, PHEs accumulated in plants may become accessible for the subsequent links of the food chain, posing a significant hazard for the environment and living organism (Hooda 2010; Nworie et al. 2019).

Remediation of soil pollution is essential to restore soil functions (Martín-Peinado et al. 2015). This frequently entails the implementation of long-term strategies focused on the application of organic and/or inorganic amendments to reduce the mobility of pollutants (Hooda 2010). Henceforth, detailed studies should be conducted to assess the interaction of PHEs with the amendment and the potential change in the behaviour of pollutants after the modification of the soil environment (García-Carmona et al. 2017).

Gypsum is an industrial mineral in global demand (Herrero et al. 2013). In 2021, gypsum world production reached 150 million tons (U.S. Geological Survey 2022). As a result, large quantities of waste material, the so-called gypsum mining spoil, are annually produced and accumulated in mining sites, resulting in both storage and environmental problems (Al-Farajat 2009; Ballesteros et al. 2017). Reusing and recycling gypsum waste is lately seen as a suitable management solution (Ahmed et al. 2011; Chandara et al. 2009) that perfectly fits the zero-waste strategy (Greyson 2007). Gypsum mining spoil contains a high proportion of gypsum (50–70%), moderately high amounts of calcium carbonate (>20%), and a mixture of fine (clay) and coarse (gravel) particles. Both gypsum and calcium carbonate have the capacity to fertilize impoverished soils, to reduce soil acidity and to immobilize certain PHEs in polluted soils (Shainberg et al., 1989; Toma et al. 1999; Adriano et al. 2004; Franzen et al. 2006; Aguilar et al. 2007; Fernández-Caliani and Barba-Brioso 2010; Sherene 2010; Chen and Dick 2011), which gives GS a good potential to be used as an amendment in polluted soils. Nevertheless, there are no references so far for the use of this novel amendment in soil remediation.

This study advances knowledge in the interaction between plants and heterogeneous mixture of polluted soil and gypsum mining spoil, with the aim of assessing for the first time the potential use of gypsum mining spoil to enhance soil properties, to reduce PHE mobility and availability in soils and, thus, to promote plant performance.

Materials and methods

Experimental design

In order to test the remediation capacity and the suitable dose of a novel amendment such as gypsum mining spoil (GS), we designed an *ex-situ* experiment before an eventual application in the field.

Soil samples were collected at the closest sector to the Aznalcóllar mine (Seville, SW Spain) in the Guadiamar Green Corridor (GGC, CMA 2003), area affected by the mine toxic spill since 1998 and where numerous restoration activities had been

implemented (Madejón et al. 2018a). Nevertheless, there are still residual polluted areas characterized by high concentrations of PHEs (mainly As, Pb, Zn, Cd, Cu, and Sb) and by the absence of vegetation (Martín-Peinado et al. 2015). For this experiment, we selected five residual plots at the closest to the mine and most polluted area, after being measured *in-situ* with a NITON XLT 792 field portable X-Ray fluorescence analyser. Considering that pollution is mainly concentrated in the topsoil layer (Aguilar et al. 2004), soil samples were collected from the uppermost 10 cm and intensely homogenized into just one composite soil sample (C_0). We also collected five samples of natural soils (Nat) in the surrounding unaffected area to be used as background values. As soil amendment, we used GS provided by Knauf-GmbH and extracted at a gypsum quarry in Escúzar (Granada, SE Spain). Both the soil and amendment samples were sieved through 4 mm to reduce gravel content which, in the case of soil samples, represents less than 10% of the total (Aguilar et al. 2004).

The experimental design was based on the addition of GS to C_0 in three different proportions (treatments): 10% GS (T1), 20% GS (T2), and 50% GS (T3). T3 is intended to test the maximum capacity of GS to neutralize pollution, which is especially important in a severe pollution episode such as the one occurred in Aznalcóllar. Moreover, such large quantities are simultaneously being tested in the search for alternative solutions based on the development of technosols (Aguilar-Garrido et al. 2022). Afterwards, we prepared 32 replicates per treatment (pot size = 6 cm × 5.6 cm × 8 cm), plus 32 control replicates (C = 0% GS) with polluted non-treated soil (C_0). After three days of incubation at room temperature, we sowed five seeds of alfalfa (*Medicago sativa* L.) in each pot, and the same experimental design was prepared for Bermuda grass (*Cynodon dactylon* (L.) Pers.). *M. sativa* and *C. dactylon* are two non-genetically related and native species present in the affected area, which are tolerant to PHEs and frequently used in pollution and phytoremediation studies (Madejón et al. 2002; Flores-Cáceres 2013). Tracking these species performance will show us whether gypsum mining spoil has the capacity to reduce soil pollution to the extent of reactivating plant emergence and whether the effect on different species can be comparable. This point is crucial to test whether the sole application of this amendment would be enough to rehabilitate this degraded ecosystem.

Finally, the 256 pots prepared (32 replicates × 4 treatments × 2 species) were randomly placed in a greenhouse equipped with an irrigation programmer, a nebulization system (30 l/h irrigation flow) and a temperature control sensor. The experiment lasted 82 days for *M. sativa* and 67 days for *C. dactylon* due to the ecological differences between both species, and pots were watered five minutes daily in order to ensure that water was not a limiting factor.

During the experiment, plant emergence and survival were monitored three times per week. Once the survival of the first seedling emerged was guaranteed, the younger and extra seedlings in a pot were clipped to avoid competence. At the end of the experiment, all plants were collected, divided into shoots and roots, washed with distilled water and dried in an oven (Memmert oven, Model 100–800) at 70 °C for 48 h. After stabilization at room temperature, we weighed biomass in a precision scale (GRAM PRECISION STA-310 S, ± 0.001 g). We also collected 5 soil samples per treatment (C_f, $T1_f$, $T2_f$, and $T3_f$) and species to characterise the effect of treatments on soil remediation.

PHE analyses in plants and soils

Plant samples were ground by means of a conventional mill. Due to the generalized low biomass produced, just one composite sample could be prepared per treatment and species in most cases. Finally, they were digested with an acidic solution (HNO_3:H_2O_2, 1:1) in a microwave XP1500Plus (Mars®) (Sah and Miller 1992) to measure PHE content in plant by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (PE SCIEX ELAN-5000 spectrometer).

Soil samples were prepared (air-dried, sieved -2 mm mesh- and finely ground with a soil mill, Retsch MM 400) to analyse their chemical properties (MAPA 1994) and main PHEs (Cu, Zn, Cd, Sb, As and Pb). Total concentrations (T) were obtained through microwave-assisted (XP1500Plus, Mars®) acid digestion (HNO_3:HF, 3:1), water-soluble fraction (S) was extracted by distilled water from soil:water extract 1:5 according to Sposito et al. (1982), and bioavailable fraction (B) was extracted using 0.05 M EDTA (pH 7) as described by Quevauviller et al. (1998). PHEs were measured

in all the extracted forms by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) (PE SCIEX ELAN-5000 spectrometer).

Data analysis

All statistical analyses were performed using R version 3.4.2 (R Core Team 2017). We assessed the effects of gypsum mining spoil (GS) on PHE immobilization in soils and on plant emergence, survival, and biomass production by applying generalized linear models (GLMs). We also used GLMs to evaluate the differences between the polluted and non-polluted soils. To fit GLMs for seed emergence, plant survival and biomass, total/soluble/bioavailable PHEs in soils, soil pH and EC, the "stats" package was used (R Core Team 2017). Model suitability was assessed by graphical exploration of the residuals (Zuur et al. 2010). After that, final models were fitted assuming gamma/gaussian distribution and inverse/identity-link function for PHE immobilization in soils and plants. For plant emergence and total survival, we fitted GLMs assuming binomial distribution and logit-link function. To evaluate differences among treatments on seedling survival rates over time, we used Cox proportional hazard models and, for data visualization, Kaplan–Meier curves (Bewick et al., 2004) using "survival" package v2.44–1.1 (Therneau, 2015). Pairwise comparisons between soil treatments in terms of PHE immobilization in soils, PHE accumulation in plants, plant emergence, survival and growth were performed with Tukey's *post-hoc* tests using "multcomp" package (Hothorn et al., 2008). Graphs and confidence intervals were obtained with R "ggplot2" v3.1.1 (Wickham 2016) and "sciplot" v1.1–1 (Morales 2017). Mean values and standard deviation were calculated by using ddply function (R "plyr" package, v1.8.4; Wickham 2011).

Results

Plant performance and PHE uptake

Seed emergence

Our results show that in the polluted non-treated soils (C), seed emergence was totally inhibited, whereas it was promoted by the addition of gypsum mining spoil (GS) at any proportion (T1, T2, T3), both for *Medicago sativa* (84% in T1, 88% in T2, and 100% in T3) and *Cynodon dactylon* (78% in T1, 94% in T2, and 100% in T3), with no significant differences among treatments.

Survival and biomass production

M. sativa showed the highest survival rate in T3 treated soils (97%), followed by T2 (64%) and T1 (30%), with statistically significant differences among all of them (Fig. 1). On the other hand, *C. dactylon* registered high survival rates (above 90%) in all treated soils, with no statistically significant differences in any case (Fig. 1). Results extracted from Kaplan–Meier survival curves and Cox proportional hazard models showed that plant survival across the experiment was rather stable for *M. sativa* in T3 and for *C. dactylon* in any treatment, while plant survival for *M. sativa* in T1 and T2 experienced a sharp decrease within the first 25 days, and then got stabilized (Fig. 1).

In terms of biomass production (all components analysed: shoot, root, and total biomass), the most effective treatments for *C. dactylon* were T2 and T3, with no significant differences between them (Fig. 2). In the case of *M. sativa,* although T3 was the treatment that most promoted growth (with significant differences), the overall biomass production for this species was rather low in this experiment (Fig. 2).

PHE uptake

As none of the seeds sown in the polluted non-treated soils (C) emerged, there was no PHE record for plant tissues in these samples. Furthermore, the low biomass obtained for *M. sativa* in all treated soils (T1, T2 and T3) and for *C. dactylon* in T1 soils, compelled us to prepare only 1 mixed sample in these cases for chemical analyses. Nevertheless, the concentrations have been included as guiding values (Table 1).

Both species retained a greater part of most PHEs in their roots, except for Zn (and Cd too for *C. dactylon*), which was similarly accumulated in roots and shoots (Table 1). In terms of percentages (Table S1), *C. dactylon* accumulated more than 80% of total Cu, Sb, As and Pb in roots in all the treated soils (with similar values). *M. sativa* accumulated more than 70% of total Cu, Cd, Sb, As and Pb in roots in T3, and

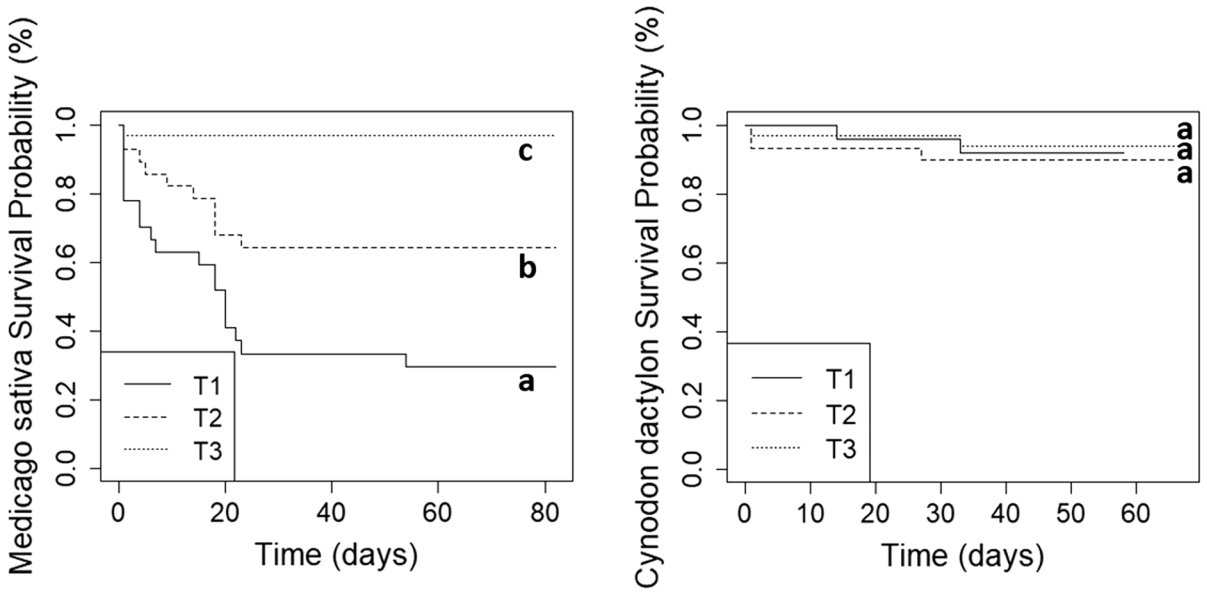

Fig. 1 Kaplan–Meier survival curves representing plant survival rates along the experiment (in days) per treatment and species. Experiment duration: 82 days for *Medicago sativa* and 67 days for *Cynodon dactylon*. Treatments: T1: 90% C_0 + 10% GS; T2: 80% C_0 + 20% GS; T3: 50% C_0 + 50% GS. C_0: contaminated soil. GS: Gypsum mining spoil. Different letters represent statistically significant differences between treatments ($p < 0.05$)

more than 60% in T2 and T1 (Table S1). The concentration of all PHEs in *C. dactylon* shoots was under phytotoxic levels (any soil treatments), whereas *M. sativa* in T1 and T2 treatments accumulated Cu, Zn, As and Pb in shoots at phytotoxic levels (Table 1).

Considering the overall accumulation of PHEs in plant tissues (PHEs in roots + PHEs in shoots), both species presented the lowest accumulation of PHEs in T3, followed by T2 (Table S2).

Soil properties and PHE content

The natural soils (Nat) collected in the unaffected area showed nearly neutral pH values (6.4 ± 0.4), low EC (0.09 ± 0.04 dS m^{-1}) and low concentration of PHEs (Tables S3). The polluted soils (C_0) collected for the experiment at the selected residual areas had a strong acidic pH (3.5 ± 0.1), high EC (2.76 ± 0.01 dS m^{-1}) and significantly higher total PHEs (Table S3 and Table S4) than Nat (with values more than 10 times higher in the case of As). Gypsum mining spoil (GS) had nearly neutral pH values (7.5 ± 0.2), high EC (2.90 ± 0.04 dS m^{-1}) and negligible quantities of PHEs in all their forms (Table S3 and Table S4), except for soluble Sb, which was significantly higher than in Nat and C_0.

At the end of our experiment (Table S5), non-treated soil samples (C_f, 0% GS) still presented a strong acidic pH (3.8 ± 0.1) and high EC (2.24 ± 0.02 dS m^{-1}), whereas in treated soils, the acidic pH was partially corrected in $T1_f$ (10% GS) and $T2_f$ (20% GS) (5.6 ± 0.2 and 6.4 ± 0.01, respectively) and neutralized (6.8 ± 0.1) in $T3_f$ (50% GS). In terms of electrical conductivity (EC), the addition of GS at any dose did not have a significant impact on salinity (EC > 2 dS m^{-1}).

With regard to PHEs, comparing initial (C_0) and final (C_f) polluted non-treated soils, leaching was significant for most PHEs (Pb excluded) after having watered C_0 daily for 12 weeks and where no plant emergence occurred (leaching > 50% of total Zn and Cd, and < 25% of total Cu, Sb and As). On the contrary, the addition of gypsum mining spoil to C_0, especially at its highest dose, promoted PHE immobilization in soil as well as the reduction of total PHEs through a dilution effect (Table 2).

When compared to C_0, PHE soluble fractions (Table 3) were significantly reduced by GS at any dose (especially in $T3_f$), except soluble Sb that was only

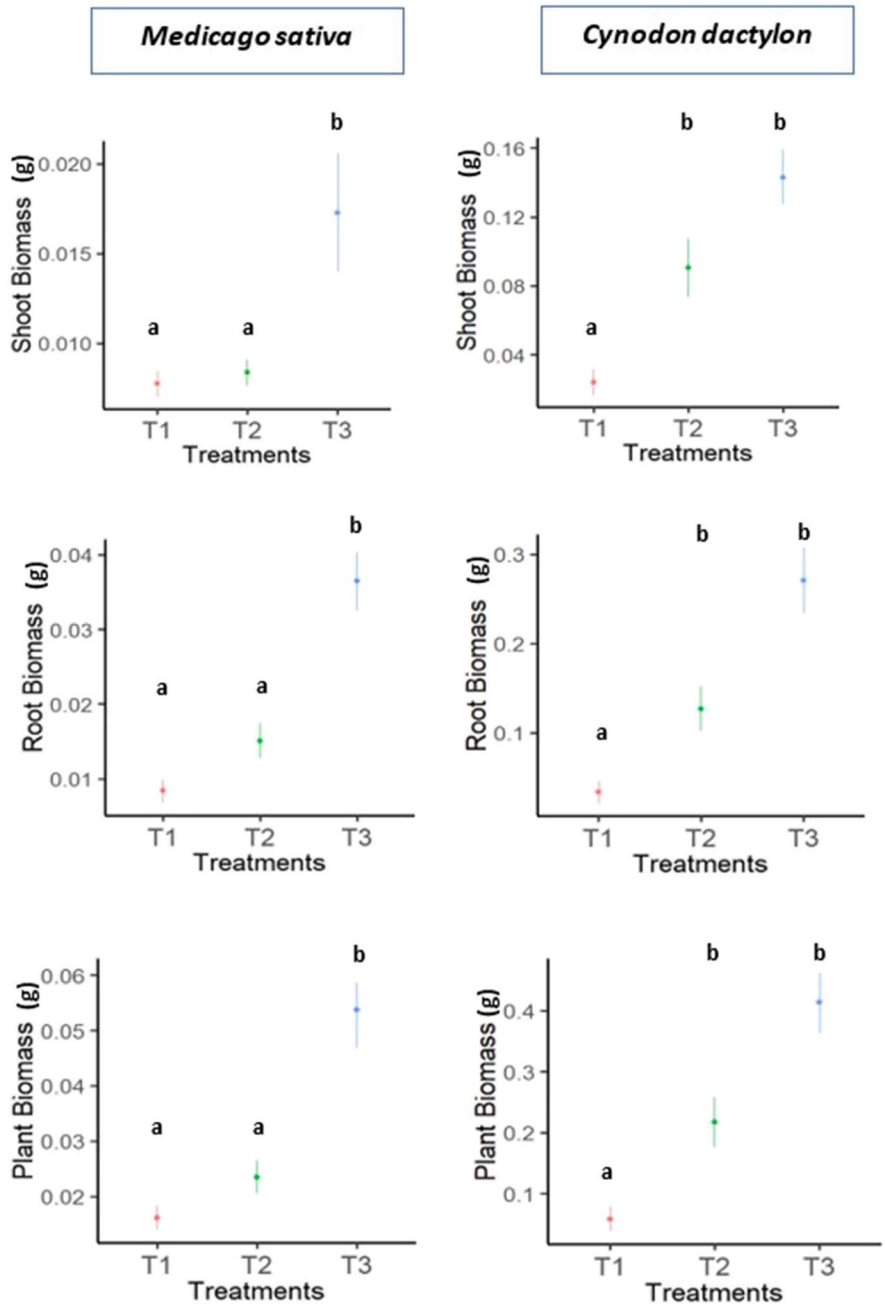

Fig. 2 Shoot, root and plant biomass (mean ± SD in g) per treatment and species (*Medicago sativa* and *Cynodon dactylon*). Treatments: T1, 90% C_0 + 10% GS; T2, 80% C_0 + 20% GS; T3, 50% C_0 + 50% GS. C_0: contaminated soil. GS: Gypsum mining spoil. Different letters represent statistically significant differences ($p < 0.05$) for the *post-hoc* Tukey tests performed after the GLMs

reduced in $T1_f$. The bioavailable fractions (Table 4) of Zn, Cd and As were reduced by GS at any dose, especially in $T3_f$; nevertheless, bioavailable Cu was only reduced in $T3_f$, and bioavailable Sb and Pb only in $T1_f$.

The ratio between soluble and total PHEs (Table S6) decreased with the addition of GS for Cu, Zn, Cd and Pb, especially in $T3_f$, and for As, especially in $T1_f$. On the contrary, this ratio for Sb progressively increased in $T2_f$ and $T3_f$ and was maintained in $T1_f$. Finally, the ratio between bioavailable and total PHEs (Table S7) was reduced in $T3_f$ for Cu (only for *M. sativa*), in $T2_f$ and $T3_f$ (both species) for Zn, in all treatments for As (both species), and in $T1_f$ (both species) for Sb. For Cd and Pb, it depended on the species and no clear results were registered in this regard, observing an increase of the ratio for Pb in the

Table 1 Mean values (±SD where applicable) of PHEs (Cu, Zn, Cd, Sb, As and Pb, in mg kg^{-1}) accumulated in roots and shoots (*Cynodon dactylon* on top and *Medicago sativa* at the bottom). Soil treatments: T1, 90% C_0 + 10% GS; T2, 80% C_0 + 20% GS; T3, 50% C_0 + 50% GS. C_0: polluted soil. GS: Gypsum mining spoil. N: total number of composite mixed samples. [a]Kabata-Pendias 2011

PHE accumulation in *Cynodon dactylon* tissues						
PHE		Soil treatments			Normal levels (mg kg^{-1} dry foliage) [a]	Phytotoxic levels (mg kg^{-1}dry foliage) [a]
		T1 ($N=1$)	T2 ($N=3$)	T3 ($N=4$)		
Cu	shoot	17,65	15,15 ± 1,02	11,82 ± 1,26	*5–30*	*20–100*
	root	82,36	78,95 ± 13,26	50,27 ± 13,17	-	-
Zn	shoot	110,63	90,37 ± 25,03	102,32 ± 19,86	*27–150*	*100–400*
	root	151,55	124,27 ± 10,47	87,21 ± 18,30	-	-
Cd	shoot	0,85	0,78 ± 0,27	0,94 ± 0,29	*0.05–0.2*	*5–30*
	root	1,14	1,05 ± 0,11	0,86 ± 0,20	-	-
Sb	shoot	0,47	0,50 ± 0,31	0,42 ± 0,26	*7–50*	*150*
	root	5,02	5,06 ± 0,46	4,14 ± 0,32	-	-
As	shoot	3,32	2,28 ± 0,56	2,82 ± 1,22	*1–1.7*	*5–20*
	root	110,3	97,29 ± 16,91	67,12 ± 31,46	-	-
Pb	shoot	3,07	1,84 ± 0,44	2,65 ± 1,53	*5–10*	*30–300*
	root	178,23	165,94 ± 31,08	91,17 ± 27,06	-	-
PHE accumulation in *Medicago sativa* tissues						
PHE		Soil treatments			Normal levels (mg kg^{-1}dry foliage) [a]	Phytotoxic levels (mg kg^{-1}dry foliage) [a]
		T1 ($N=1$)	T2 ($N=1$)	T3 ($N=1$)		
Cu	shoot	47,34	27,43	14,99	*5–30*	*20–100*
	root	89,48	57,44	64,94	-	-
Zn	shoot	242,53	129,8	88,19	*27–150*	*100–400*
	root	276,27	128,89	135,57	-	-
Cd	shoot	2,02	0,81	0,38	*0.05–0.2*	*5–30*
	root	6,25	2,52	2,73	-	-
Sb	shoot	9,86	5,2	1,6	*7–50*	*150*
	root	12,79	8,33	4,52	-	-
As	shoot	35,51	21,44	6,64	*1–1.7*	*5–20*
	root	79,42	49,94	40,88	-	-
Pb	shoot	57,01	29,87	10,42	*5–10*	*30–300*
	root	143,65	88,42	71,21	-	-

soils where *M. sativa* grew and an increase for Cd in the soils with *C. dactylon*.

Discussion

Plant performance and PHE uptake

Seed emergence

In this *ex-situ* experiment, none of the species could emerge in the polluted non-treated soils (C) due to the strong surface crust, the acidic pH, and the high concentration of salts and PHEs, which resulted in a lack of available essential nutrients. For instance, Delgado-Caballero et al. (2017) observed that low pH together with high concentrations of Cd, Pb and Zn could increase the solubility and toxicity of these elements, inhibiting seed germination (Undersander et al. 2011; Tiller and Merry 1981). In this context of pollution and acidity, gypsum mining spoil (GS) had a crucial role to reinitiate plant colonization, which had been arrested for 20 years. Thus, after having amended polluted soils with GS at any proportion,

Table 2 Mean values (±SD) of total content (mg kg^{-1}) of PHEs (Cu, Zn, Cd, Sb, As and Pb) present in non-treated soil samples before (C_0) and after the experiment (C_f) and in treated soil samples at the end of the experiment ($T1_f$, $T2_f$, $T3_f$); Letter "T" before PHEs refers to total content. Treatments: C, non-treated soil (100% C_0); T1, 90% C_0 + 10% GS; T2, 80% C_0 + 20% GS; T3, 50% C_0 + 50% GS. C_0: polluted soil. GS: Gypsum mining spoil. Nat: Reference levels of total PHEs for unaffected soils within the Guadiamar Green Corridor. NGR (regional thresholds to declare potentially polluted soils for agricultural use in Andalusia, BOE 2015). N: number of samples. Different letters (columns) represent statistically significant differences ($p < 0.05$) for the *post-hoc* Tukey tests performed after the GLMs. [1] < LOD = Under limit of detection

Sample	N	TCu	TZn	TCd	TSb	TAs	TPb
Total PHEs (mg kg^{-1}) in soil samples with *Cynodon dactylon* seedlings							
Nat	5	50.29 ± 22.25	81.08 ± 12.99	< LOD [1]	< LOD [1]	17.35 ± 7.63	46.69 ± 31.31
NGR	5	595	10,000	25	90	36	275
C_0	5	103.50 ± 4.25 c	220.20 ± 11.45 c	0.77 ± 0.03 d	68.37 ± 4.56 c	198.53 ± 11.57 c	240.14 ± 17.71 ab
C_f	5	88.92 ± 3.55 b	69.80 ± 9.34 a	0.21 ± 0.04 a	67.24 ± 5.10 bc	155.36 ± 11.54 b	264.49 ± 14.65 b
$T1_f$	5	93.78 ± 4.77 b	150.50 ± 11.90 b	0.47 ± 0.04 bc	65.69 ± 3.62 bc	160.05 ± 10.70 b	264.31 ± 15.59 b
$T2_f$	5	88.96 ± 4.43 b	152.77 ± 3.12 b	0.49 ± 0.02 c	61.12 ± 1.75 b	149.45 ± 6.97 b	253.38 ± 8.73 ab
$T3_f$	5	77.65 ± 7.06 a	136.18 ± 13.05 b	0.42 ± 0.04 b	53.37 ± 4.62 a	125.13 ± 14.31 a	227.19 ± 28.41 a
Total PHEs (mg kg^{-1}) in soil samples with *Medicago sativa* seedlings							
Nat	5	50.29 ± 22.25	81.08 ± 12.99	< LOD [1]	< LOD [1]	17.35 ± 7.63	46.69 ± 31.31
NGR	5	595	10,000	25	90	36	275
C_0	5	103.50 ± 4.25 b	220.20 ± 11.45 c	0.77 ± 0.03 c	68.37 ± 4.56 c	198.53 ± 11.57 c	240.14 ± 17.71 b
C_f	5	97.42 ± 11.36 b	99.82 ± 10.04 a	0.39 ± 0.14 a	63.65 ± 7.02 bc	194.02 ± 18.68 bc	242.82 ± 45.87 b
$T1_f$	5	100.29 ± 6.46 b	225.60 ± 16.75 c	0.73 ± 0.03 c	61.32 ± 5.35 bc	185.53 ± 11.05 bc	230.98 ± 16.19 b
$T2_f$	5	90.21 ± 6.27 b	220.88 ± 14.88 c	0.68 ± 0.06 bc	55.77 ± 2.84 b	169.18 ± 9.69 b	208.39 ± 11.82 b
$T3_f$	5	72.24 ± 11.38 a	179.41 ± 32.05 b	0.54 ± 0.11 b	42.59 ± 7.99 a	132.35 ± 25.65 a	159.84 ± 31.64 a

Table 3 Mean values (±SD) of soluble PHEs (Cu, Zn, Cd, Sb, As and Pb) (mg kg^{-1}) in non-treated soil samples before (C_0) and after the experiment (C_f) and in treated soil samples at the end of the experiment ($T1_f$, $T2_f$, $T3_f$); Letter "S" before PHEs refers to soluble fraction. Treatments: C, non-treated soil (100% C_0); T1, 90% C_0 + 10% GS; T2, 80% C_0 + 20% GS; T3, 50% C_0 + 50% GS. C_0: polluted soil. GS: Gypsum mining spoil. N: number of samples. Different letters (columns) represent statistically significant differences ($p < 0.05$) for the *post-hoc* Tukey tests performed after the GLMs

Sample	N	SCu	SZn	SCd	SSb	SAs	SPb
Soluble PHEs (mg kg^{-1}) in soil samples with *Cynodon dactylon* seedlings							
C_0	5	123.72 ± 7.12 b	185.81 ± 16.84 c	0.54 ± 0.04 e	0.06 ± 0.01 b	0.12 ± 0.01 c	0.04 ± 0.01 b
C_f	5	3.38 ± 2.48 b	16.17 ± 9.31 c	0.14 ± 0.10 d	$0.02 \pm 3.30E^{-3}$ a	0.05 ± 0.01 ab	$2.60E^{-3} \pm 4.30E^{-3}$ a
$T1_f$	5	0.16 ± 0.01 a	0.12 ± 0.05 b	$9.00E^{-3} \pm 3.00E^{-3}$ c	0.10 ± 0.01 c	$0.04 \pm 4.70E^{-3}$ a	$1.00E^{-4} \pm 1.00E^{-4}$ a
$T2_f$	5	0.15 ± 0.02 a	0.06 ± 0.01 a	$4.00E^{-3} \pm 2.00E^{-4}$ b	0.16 ± 0.01 d	$0.06 \pm 4.70E^{-3}$ b	$3.00E^{-4} \pm 4.00E^{-4}$ a
$T3_f$	5	0.11 ± 0.02 a	0.05 ± 0.03 a	$2.00E^{-3} \pm 1.00E^{-3}$ a	0.16 ± 0.02 d	0.06 ± 0.01 b	$4.00E^{-4} \pm 6.00E^{-4}$ a
Soluble PHEs (mg kg^{-1}) in soil samples with *Medicago sativa* seedlings							
C_0	5	123.72 ± 7.12 c	185.81 ± 16.84 d	0.54 ± 0.04 c	0.06 ± 0.01 b	0.12 ± 0.01 c	0.04 ± 0.01 b
C_f	5	2.44 ± 1.41 b	8.34 ± 3.42 c	0.08 ± 0.02 b	$0.02 \pm 1.90\ E^{-3}$ a	0.05 ± 0.01 b	$2.40E^{-3} \pm 1.60E^{-3}$ a
$T1_f$	5	0.13 ± 0.02 a	0.33 ± 0.15 b	$1.30E^{-2} \pm 3.00E^{-3}$ a	0.08 ± 0.02 b	$0.03 \pm 2.30E^{-3}$ a	$2.80E^{-3} \pm 1.00E^{-3}$ a
$T2_f$	5	0.15 ± 0.01 a	0.08 ± 0.01 a	$4.00E^{-3} \pm 1.00E^{-3}$ a	0.17 ± 0.01 c	$0.05 \pm 4.19E^{-3}$ b	$2.40E^{-3} \pm 2.80E^{-3}$ a
$T3_f$	5	0.11 ± 0.03 a	0.06 ± 0.02 a	$2.00E^{-3} \pm 4.13E^{-4}$ a	0.17 ± 0.01 c	0.05 ± 0.01 b	$2.00E^{-4} \pm 1.00E^{-4}$ a

high emergence rates were observed for both model species (*Medicago sativa* and *Cynodon dactylon*). This could be mainly due to the improvement of soil properties, including pH increase (with values above 4), what would have promoted the reduction of PHE toxicity by decreasing their bioavailability, being of

Table 4 Mean values (±SD) of bioavailable PHEs (Cu, Zn, Cd, Sb, As and Pb) (mg kg^{-1}) in non-treated soil samples before (C_0) and after the experiment (C_f) and in treated soil samples at the end of the experiment ($T1_f$, $T2_f$, $T3_f$); Letter "B" before PHEs refers to bioavailable fraction. Treatments: C, non-treated soil (100% C_0); T1, 90% C_0 + 10% GS; T2, 80% C_0 + 20% GS; T3, 50% C_0 + 50% GS. C_0: polluted soil. GS: Gypsum mining spoil. N: number of samples. Different letters (columns) represent statistically significant differences ($p < 0.05$) for the post hoc Tukey tests performed after the GLMs

Sample	N	BCu	BZn	BCd	BSb	BAs	BPb
Bioavailable PHEs (mg kg^{-1}) in soil samples with *Cynodon dactylon* seedlings							
C_0	5	40.06 ± 1.36 b	166.87 ± 4.69 e	0.62 ± 0.02 c	0.54 ± 0.06 b	3.90 ± 0.18 d	0.55 ± 0.05 a
C_f	5	34.92 ± 3.75 ab	12.57 ± 3.71 a	0.17 ± 0.05 a	0.54 ± 0.21 b	1.47 ± 0.18 bc	0.46 ± 0.08 a
$T1_f$	5	35.83 ± 3.36 ab	73.34 ± 13.74 d	0.51 ± 0.05 b	0.17 ± 0.05 a	1.29 ± 0.18 b	0.39 ± 0.12 a
$T2_f$	5	37.33 ± 6.06 b	45.92 ± 8.33 c	0.51 ± 0.07 b	0.45 ± 0.09 b	1.75 ± 0.32 c	0.55 ± 0.16 a
$T3_f$	5	28.72 ± 5.65 a	31.25 ± 5.80 b	0.42 ± 0.06 b	0.51 ± 0.08 b	0.68 ± 0.14 a	0.47 ± 0.02 a
Bioavailable PHEs (mg kg^{-1}) in soil samples with *Medicago sativa* seedlings							
C_0	5	40.06 ± 1.36 b	166.87 ± 4.69 c	0.62 ± 0.02 c	0.54 ± 0.06 b	3.90 ± 0.18 c	0.55 ± 0.05 bc
C_f	5	35.84 ± 3.48 b	17.18 ± 10.46 a	0.22 ± 0.12 a	0.39 ± 0.05 b	1.50 ± 0.33 b	0.43 ± 0.06 ab
$T1_f$	5	37.32 ± 3.32 b	55.69 ± 5.93 b	0.53 ± 0.04 c	0.23 ± 0.04 a	1.50 ± 0.33 b	0.40 ± 0.05 a
$T2_f$	5	40.13 ± 0.71 b	57.71 ± 6.53 b	0.58 ± 0.02 c	0.49 ± 0.01 b	1.99 ± 0.11 b	0.64 ± 0.19 c
$T3_f$	5	22.49 ± 6.38 a	25.22 ± 7.52 a	0.35 ± 0.09 b	0.42 ± 0.15 b	0.63 ± 0.12 a	0.54 ± 0.07 bc

particular importance the reduction of As, Cd, and Pb bioavailable fractions (Kabata-Pendias 2011; Delgado-Caballero et al. 2017). Seed germination and emergence are the very first and crucial stages in plant cycle (Fenner and Thompson, 2005); however, after emergence, survival and growth must be monitored to evaluate the effectiveness of this amendment.

Survival and growth

In this experiment, gypsum mining spoil proved to favour plant survival and growth, similarly to what was observed by Madejón et al. (2006), where the application of inorganic amendments to polluted soils enabled seedling growth under similar conditions. Moreover, both survival and growth were higher in the treatments with a greater proportion of gypsum mining spoil. In fact, plant survival decreased sharply in the case of *M. sativa*, where a low proportion of amendment was applied. Similarly, seedling growth was low for both species in the treatment with the lowest dose of gypsum mining spoil. These could have been due to the excessive concentration of PHEs accumulated in plant tissues, especially in the case of *M. sativa* which accumulated PHEs at phytotoxic levels (Kabata-Pendias, 2011), causing not only a limited growth but also plant death (Chibuike and Obiora 2014; Kabata-Pendias 2011). In this sense, these negative effects could have been especially important in the case of As, whose deleterious effects have been previously reported (Madejón et al. 2002; Kabata-Pendias 2011; Kumpiene et al. 2019).

The observed differences in the survival of the two model species highlight the importance of using tolerant species to remediate heavily polluted soils (Nirola et al. 2016).

PHE uptake

According to our results, the addition of gypsum mining spoil (calcium-rich amendment, Ballesteros-Jiménez 2018) benefited the performance of both species by limiting PHE uptake, especially at the highest dose of amendment, where both species recorded the highest survival and growth and the lowest PHE accumulation, probably due to the protective action of calcium (Carbonell et al., 1998). As calcium is one of the main antagonistic elements against some PHE sorption and metabolism (i.e. Pb), its presence in the soil solution enhances the selectivity in the uptake of metabolic important elements against unwanted ones (Kabata-Pendias, 2011). Moreover, sulphur may have also reduced the availability of some PHEs such as arsenic (Kabata-Pendias, 2011). Being the incorporation of PHEs to the food web and

their biomagnification a great environmental concern, these protective effects offered by the amendment are of paramount relevance (Gall et al. 2015). Comparing PHE accumulation in tissues, gypsum mining spoil addition appeared to mainly retain PHEs in plant roots, reducing the potential risk for the ecosystem (Freitas et al. 2004; Kumpiene et al. 2019). *C. dactylon* accumulated most Cu, Sb, As and Pb in their roots at any dose of gypsum mining spoil, suggesting this species could act as a phytostabilizer (Abou-Shanab et al., 2007; Sekabira et al. 2011). *M. sativa* mostly accumulated Cu, Cd, Sb, As and Pb in its roots, especially in the soils with the highest dose of amendment, indicating that this species could be dose-dependent. In terms of Zn immobilization in roots, the addition of gypsum mining spoil was less effective for both species (and Cd too for *C. dactylon*), making this element more bioavailable for herbivores. Despite the positive effects of gypsum mining spoil on the overall performance of both species, *M. sativa* still accumulated excessive amounts of most PHEs in its tissues, what could have limited this species growth and survival, and what would pose a risk for the food chain (Kabata-Pendias, 2011).

Observing the strong differences between the two sown native species in terms of plant performance and PHE uptake, the sole application of soil amendment in such a pollution scenario could result in a biodiversity lower than expected. Under these circumstances, sowing with endemic species after soil remediation would be advisable to both help retain pollutants in soil and to ensure the recovery of biodiversity (Madejón et al. 2018b).

Soil properties and PHE content

More than 20 years after the accident, the polluted soils of this experiment are still characterized by a strong acidic pH, salinity and high concentrations of PHEs. The addition of gypsum mining spoil increased pH towards neutrality, but no change was observed on EC values, probably because the solubility of the gypsum present in the amendment may have resulted in more available salts (Casas-Castro and Casas-Barba 1999).

The addition of gypsum mining spoil, especially at its highest dose, produced a dilution effect of total PHEs. Nevertheless, total concentrations of Cu, Cd and Zn in treated soils were still substantially higher than the background concentrations in the surrounding unaffected soils (Nat). Otherwise, total concentrations of As far exceeded the regional threshold for agricultural soils (NGR, BOE 2015) (36 mg kg^{-1}), and the concentrations of Sb and Pb were still too close (Sb = 90 and Pb = 275 mg kg^{-1}), suggesting that further measures could be required whether solubility and bioavailability of PHEs were also high.

Comparing non-treated initial (C_0) and final (C_f) polluted soils, leaching was very significant for the most mobile elements (Zn and Cd) in non-treated soils after having watered C_0 daily for 12 weeks and where plant emergence did not occur, what poses a high environmental risk (Page et al. 2014). On the contrary, the addition of gypsum mining spoil promoted the so needed PHE immobilization in soil. Thus, soluble and bioavailable forms of Cu, Zn and Cd were reduced with the addition of the amendment due to pH rise (Hooda 2010; Kabata-Pendias 2011). Moreover, the presence of gypsum mining spoil could have promoted the formation of Al-hydroxy polymers in the polluted soil, immobilizing them (Garrido et al. 2005). As well, Ca and S seem to have significantly reduced Zn solubility (Kabata-Pendias, 2011). Soluble As did not increased along with pH as it could be expected (Hooda 2010), but decreased with the lowest dose of gypsum mining spoil. Moreover, bioavailable As decreased in all treated soils, especially with the highest dose, probably because arsenate toxicity diminished as a result of its adsorption by iron hydroxysulphates (O'Neill 1995). Therefore, gypsum mining spoil proved its effectiveness in reducing the toxicity of Cu, Zn, Cd and As. On the contrary, its effect on Sb and Pb was controversial. Bioavailable Sb only descended with the lowest dose of the amendment and soluble Sb increased with the addition of higher doses. This fact could have been promoted by both the presence of organic matter (Nakamaru and Martín-Peinado, 2017) as a result of seed emergence and growth, and by a significant addition of soluble Sb present in the amendment. Lead bioavailable fraction did not present any change in most of the amended soils. Nevertheless, according to Pb bioavailability ratio, Pb bioavailability increased in the treatments with higher doses of gypsum mining spoil. However, Pb solubility was reduced with any dose of the amendment; in this sense, it has been reported that calcium ions could reduce Pb bioavailability by

direct competence (Li et al. 2014), that sulphate ions could promote the precipitation of Pb as anglesite ($PbSO_4$) (Rehman et al. 2017), and that the formation of Al-hydroxy polymers could promote Pb sorption (Garrido et al. 2003).

Despite the controversial behaviour of As, Sb and Pb in soil depending on the dose of GS, if we also focus on the results of PHE uptake, the more GS we added to the polluted soil, the less PHEs were accumulated in plants. Moreover, in the case of *Medicago sativa*, the higher doses of GS in the soil, the higher retention of these elements were found in roots too, probably because there was a more balanced composition of nutrients in the soils treated with the amendment as a result of PHE immobilization (Kabata-Pendias, 2011).

According to our results, the potential toxicity of some PHEs seem to depend on the dose of gypsum mining spoil; consequently, the right dose should be carefully studied, especially where complex mixtures of PHEs are present (Clemente et al. 2012; Madejón et al. 2018a; Simón et al. 2010). Nonetheless, considering plant performance and soil properties altogether, we can state that the addition of gypsum mining spoil brought a clear benefit, especially where 20% and 50% of the amendment was added to this polluted soil.

Applicability

PHE fixation is considered among the most effective treatments for a wide range of polluted soils (Vangronsveld and Cunningham, 1998), since chemical immobilization prevents the transport of pollutants into deeper soil layers and groundwater (Querol et al 2006). In this sense, considering our result, gypsum mining spoil could be a promising amendment material for the remediation of PHE-polluted soils.

Compared to other inorganic amendments frequently used in remediation of soils polluted with PHEs such as lime (Clemente et al. 2006; Madejón et al. 2006; Pérez-de-Mora et al. 2006), Wallace and Wallace (1995) observed that gypsum, the main component of gypsum mining spoil, was more effective in improving acid soils, especially because gypsum can reach the subsoil where lime cannot penetrate. On top of that, and contrary to organic amendments (McGrath et al. 1995), gypsum mining spoil has negligible quantities of PHEs and no pathogens.

The combination of gypsum mining spoil with organic amendments could improve its potential in soil remediation. In this sense, Alvarenga et al. (2008) showed that organo-mineral amendments could decreased Cu, Pb, and Zn mobile fractions in mining soils, and Jiménez-Moraza et al. (2006) observed the immobilization of Zn and Cd after the application of sugar-beet lime which, according to our results, were the two most bioavailable elements for herbivores as they accumulated equally in roots and shoots.

As gypsum is a mineral in global demand (Herrero et al. 2013), and its extraction through mining produces great amounts of gypsum mining spoil (Ballesteros-Jiménez 2018), the use of this waste as amendment would help to overcome this environmental issue. Moreover, the costs associated to the use and management of gypsum mining spoil are usually low, and as no further processing is required, the expenses of this waste material are mainly related to its transport, what should be reflected in reduced market prices in comparison to other gypsum derived products such as phosphogypsum (Campbell et al. 2006). In this vein, further studies should be conducted on the applicability of this material, including the economic viability of its commercialisation as amendment for environmental and agricultural applications.

Conclusions

Gypsum mining spoil is a waste material rich in gypsum and calcium carbonate, so that it presents a high potential as an amendment of soils polluted with potentially harmful elements (PHEs).

Based on our study, gypsum mining spoil appears to have positive effects on the remediation of soils polluted with PHEs and the recovery of their vegetation. The presence of gypsum mining spoil at any dose, and especially at 50%, enhanced seed emergence, biomass production (growth) and survival rates in our model species. This is directly related to the reduction of soil acidity, soil crust formation and the availability of potentially harmful elements. Moreover, it appears to reduce PHE uptake and promotes its retention mainly in plant roots, reducing the potential risk for ecosystems.

In comparison to other soil amendments, the use of gypsum mining spoil would also bring the following benefits: i) Its relatively low solubility rates make it a

great source of calcium over time; ii) It has negligible quantities of potentially harmful elements and no pathogens described; iii) It does not need to be processed prior to its application, reducing time and costs.

The next step would be to test the effectiveness of gypsum mining spoil in the field and assess its effects on a wider range of native plant species. As well, further studies should be conducted to enhance the potential of gypsum mining spoil as amendment of polluted soils. In this vein, it would be interesting to test a new mixed amendment containing gypsum mining spoil and an organic matter-rich amendment (i.e. olive mill waste compost, vermicompost, manure, etc.).

In summary, the relevance of this paper lies in the fact that, according to our findings, gypsum mining spoil could be used as a novel and effective amendment, when applied in the right dose, to recover soils polluted with potentially harmful elements and their associated vegetation. Moreover, the use of gypsum mining spoil as soil amendment, will simultaneously help mitigate two urgent environmental issues: (i) the sustainable management of mining waste material; and (ii) the effective remediation of degraded soils polluted with potentially harmful elements.

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