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Assessment of the phytoremediation effectiveness in the restoration of uranium mine tailings

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ABSTRACT

Uranium (U) contamination due to mining and metallurgical operations is a serious problem and poses a high potential threat to humans and other organisms. The application of amendments and/or plants, known as assisted phytoremediation, can accelerate the adsorption, complexation and precipitation processes in soil, and therefore can facilitate the restoration of U mining areas. This study located in the central-west of peninsular Spain was focussed on two different U mine waste dumps (MWD), where the assisted phytoremediation process was implemented. At each MWD, an area of 10 ha (approx.) was selected for sugar lime (SL) application, at a rate of 75 T ha $^{-1}$. In addition, after SL addition, common grasses *Cynodon dactylon, Secale cereale*, and the leguminosae *Vicia sativa* were seeded. Evolution of soil physico-chemical properties and plant development was monitored for 69 months in Top, Medium and Low areas of both MWDs. The main results indicated a positive effect of the amendment, improving the development of the spontaneous and induced vegetation and the accumulation of soil organic matter. Values of pH were maintained in the range of the neutrality during all the experimentation period. In general, total concentrations of trace elements (including U) presented a high variability. Values were similar in the different studied areas of each MWD (Top, Medium and Low) and did not change in time. Trace elements contents were, in general, higher than those found in non-contaminated soils. However, U and Mn availability decreased with time, as well as the accumulation of trace elements (especially U and Mn) in the studied plants. Moreover, these contents were lower than the permitted limit for animal consumption. In conclusion, application of phytoremediation contributed to the stabilization of U within the soil-plant system, mainly by its positive effects on vegetation cover and the consequent increase in soil organic matter.

1. Introduction

Surface mining worldwide directly results in high disturbances to the ecosystems. These mining activities can directly affect large areas of natural vegetation and can cause indirect environmental impacts due to erosion, run-off and loss of diversity in the surroundings (Doley and Audet, 2013). Today, all countries with a recognized mining prestige look back at their old mining facilities, developing an important task for their reconversion and recovery. Viable and efficient restoration actions of these mined sites are mandatory to reduce negative environmental impacts. The rehabilitation implies the restitution of the original ecosystem in terms of soil and hydrology, diversity of flora and fauna species (Madejón et al., 2021). The final aim is to restore vital ecological processes in such a way that the resulting ecosystem will be capable of self-sustaining (Hobb and Norton, 1996).

In particular, Uranium (U) contamination due to mining and metallurgical operations is a serious problem and poses a high potential threat to humans and other organisms. Uranium is a threatening radionuclide due to its radioactivity and its toxicity (Antunes et al., 2007; Bai et al., 2010), posing a risk to health when incorporated especially in aqueous forms (Bhalara et al., 2014; Schöner et al., 2009). Uranium available in the environment can reach humans through the

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food chain, causing several health disorders (Craft et al., 2004; Xie et al., 2008).

Uranium dynamics in soil has been extensively studied and found to be driven by the interaction with different soil components (Chen et al., 2021). Among these components, organic matter has an important influence on U dynamics since certain organic compounds may effectively immobilize it, limiting the potential movement of U through the biosphere (Fuller et al., 2020; Ma et al., 2020). The adsorption of U to organic matter has received substantial research attention as a mechanism of U retention in soils. For that reason, organic amendments could decrease U mobility. Moreover, other inorganic amendments have also been used to adsorb U in soil reducing its availability and solubility (Zhang et al., 2015; Gavrilescu et al., 2009).

The assisted phytoremediation consists on the application of amendments and plants that can accelerate the adsorption, complexation and precipitation processes, which occur naturally in the soil, and facilitate the retention of trace elements (included U). Thus, the application of amendments is usually a successful technique as first step in the restoration of contaminated and degraded mining soils (Kumpiene et al., 2019; Palansooriya et al., 2020). This technique can be considered as a soft or low impact rehabilitation technique, since the incorporation of amendments followed by the establishment of plants constitutes a more natural and respectful approach with the natural environment than the classic methods based exclusively on physicochemical processes (Madejón et al., 2018a). The main advantages of phytoremediation are: 1) it is economically feasible, simple to manage, and with low costs of installation and maintenance, 2) it is an environment and eco-friendly technique, which reduces exposure of of the ecosystem to the contaminants, 3) it can be applied over a large-scale field, 4) it prevents erosion, reducing the risk of spreading of contaminants, and 5) it can also improve soil fertility by releasing various organic matters to the soil (Yan et al., 2020).

Among inorganic soil amendments, the sugar beet lime (SL) has been used since ancient times to correct the acidity and the phytotoxicity of Al in acid soils, mainly due to its high content of active limestone (García-Navarro et al., 2009). In addition, the SL contains organic matter and several essential micronutrients (Vidal et al., 2006). The high Ca content of the sugar foam is mainly due to the presence of Ca in the form of slaked lime (Ca (OH_2)) and, to a lesser extent, as calcium carbonate ($CaCO_3$) (Espejo, 2001). Slaked lime progressively reacts with atmospheric carbon dioxide to produce $CaCO_3$. This carbonation occurs at a rate that depends on the particle size, porosity, and water content of the SL. This gradual carbonation process makes the durability of the product more effective with time. For all this, the SL has also been used as an amendment in the assisted recovery of contaminated soils. For example, SL has been used successfully in the restoration of the Guadiamar Green Corridor that was created after the Aznalcóllar mining spill in 1998 (Madejón et al., 2018b). Several studies carried out in this area (Clemente et al., 2015; Madejón et al., 2010) have demonstrated, at different scales, the efficacy of this by-product in the remediation / stabilization of soils contaminated by trace elements.

On the other hand, the use of vegetation covers on unstable degraded land, such as mine spoils and tailings facilities, has been proved to provide an in-situ cost-effective and environmentally sustainable method of stabilizing and reclaiming waste lands (Mendez and Maier, 2008; Madejón et al., 2018a). The use of vegetation to reduce erosion and to provide long-term structural support is known as phytostabilization. Revegetation of mine sites is a well-documented tool for effective surface stabilization, as tailings are usually almost completely devoid of vegetation cover, which increases the likelihood of serious pollution resulting from wind and water erosion of the bare tailings surface (Pérez-de-Mora et al., 2011). For the remediation of large-scale and low U-polluted soils, it is assumed that phytoremediation and assisted remediation would be effective technologies (Abreu et al., 2014; Tan et al., 2019). Nevertheless, these technologies need long-term monitoring and supervision because they have barely been demonstrated on a full-scale basis.

Previous tests carried out on columns and collectors showed promising results from the application of SL on mining soils contaminated by U, especially due to the improvement of soil conditions and the increase of the pH of the runoff waters (Madejón et al., 2014). However, validation of these results in field experiments is needed to demonstrate the advantages and the feasibility of the use of SL. In this work, we performed an in-situ remediation experiment in two U waste mine dumps (MWD), where we tested the long-term effectiveness of SL application and revegetation for the stabilization of U in the soil-plant system. We hypothesized that the assisted phytoremediation could help to increase soil fertility and the buffering capacity of the degraded and shallow soils of the dumps, and to improve the development of vegetation. To test this hypothesis, sampling of soil, plants and runoff water in these two dumps were periodically performed during 69 months to analyse several physico-chemical indicators and to evaluate the efficiency of the restoration measures.

2. Materials and methods

2.1. Study area

The study area is located in the region of Ciudad Rodrigo (29 T 702113 4,501,299), which constitutes a territory of large extent in western Salamanca Province, in the central-west of peninsular Spain (Fig. 1). This region is located 700–800 m above sea level. The climate is sub-humid, temperate, and dry in the summer, with an annual precipitation of 500–600 mm, an average temperature of ~12 °C and annual evapotranspiration of more of 1000 mm. There are large interannual differences and significant heterogeneity in the spatial distribution of rainfall (Fig. S1). Rainfall is more abundant in autumn (October and November) while the summer is dry or very dry (July and August with values below 15 mm). Evaporation is very high (1200–1400 mm per year), with a considerable water deficit from May to September, which reaches its maximum in July. Although during the rest of the year rainfall is greater than evaporation, it is not enough to alleviate the water loss during summer, with a deficit of 800 mm per year (Aran et al., 2020). The moisture and temperature regimes of the soils are xeric and mesic, respectively. The climatophilous vegetation of this area corresponds to supra-Mediterranean silicic oak, which rarely appears in the form of dense forest, but normally occurs in the form of sparse woodland (Santos-Francés et al., 2018).

2.2. Mining activities

During the exploitation period (1975–2000) more than 80 Mt. of rock were extracted, through open-pit mining and the bank system. Once production was completed, a comprehensive decommissioning program began in January 2000, in order to recover radiological and environmental conditions similar to those existing at the site before mining began. The program included the dismantling of the plant for the manufacture of U concentrates (including its static leaching beds and sludge dikes), and the definitive restoration of the mining operations (shafts, dumps and associated structures and facilities).

2.3. Experiment design

Two different waste dumps (MWD) in the mine were selected for the experiment, hereafter denoted as MWD A and B, respectively. Materials from the uranium deposit debris from the Tertiary of the Ciudad Rodrigo trench (arcoses, with intercalations of gravels and pebbles) and from the Pliocene and/or Quaternary (materials type “rañas”, colluvions and current alluvial deposits of the Águeda river) can be found. Soil in MWD-A had a thickness between 10 and 15 cm in average. Soil in MWD-B was even shallower and with a coarser texture than in MWD-A. Moreover, the MWD-B, was characterized by steeper slopes. At each MWD, an area

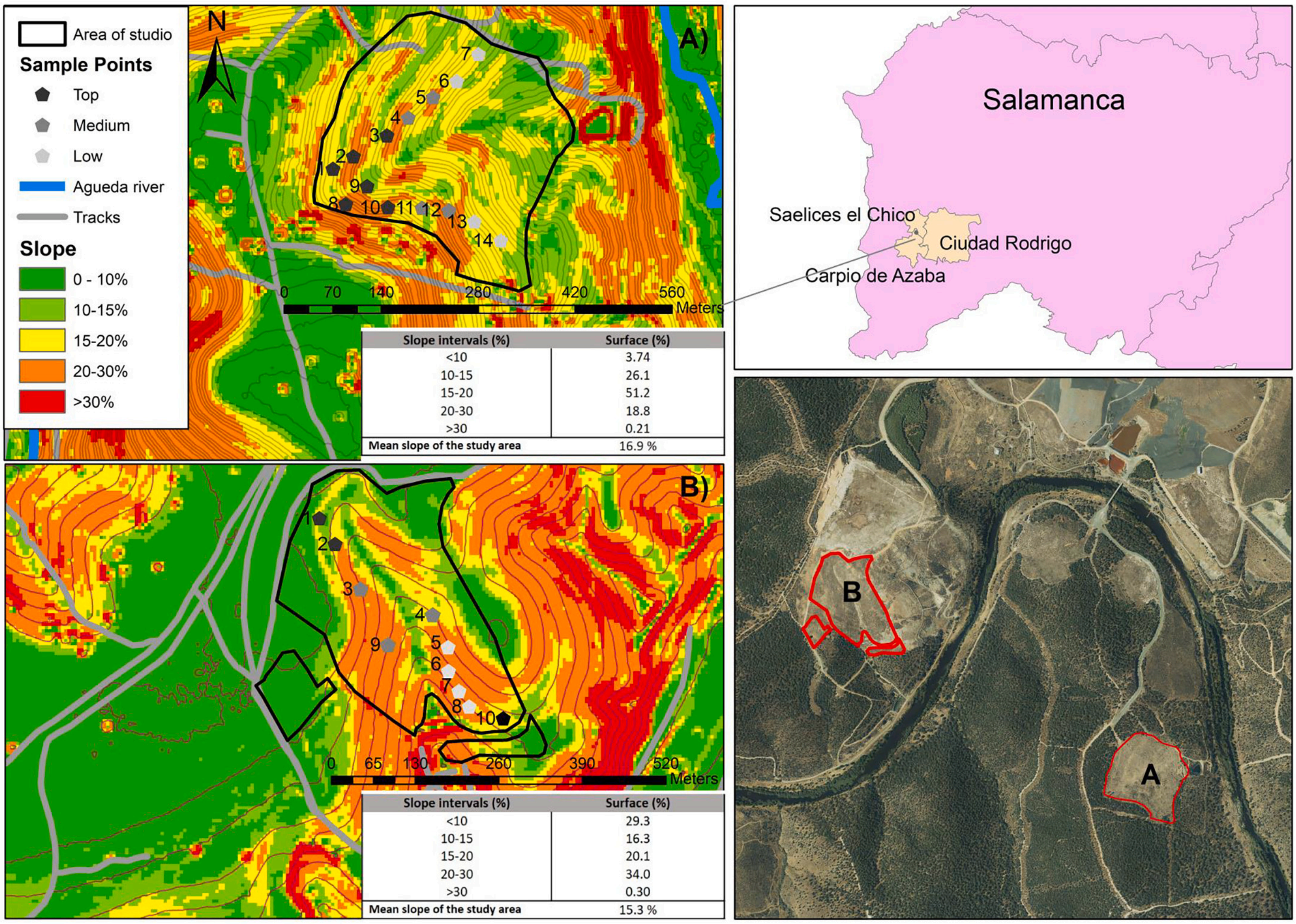

Fig. 1. Location map of the remediated study area "Saelices el Chico". A) Location of the mine waste dump A and their sampling points B) Location of the mine waste dump B and their sampling points.

of 10 ha (approx.) was selected for the SL application, at a rate of 75 T ha^{-1}. In addition, after the amendment application common grasses *Cynodon dactylon, Secale cereale*, and the leguminosae *Vicia sativa* were seeded. Both activities, SL application and seedling, were carried out at the end of November 2013 in the MWD-A and at the end of April 2014 in the MWD-B.

Sugar lime (SL) is a residual material from the sugar beet manufacturing process with 70–80% (dry basis) of $CaCO_3$. Apart from its high calcium carbonate content (pH around 9) it contains also a considerable amount of OM (7.5%) and other nutrients such as P, Mn, and Fe (Clemente et al., 2015).

2.4. Sampling: soil, water and plants

Fourteen and nine sampling points were established in MWD-A and MWD-B respectively. We distributed these sampling points across the studied area according to the slope of each waste dump: Top (Number of samples (N), N = 6 for dump A and N = 2 for B) (T), Medium (N = 4 for A and B) (M), and Low (N = 4 for A and N = 3 for B) (L) (Fig. 1).

Soil sampling at each selected point was carried out at 0–15 cm depth (sometimes at 0–10 cm, especially at MWD-B, if reaching the rock before). For each point three soil cores were taken (using a spiral auger of 2.5 cm diameter) to make a representative composite sample. Sampled soils were stored in plastic bags and transported to the laboratory for analysis. Sampling was carried out 7 times since SL addition in MWD-A and 6 times since SL addition in MWD-B, from February 2014 to October 2019 (Table 1).

The development of the vegetation in the revegetated areas was monitored during the period of study. Vegetation cover (using a 0.03 × 0.03 m quadrant) and plant richness was also recorded at each sampling point. In addition, most common plants were sampled for their trace element content analysis. Identification of the different plant species was checked in the laboratory. Depending on the sampling time and phenological state of the plant, plants were identified to the species, genus or family level.

The runoff waters from these two areas were collected directly from their influence ponds. Sampling times for soil, plants and water throughout the experimentation are listed in Table 1.

2.5. Chemical analyses of soil, plant and pond water

Soil samples were dried at 40 °C and then sieved to <2 mm for analysis. Soil pH was measured in a 1 M KCl extract (1:2.5, m/v) (Hesse, 1971) using a pH meter (CRISON micro pH 2002). Organic Matter (OM) was determined by dichromate oxidation and titration with ferrous ammonium sulphate, according to Walkley and Black (1934).

The carbonate determination was carried out using the Bernard calcimeter. The method is based on the reaction of hydrochloric acid (HCl) with calcium carbonate ($CaCO_3$), causing the evolution of carbonic anhydride (CO_2). This gas displaces a volume of an indicator liquid that is quantified.

For trace elements analyses, including U, the soil samples (2 mm)

Table 1
Sampling schedule for the collection of data on soil properties, vegetation development, trace element accumulation in plants and chemistry of pond water.

MWD	Sampling	Date	Soil	Vegetation cover	Plant richness	Plant analysis	Pond water
A	1	12/02/2014	X				X
	2	03/06/2014	X	X	X		X
	3	01/10/2014	X			X	X
	4	02/06/2015	X	X			
	5	21/10/2015	X			X	X
	6	08/04/2016	X	X	X	X	
	7	17/10/2019	X	X	X	X	X
B	1	03/06/2014	X		X		X
	2	01/10/2014	X	X		X	X
	3	02/06/2015	X	X			
	4	21/10/2015	X			X	X
	5	08/04/2016	X	X	X	X	
	6	17/10/2019	X	X	X	X	X

were ground and sieved at <60 µm. Pseudototal concentration was determined digesting them with a mixture of concentrated HNO_3 and HCl (1:3, 'aqua regia') in a microwave oven (Microwave Laboratory Station Mileston ETHOS 900, Milestone s.r.l., Sorisole, Italy). Meanwhile, their available concentrations were estimated after extraction with a 0.01 M $CaCl_2$ solution at a rate 1:10 (Houba et al., 2000). Trace elements in all the extracts were determined by ICP-OES (inductively coupled plasma-optical emission spectrometry) using an IRIS Advantage spectrometer (Thermo Jarrel Ash Corporation, MA USA). The accuracy of the analytical method was determined using the reference NIST 2711a obtaining recovery rates of 85%–110%.

Plant material was washed for 15 s with 0.1 M HCl, followed by a 10-s washing with distilled water, and then oven-dried at 70 °C. Dried plant material was ground by passing them through a 500-µm stainless steel sieve. Trace elements in plants were extracted by wet oxidation with concentrated HNO_3 in a microwave oven. The extracts were measured by inductively coupled plasma optical emission spectrometry (ICP-OES). The quality of the analyses was assessed using reference material NIST SRM 1573a, and the obtained recovery rates were between 95 and 105%.

Water pH and EC were directly measured with a CRISON pH-meter and conductivimeter. For trace elements concentration in the water, samples were filtered using a 0.45-µm filter, and 25 ml of this filtrated water were collected and 0.2 ml HCl were added.

2.6. Data analysis

Means and standard errors were determined for all variables, except for available trace elements in soils, which showed high variability, and results were showed as median values. Differences among the three topographical zones and the evolution in time of each zone in soil chemical composition were tested by one-way ANOVA and Tukey posthoc test ($p < 0.05$). Before the ANOVA analysis, data normality (by Kolmogrov-Smirnov test) and homogeneity of the variance (by Levene test) was tested. When data were non-normal, even after a logarithmic transformation, Kruskal-Wallis test and Mann-Whitney tests were used for mean comparison. To compare soil total content between the initial and final sampling a Student's *t*-test ($p < 0.05$) was used to assess significant differences. All analyses were performed using the program IBM SPSS Statistics 27.0 for Windows (SPSS, Chicago, IL, USA).

3. Results

3.1. Evolution of soil physical and chemical properties

3.1.1. pH, OM and $CaCO_3$

In MWD-A, the initial soil pH value, before SL addition was between 6.6 and 6.7. After the amendment application, soil pH increased to values between 7.7 at the L zones of the dump and to 7.3 at the T zones (Fig. 2a). In the case of this sampling (and only for this time), significant differences among the three zones were observed ($p < 0.05$). These values decreased during the first nine months after the application, especially at M zones reaching values of 6.8. However, these values increased again 17–21 months after the beginning of the experiment. A new slight decrease was observed in the three zones at the end of the experimentation period. For the three studied zones pH values showed significant differences in time ($p < 0.001$). In all cases, values remained neutral (7.0), higher than the initial values and similar along all the zones of the MWD-A.

pH values of 6.3–6.5 were recorded in MWD-B, before the implementation process (Fig. 2b), and increased to pH values of 6.7 at M and L zones, and to 7.2 at zone T after one month from SL application. Over the study, values increased during the first two years. For the three studied zones pH values showed significant differences in time ($p < 0.01$ for T and M zones and $p < 0.001$ for L zone). At the end of the study (after 65 months), values were still neutral (7.2), even higher than in MWD-A. Significant differences were among zones at each sampling were only observed at the sampling 4 ($p < 0.05$).

Organic matter (OM) contents increased through the experimental period at both dumps (Fig. 2c, d). In the case of MWD-A, from an initial average of 1.0% of OM, values of 1.2% were recorded after one moth from the beginning of the study in the three zones (Fig. 2c). Moreover, this value increased by 0.5 points during the first two years. After this sampling time (27 months from application), OM continued increasing only at the L zones, reaching values of OM of 2.5%. For this parameter, significant differences in time were observed for T and L zone ($p < 0.05$). However, among the three studied zone there no differences at each sampling.

In MWD-B, the initial OM contents were very low (< 0.5%), and increased significantly to 1.0% after one month of monitoring. In this dump, a constant increment with time, from 1.0% OM until values close to 3%, was observed after two years at T and L zones (Fig. 2d). At the end of the study, maximum values, up to 2.0%, were found in all the zones. In this case, the three studied zones showed significant differences in OM content in time (For T and M zones $p < 0.01$, and for L $p < 0.05$).

Carbonate content presented a high variability over time in both dumps (Fig. 2 e, f). This is why significant differences were not observed in time. In the MWD-A, values fluctuated between 5% in the T to 7% in the L zone. Through the course of the study values varied without any clear pattern, and at the last sampling in the L zone values were around 6.5% while at the M zone values decreased to 3% (Fig. 2e). In MWD-B the variability at the beginning of the study at T zone was also very high (from 4.2 to 11%), while at the M and the L zones $CaCO_3$ content was around 4% (Fig. 2f). Finally, in the last sampling values were between 5 and 6% at the M and T zones and decreased to 3% in the L zone. In this last sampling, significant differences among zones were recorded at both MWDs ($p < 0.05$).

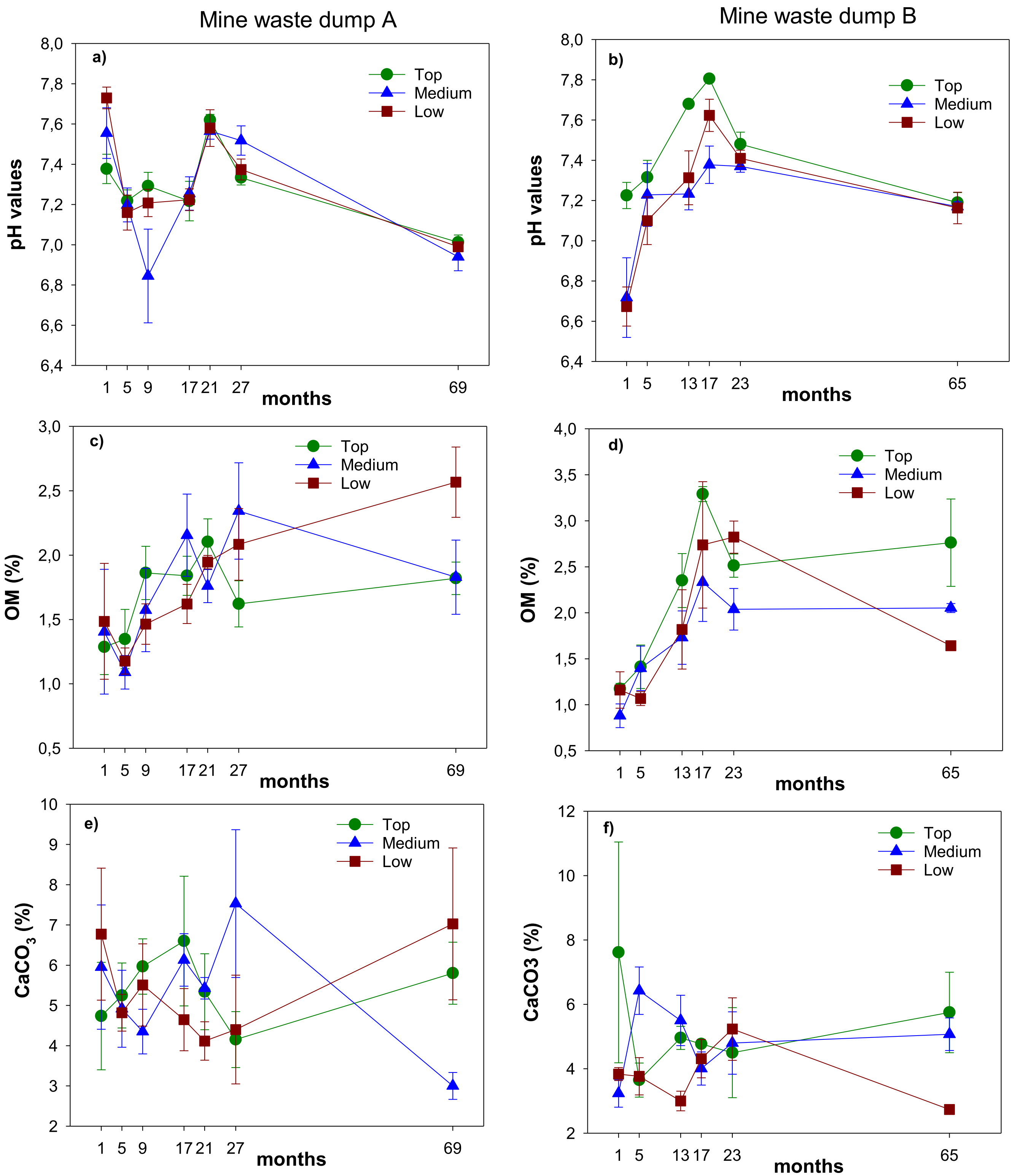

Fig. 2. Evolution over time of pH (a, b), OM (c, d) and CaCO3 (e, f) in both waste mine dumps (mean values and standard error) in each studied zone (Top, Medium and Low).

3.1.2. Uranium and other trace elements in soils

At both MWDs pseudototal values of trace elements showed a high variability and did not differ significantly among the three zones (Table 2). Uranium contents in the MWD-A in the initial sampling were in a range (in mg kg^{-1}) of 2.70–70.9, meanwhile at the final sampling the ranges at the three zones were 10.6–157. Maximum U values were found at the M zone in both samplings, especially at the final one.

In the MWD-B, initial U range (in mg kg^{-1}) at the three studied zones were between 6.4 and 492, meanwhile at the end of the study values were 29.0–309. The highest values recorded in this MWD were found at the T and M zone.

Manganese ranges (mg kg^{-1}) in the MWD-A at the initial sampling

Table 2
Pseudototal trace element concentrations (mg kg^{-1}) in soils from both mine waste dumps at the initial and the final samplings. Mean values and standard error in parenthesis.

M.W·D	Zone	Sampling	As	Cd	Co	Cu	Fe (%)	Mn	Ni	Pb	U	Zn
A	Top	Initial	28.1	0.15	7.20	16.6	1.58	231	16.4	10.9	30.8	34.8
			(0.34)	(0.04)	(1.92)	(0.13)	(0.06)	(55.8)	(4.54)	(0.21)	(11.7)	(1.58)
		Final	26.3	0.26	10.2	35.1	2.63	539	32.9	16.8	77.6	97.9
			(2.30)	(0.03)	(1.49)	(8.98)	(0.38)	(81.9)	(3.12)	(1.95)	(56.0)	(33.5)
	Medium	Initial	44.6	0.18	9.70	24.4	2.57	266	22.3	17.32	39.7	48.1
			(1.43)	(0.05)	(2.31)	(8.08)	(0.62)	(72.2)	(8.08)	(3.94)	(16.7)	(12.3)
		Final	23.5	0.20	5.95	28.2	2.06	295	24.1	13.3	51.1	44.5
			(1.90)	(0.02)	(0.85)	(5.75)	(0.32)	(28.0)	(4.80)	(4.43)	(35.4)	(3.79)
	Low	Initial	31.9	0.20	6.89	11.3	1.59	190	12.9	10.65	11.1	36.4
			(3.05)	(0.05)	(1.07)	(0.86)	(0.11)	(31.3)	(1.72)	(0.76)	(2.52)	(5.01)
		Final	20.6	0.20	7.53	26.2	2.03	339	32.0	13.2	27.5	53.2
			(2.66)	(0.03)	(1.70)	(3.03)	(0.20)	(32.8)	(2.04)	(2.08)	(7.53)	(6.66)
B	Top	Initial	18.1	0.66	5.61	87.9	1.98	214	15.7	16.0	344	55.3
			(1.6)	(0.53)	(0.23)	(38.4)	(0.32)	(18.2)	(1.45)	(0.20)	(153)	(5.28)
		Final	19.3	0.17	10.1	46.7	3.17	1063	44.1	14.6	147	191
			(4.39)	(0.06)	(2.26)	(8.78)	(0.60)	(606)		(3.00)	(139)	(100)
	Medium	Initial	47.5	0.35	14.8	35.1	2.80	454	42.7	14.6	142	89.1
			(11.5)	(0.03)	(3.69)	(5.66)	(0.59)	(133)	(8.70)	(2.17)	(34.8)	(16.2)
		Final	60.9	0.40	15.3	49.8	4.28	1310	52.8	15.6	144	141
			(7.09)	(0.03)	(0.83)	(6.50)	(0.27)	(497)		(2.24)	(84.0)	(20.5)
	Low	Initial	38.0	0.38	17.6	43.5	4.38	574	52.0	18.0	58.6	130
			(5.50)	(0.16)	(2.51)	(6.41)	(0.30)	(83.4)	(7.99)	(2.69)	(23.8)	(12.7)
		Final	26.9	0.23	7.94	30.5	3.57	486	4.00	18.7	34.3	84.1
			(2.62)	(0.02)	(2.97)	(14.7)	(0.84)	(109)		(5.24)	(29.0)	(22.1)
Background soils[1]			18.3	0.15	13.4	16.4	1.99	698	29.2	18.9	28.9[2]* 71.2[2]**	58.5
Mediam values in Europe[3]			6.0	0.145	7.0	12.0		382	14	15	2.0	48

[1] Aran et al., 2020.
[2] Santos-Francés et al., 2018. * for granite rock, ** for slate.
[3] Alloway, 2013.

were 175–430, and the final ranges were 270–412. For the MWD-B, Mn ranges at the initial sampling were 196–821; for the final sampling mean values ranged between 162 and 2272 mg kg^{-1}. As for the case of U, higher concentrations of Mn were found at the MWD-B reaching values near 2770 mg kg^{-1}. This element reached maximum values at M and T zones (Table 2).

The rest of the studied trace element concentrations in MWD-A were, in general, higher than mean values established for background soils in this area (Table 2). The variability of the data was remarkable (see standard error) due to the heterogeneity of the wide study area of both MWDs (around 10 ha; Table 2). Levels of trace elements were in a similar range or higher in the MWD-B. Regarding the differences between the different divided zones of study (T, M, L), we could not find any clear pattern that could indicate a mobilization of these elements from the top to the bottom of the dump. The same fact was observed for metal contents comparing the initial values with those recorded five years later (final sampling). Both dumps showed similar metal concentrations in soils over time, although the variability of metals at each dump was high.

The positive effect of assisted phytoremediation was more evident in available concentrations, reducing this fraction of Mn and U (Fig. 3). In the beginning of the study available U at the MWD-A reached values of 7 mg kg^{-1} in the Top zone, although in the M and the L zones values were around 2 mg kg^{-1}. However, at the end of the study U available concentrations in all the zones decreased to values around 0.5 mg kg^{-1}. On the other hand, U available at MWD-B was much higher than in MWD-A (14 mg kg^{-1} in Top zone in the first sampling, and close to 16 mg kg^{-1} at the M zone after 13 months of SL application). Despite the high concentrations in the first two samplings, availability decreased with time until values of around 1.0 mg kg^{-1} in all zones in the last sampling. As for U, the highest concentrations of available Mn were found at MWD-B, but these values presented a high variability during the first two years (Fig. 3). After 23 months, however, these contents decreased and at the end of the study all values were $\leq$ 1.0 mg kg^{-1}.

The availability of the rest of trace elements showed a high heterogeneity; therefore, median values are shown in Table S1. In general, available trace element concentrations were very low, lower than 1 mg kg^{-1}, except for Fe in some samplings.

3.2. *Evolution of vegetation*

3.2.1. *Vegetation cover and plant richness*

Vegetation cover was monitored four times during the experiment, taking into account the time needed for plant development. The first plant data was collected five months after seeding and the fourth observation was recorded at the end of the study, after 65–69 months. In general, vegetation cover increased over the course of the experiment (Fig. 4), and due to better soil conditions, the vegetation cover was always higher in MWD-A than in MWD-B. In MWD-A, vegetation cover was similar in the T and the M zones (50%) and slightly higher in the L zone (55%) five months after the beginning of the study. In the L zone, vegetation cover increased until percentages of 82%, 17 and 27 months after the start of the experiment, and by the end of the study vegetation covered 95% of soil surface. In the M, and especially in the T zone, the plant development was slower but, in both zones, values recorded after 69 moths reached 85% of vegetation cover.

In the MDW—B, the highest vegetation cover was also found at the L zone. However, during the first two observations percentage was around 53%, reaching values of 90% at the end of the study. In the M zone, the initial vegetation cover was around 30%, increasing to 50% and reaching values of 83% in the last observation. Finally, the lowest vegetation development was found at the T zone of this dump (values of 5% in the first sampling). However, an important increase in time was observed until values of 60% at the end of the study (Fig. 4).

The plant species richness was recorded in 2014, 2016 and 2019 (Fig. 5). As mentioned above, three different species were seeded at the start of the experiment, *C. dactylon, S. cereale* and *V. sativa*. Among them, *S. cereale* proliferated at both MWDs, especially at MWD-B, where it was the dominant species, being the only plant species in 2014 and 2016. Nevertheless, *V. sativa* was also found in 2014 at the M zone.

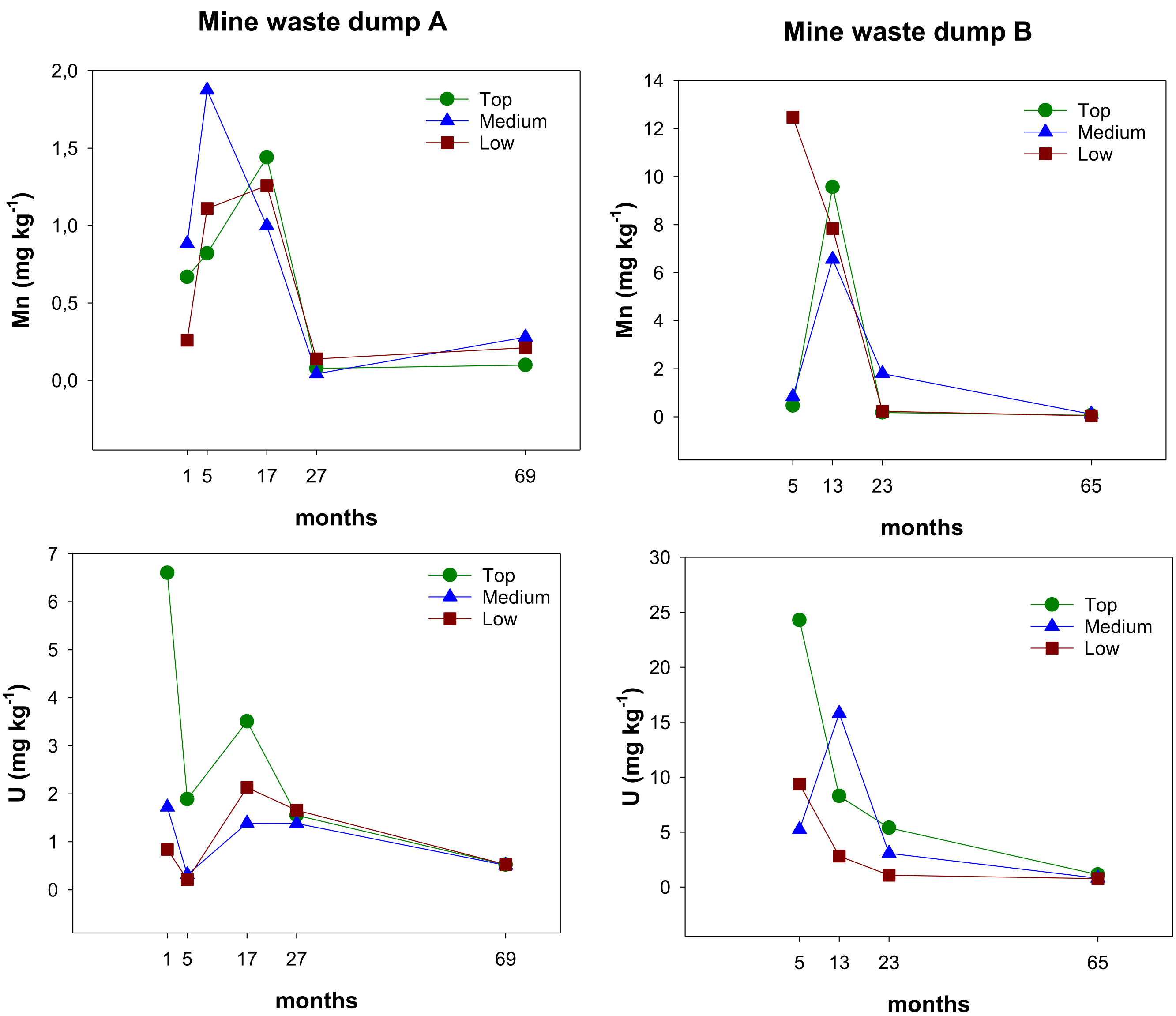

Fig. 3. Median values of availability of Mn and U over the studied time in each studied zone (Top, Medium and Low) for MWD-A and B.

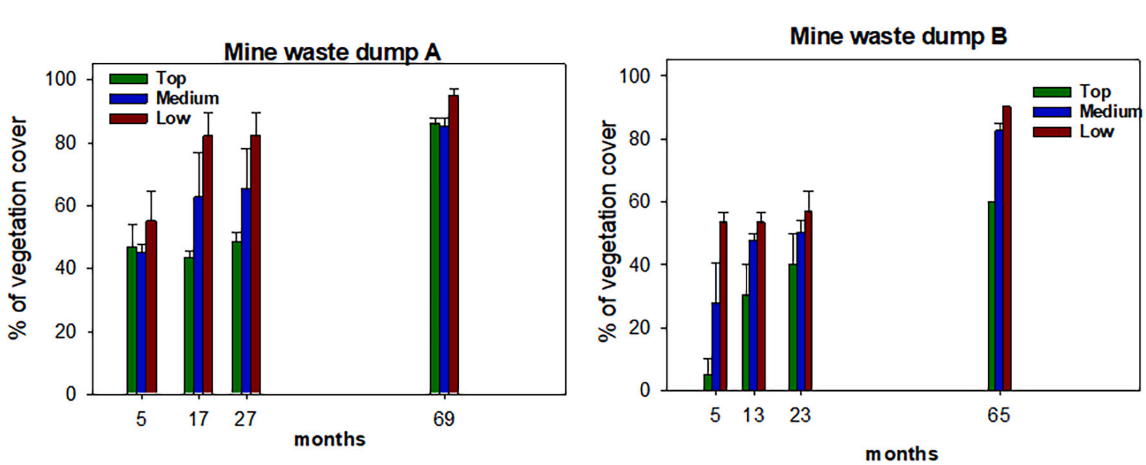

Fig. 4. Evolution over time of vegetation cover percentage (mean values and standard error) in each studied zone (Top, Medium and Low) for MWD-A and B.

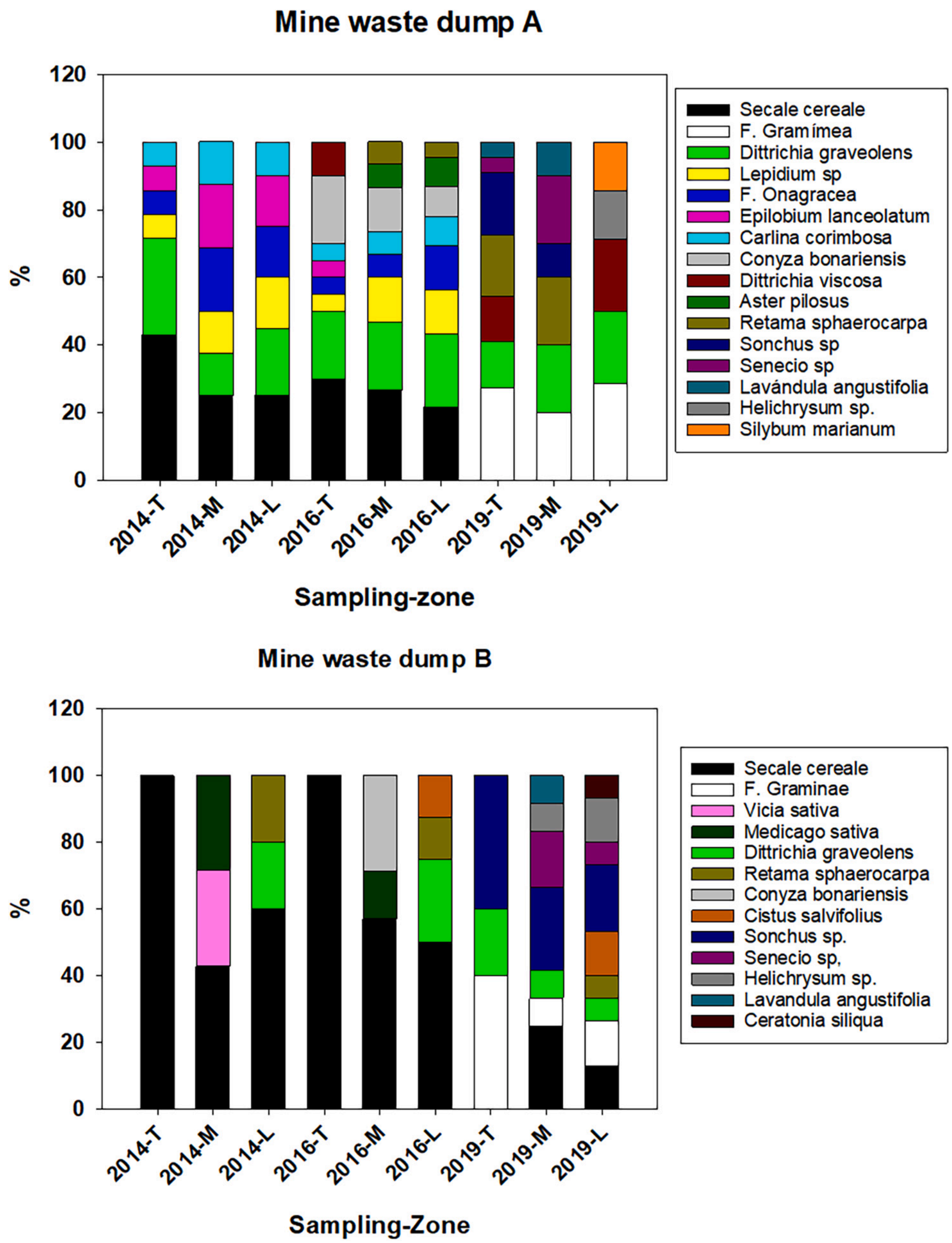

Fig. 5. Percentage of abundance of the main soil, at the three established zones (Top, Medium and Low) of MWD-A and B.

In the MWD-A different plant species established in all zones during the whole experimentation period; in fact, at least six different species were able to grow in each zone (8 different species in 2016). In 2014 and 2016 the most common species were *S. cereale*, *Dittrichia graveolens* and *Lepidium* sp., among others. In these two samplings, the richness was similar in all of the three zones. In 2019, a change in plant composition, in comparison with previous samplings, was observed. Different species of Graminae proliferated (Fig. 5). The only common species during the study was *D. graveolens*.

Plant richness in MWD-B was scarce in the first two samplings (in 2014 only one species at T zone and 2 species at M and L zones were observed; in 2016 only in L zone vegetation diversity included 3 species). However, in 2019, vegetation diversity increased, especially at the M and the L zones, where six and nine plant species were respectively recorded.

3.2.2. *Trace element contents in plants*

Samples of the plant species growing at both MWDs were taken at four different times through the study (years 2014, 2015, 2016 and 2019). Concentrations of trace elements in the aboveground biomass are shown in Table 3 (plants of the *Graminae* family) and Table S2 (other plant species, except *Graminae* plants).

For *Graminae* species, trace element concentrations (except U and Mn) were, in general, in the range of normal levels for plants, except in some cases for Co, Cr and Ni. Despite these exceptions, these values were in general far from toxic to plants and animals (Table 3). Uranium contents in *Graminae* species in both MWDs were similar and reached the highest values in the first sampling (2014). In this sampling, maximum values were found in *Agrostis* sp. (1.82 mg kg^{-1}) in MWD-A, and in *S. cereale* in MWD-B (1.72 mg kg^{-1}), being above the normal levels for plants. Concentrations of U were in the same range between MWDs and zones. In the last sampling (2019), U concentrations in *Graminae* decreased, especially in MWD-A (0.17 mg U kg^{-1}). Manganese concentrations in *Graminae* plants were, in general, higher than normal levels in plants. These values were similar in the two MWDs and among zones. The highest concentration of this element was found in the 2015

Table 3
Trace elements contents (mg kg^{-1}) in the most abundant plants of the *Graminae* family. Normal levels for plants, phytotoxic levels and tolerable level for cattle are indicated according to Chaney, 1989.

Sampling	MWD	Zone	Species	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	U	Zn
2014	A	Top	*Secale cereale*	0.40	0.03	0.67	0.36	3.16	50.5	223	1.29	0.24	0.65	19.6
		Medium	*Cynodon dactylon*	0.48	0.24	0.70	0.44	2.75	38.8	344	7.75	0.30	1.36	12.9
		Low	*S. cereale*	0.41	0.03	0.69	0.77	3.25	41.3	211	1.23	0.48	0.49	19.4
			Agrostis sp.	0.77	0.03	0.78	0.87	2.20	169	316	5.83	0.43	1.82	12.7
	B	Top	*S. cereale*	0.49	0.01	0.20	0.23	4.14	36.5	83	1.28	0.19	0.59	16.5
		Medium	*S. cereale*	nd	0.13	1.09	0.31	5.11	62.3	251	2.63	0.18	1.73	38.5
		Low	*S. cereale*	0.31	0.17	1.87	0.24	5.15	62.6	397	5.44	0.20	0.71	31.8
2015	A	Top	*S. cereale*	0.01	0.12	0.50	1.05	7.86	162	568	2.31	0.42	0.93	35.5
		Medium	*S. cereale*	1.11	1.28	3.57	1.54	8.16	593	780	6.29	0.44	0.85	57.4
			S. cereale	0.06	0.06	0.54	1.36	8.53	82.1	609	1.86	0.63	1.03	58.6
		Medium	*S. cereale*	0.09	1.49	2.31	1.03	9.32	143	517	14.1	0.48	1.25	51.6
	B	Low	*S. cereale*	0.11	0.11	0.54	0.57	13.6	104	286	1.73	0.57	1.56	54.0
2016	A	Top	*F. Graminae*	0.80	0.15	0.41	–	3.35	97.4	192	3.57	0.29	0.89	13.3
		Medium	*F. Graminae*	0.50	0.15	0.03	–	4.15	52.9	392	7.16	0.40	0.75	14.6
		Low	*F. Graminae*	0.79	0.18	0.10	–	5.03	64.2	71.3	2.61	0.06	1.10	14.9
	B	Top	*S. cereale*	0.61	0.05	0.15	–	3.98	40.5	86.9	1.32	0.17	0.75	17.9
		Medium	*S. cereale*	0.61	0.11	0.81	–	4.13	64.4	288	3.38	0.36	1.53	22.9
		Low	*S. cereale*	0.73	0.14	0.51	–	3.64	49.3	273	4.17	0.17	1.33	17.6
2019	A		*F. Graminae*	0.14	0.26	0.25	1.08	7.38	114	182	3.79	nd	0.17	28.9
	B		*S. cereale*	0.83	0.89	0.38	1.24	7.20	181	114	4.48	0.12	0.61	37.7
Normal levels in plants				0.01–1	0.1–1	0.01–0.3	0.1–1	3–20	30–300	15–150	0.1–5	2–5	0.01–0.03*	15-150
Toxic levels for plants				3–10	5–700	25–100	20	25–40	–	400–2000	50–100	–	0.05–2*	500-1500
Maximum tolerable levels for cattle				50	0.5	10	3000	100 (25)	100 (500)	1000 (400)	50	30	–	500 (300)

* Wang et al., 2019.

sampling, reaching values higher than 500 mg kg^{-1}. However, concentrations in *Graminae* decreased close to normal levels in the last sampling (Table 3).

Trace element concentrations in other species are shown in Table S2. These values were within the normal range for plants. The exception was Cd, because for most of these plants (Cistus *salvifolius* and *Sonchus* sp., *Dittrichia* sp. and *Helichrysum* sp.) values were higher than 1 mg kg^{-1}, considered as the upper level of the normal range in plants (see Table S2). For these species, U concentrations were higher than in *Graminae*, especially in both *Dittrichia* species, in which values above 1 mg kg^{-1} were recorded. Manganese in these other species did not present a clear pattern, and concentrations were dependent on the plant species, reaching concentrations higher than 500 mg kg^{-1} occasionally in *Conyza bonariensis, Cistus salviifolius, Helichrysum* sp. *and Lavandula angustifolia.*

3.3. Uranium and other trace elements in the collected pond waters

It should be noted that analysis of water from the ponds were carried out when these waters were available in the basin or in the area of influence of the dumps (Table 4).

In MWD-A, the pH values decreased approximately by one point between the first and the third sampling, and then these values remained close to neutrality. In October 2015, a considerable increase in pH was observed, and no more water was received in the reservoir until March 2019, when the pH values decreased considerably to slightly acidic values. In general, trace element content of water decreased over time (Table 4). The content of As was mostly below the detection limit of the used method. For Mn and U a concentration decrease was observed during the first four samplings, especially for Mn, which concentration decreased in four units. Even in the last sampling, when pH values were slightly acidic, the metal concentrations, including, U and Mn were very low. Manganese concentrations were close to the limits allowed for the discharge of the water pond (0.4 mg L^{-1}).

In MWD-B, pH in the water remained quite stable over the first four samplings and with slightly acid values. Trace elements concentrations also remained constant and low. In the case of U, values below 1 mg L^{-1} were found in the first four samplings. Undoubtedly, the most controversial element present in the water was Mn, observing quite high values in the second and the last sampling. In any case, Mn concentrations fell considerably, and even in the March 2019 sampling they were lower than 1 mg L^{-1}. However, results of the last sampling (October 2019) should be presented apart. In this sampling, a considerable decrease in water pH was observed, as well as an increase in all the metal

Table 4
pH, electrical conductivity (EC) and trace elements in waters from the pond at the two MWDs at during the study. d.l: detection limit.

MWD	Sampling	pH	EC	Al	As	Cd	Co	Cr	Cu	Ni	Pb	Zn	Fe	U	Mn	S
			mS cm^{-1}	µg l^{-1}										mg l^{-1}		
A	Feb.2014	7.38	1.05	120	<d.l.	2.7	94.0	1.42	9.30	204	<d.l.	30.0	20.0	0.46	4.56	124
	May2014	7.52	1.21	15.0	<d.l.	<d.l.	21.0	<d.l.	10.2	75.0	<d.l.	110	22.0	1.39	2.67	785
	Oct. 2014	6.25	1.16	<d.l.	<d.l.	<d.l.	8.00	<d.l.	<d.l.	49.0	12.0	<d.l.	<d.l.	0.14	1.69	226
	Oct. 2015	8.60	1.41	200	<d.l.	<d.l.	<d.l.	<d.l.	<d.l.	65.0	<d.l.	30	18	0.32	0.58	167
	Mar.2019	5.87	1.51	194	<d.l.	3.0	24	2.0	3.0	224	11.3	102	<d.l.	0.076	2.63	424
B																
	May 2014	6.95	1.99	20	<d.l.	<d.l.	5	<d.l.	12.5	28	<d.l.	120	20.0	0.53	0.47	640
	Oct. 2014	6.7	2.1	12	<d.l.	12.5	160	<d.l.	9.0	390	18.0	137	<d.l.	0.72	27.0	658
	Octt.2015	5.07	2.01	175	<d.l.	4	100	<d.l.	3.0	280	52	380	90.0	0.23	4.39	144
	Mar.2019	6.08	1.94	194	<d.l.	7	17.0	3.0	4.0	46.0	75	33.0	171	0.48	0.86	584
	Oct. 2019	3.99	2.88	36.3 10^{3}	<d.l.	31	1650	22	220	2830	60	3000	990	2.07	51.7	1081

concentrations. This increment is particularly striking, considering that in the previous sampling, 6 months before, metal concentrations were very low. The conditions of drought occurred in the last months of the experimentation meant that new water samples from this pool were not available for analysis. Finally, data should be considered with caution because ponds were not covered and this water is affected by other inputs/outputs like the rain or evaporation.

4. Discussion

4.1. Soils

Finding solutions to the mobility of trace elements through the ecosystems is one of the main challenges for mining restoration. Ideally, solutions should be cost-effective and applicable to the usually large extensions affected by mining. In this work, we have evaluated the usefulness of the assisted phytoremediation for the restoration of a U mine, by monitoring the evolution of soils, vegetation cover and runoff waters over almost five years. We expected that the addition of this amendment rich in nutrients would promote in time the development of spontaneous and induced vegetation, and thus would accelerate the processes of formation of an organic horizon from plant debris. This is part of the phytostabilization approach, and it seems to be the most feasible option for the management of large areas contaminated by trace elements to immobilize metals in the soil. The increase in vegetation reduces soil erosion and dispersion of contaminants (Madejón et al., 2018a). In addition, this amendment helps to maintain soil pH close to neutrality, causing a buffering effect that is essential for the dynamics of nutrients and trace elements in the soil.

Soil pH governs the equilibrium among solubility, adsorption, and desorption of U and other trace elements, such as Mn, in soils (Fu et al., 2019). The pH decreased through the studied period; however, the final pH was still above 7.0 at both MWDs. This result confirms the utility and durability of SL under these conditions. In fact, González-Fernández et al. (2012) calculated that the useful life of this amendment could reach up to 9 years with additions of less than 10 T ha^{-1}. Moreover, under real field conditions in the Guadiamar Green Corridor in Spain, the effectiveness of this amendment has been proved for at least 12 years, using doses of 30 T ha^{-1} (Madejón et al., 2018a).

The increase of OM content in soil is a main factor to reduce trace element availability in soil and to improve soil fertility for plant development, which contributes to reduce soil erosion. Although the organic matter content of SL is not as high as that of an organic amendment, an important increase of this parameter was recorded in both MWDs. This is an indirect effect caused by the application of SL, already reported by other authors (Pérez-de-Mora et al., 2006), and it is explained by the positive effect of SL on plant establishment, as the amendment clearly helped to the development of spontaneous and induced vegetation. After the growth and die back of the annual plant species, the soil becomes rich in plant residues, which progressively creates a thick organic mulch. This is especially interesting in this particular case, where the soil was highly degraded, totally or partially devoid of its surface litter and upper soil horizon. The soils affected by this type of contamination often have low biological activity, and limited soil functioning (Pardo et al., 2011). Organic matter is vital to soil quality and regulation of important soil functions enhancing fertility, structure and water retention (Bastida et al., 2018). Therefore, there is a growing recognition that soil organic matter is a key property of ecosystems to understand their stability in the face of global change (Bastida et al., 2019).

The monitoring of $CaCO_3$ in time was performed to assess the presence of SL in soil. This parameter was studied to try to control the traceability of this product and to elucidate whether there were significant losses due to runoff. Although the application of the SL was carried out in a methodical way and with an intense spreading work, patchy areas and a high heterogeneity in $CaCO_3$ were observed. A proximate calculation of SL losses indicated that despite this variability and after 69 months of monitoring, no significant product losses were observed.

Pseudototal content of trace element may give information concerning possible enrichment of contaminants in the soil, but its analysis is not enough for estimating their mobility and availability for ecological processes. Instead, determination of the bioavailable fraction might be more useful for environmental protection and ecological risk assessment. For that reason, determination of bioavailable trace elements is one of the key factors to evaluate the biogeochemical behaviour of these contaminants in the soil-plant system, which has to be a concern in soil remediation processes (Bone et al., 2017; Selvakumar et al., 2018). In this study, the availability of U and Mn, the most problematic elements, decreased over time, indicating the effectiveness of the phytoremediation process for the stabilization of these elements into the soil (phytostabilisation). The U and Mn availability is strongly dependent on soil chemical properties (e.g., Eh, soil pH, and SOM), (Papanicolaou et al., 2010; Bone et al., 2017) and the increase in organic matter is responsible of this stabilization. Organic matter contains diverse functional groups (e.g., carboxylic, hydroxyl, phenolic, aliphatic, aromatic, and aromatic groups), that can bond U ions (Cumberland et al., 2016), and therefore organic matter has a critical role in the mobility of U in soils (Tinnacher et al., 2013; Bone et al., 2017).

Although the availability of Mn and U decreased throughout the experimentation period, it is true that the concentrations found in the influence pond of MWD-B (Table 4) in some of the samplings exceeded the values allowed for its discharge. These high values obtained could be related with the characteristics of the southern zone of the dump (not analysed in this study), where there was a very scarce layer of soil and a total absence of vegetation. In this particularly harsh area, it is possible that the applied dose was not enough to improve soil conditions, leading to a high leaching and runoff of metals. In this anomalous area other actions would be necessary to continue with the recovery of the dump, such as the addition of a new SL dose or the application of new materials similar to those that are being used for the construction of the artificial soils (Technosols), and a new planting of vegetation. In any case, it would be interesting to continue with long-term studies to verify these values and to evaluate the effectiveness of the actions taken.

4.2. Vegetation development

The spontaneous colonization of mine dumps is a very slow process due to the adverse soil conditions, and thus revegetation of these sites requires human intervention (Yan et al., 2018). Benefits of vegetation in these sites are broad, such as protection against erosion by wind and water, and reduction of dispersion of contaminants (Pérez-de-Mora et al., 2011). Therefore, restoration of the vegetation cover can control soil pollution, improve the visual impact, and reduce the threats to human beings (Wong, 2003), having an important role in soil conservation (Tambunan et al., 2017). In our study, the increase of vegetation cover with time at the two MWDs indicated an improvement of soil fertility due to phytoremediation, which boosted vegetation growth. Similar results using SL as an amendment were observed by Xiong et al. (2015) in Mediterranean soils contaminated with trace elements. Moreover, the highest percentage of vegetation cover of all the study was found in the lower zones. This fact is usually related with the transport and accumulation of soil nutrients from top to bottom; this produces higher vegetation cover at the bottom zones due to the increment in soil fertility (Maltez-Mouro et al., 2005).

Regarding the election of plant species for revegetation purposes in rehabilitated mine areas, species with a high tolerance to potentially toxic metals are generally planted to ensure that plants can resist soil conditions. After this step, the progressive growth of pioneer plants promotes the accumulation of organic matter, and as soil fertility increases new plants begin to appear, increasing plant richness as succession progresses (Bu et al., 2014). This pattern was clearly observed even in the MWD-B, where initial soil conditions were very adverse (Fig. 4).

Among the three sown species, only *S. cereale* was able to grow under these conditions at both MWDs and showed the lowest levels of trace elements, indicating their suitability for remediation purposes. The establishment of *C. dactylon* was not successful, despite other studies have showed the ability of *C. dactylon* to grow even in highly contaminated substrates, such as sludge containing high levels of metals (Madejón et al., 2006).

4.3. Trace elements in plants and toxicity risk

Plants from the *Graminae* family (Table 3) showed values of trace elements (except for U) within the normal levels for plants, and below toxic levels for plants and animals. These data showed the suitability of the use of this group of plants for remediation purposes, because, in general, their accumulation of trace elements in aboveground biomass is low. This is of a high importance given that these plants are usually consumed for grazing animals. However, values of U in *Graminae* plants were higher than the lower limit of the range that could be toxic for plants, according to Wang et al. (2019), although they were not close to the upper limit of 2 mg kg^{-1}. Nevertheless, the levels of U reported to provoke phytotoxicity are quite variable among different studies and depend on plant species and soil conditions (Sheppard et al., 2005). For example, sunflower plants can grow in certain soils with U concentrations of >100 mg kg^{-1} without showing toxicity (Shahandeh and Hossner, 2002), and concentrations of U below 300 mg kg^{-1} had no effect on a range of plants species (Sheppard et al., 1992). These concentrations are much higher than those found in the soils from the present study.

The U contents found in *Graminae* plants in our study are similar or lower that those reported for other mining areas. Favas et al. (2016) showed that *Graminae* plants growing in an abandoned mine located in the important uriniferous region of Beiras (Central Portugal) presented concentration of U between 0.2 – - 6.0 mg kg^{-1}. However, these authors pointed out that these plants accumulate the most important amount of this element in roots. This fact reinforces the use of these plants for phytoremediation of soils polluted with U, since even though certain amounts of U can be accumulated aboveground most of this element is excluded at the root level. Additional research is needed to evaluate whether this U accumulation could, although low, can have any implication for the food web.

Finally, it is important to remark that rainfall can influence the dissolution of U in soil, making it more bioavailable, and therefore rainfall can be a determinant factor in the absorption of this element by plants. This fact has been considered in an area close to the site of this study (Gil-Pacheco et al., 2021). Therefore, the variability of U accumulation in plants reported here could be also related to the inter-annual variability of rainfall.

The rest of the studied plants (Table S2) only showed values above normal levels for Cd, Mn and U. In the case of Cd, although its concentration and availability in soils was moderate, the two *Dittrichia* species, *C. salviifolius, Helichrysum* sp. and *Sonchus* sp. tended to its accumulation. The capacity of *D. viscosa* to accumulate Cd into leaves had also been observed in some Mediterranean sites affected by long-term mining activities (Domínguez et al., 2017; Gómez-Ros et al., 2013). Similar results for *Cistus* species were found in other study for *Cistus ladanifer* although the content of Cd in soils was low (Madejón et al., 2021).

Manganese was the only element with concentrations in some samplings that could be in the range of toxicity for animals. However, essential metals such as Mn and Cu are generally less toxic than nonessential metals such as Cd and Pb (Yasutake and Hirayama, 2002). Moreover, at the end of the study only *L. angustifolia* reached concentrations of Mn above toxic levels. Rossini-Oliva et al. (2019) also found high levels of Mn in other species of *Lavandula.*

Regarding U accumulation by these non-grass species, Favas et al. (2016) found concentrations of U in the aerial parts of species of *Cistus*, *Lavandula* and *D. viscosa* similar to those obtained in this study. That study also showed that U content in the aerial part of numerous plants from different families was lower than that found in the roots, so that the transfer of U to aboveground biomass is partly excluded. As discussed above, this is a positive feature as prevents a high accumulation of U through the food web of the area. Interestingly, the concentrations in some woody plant species, such as *Lavandula*, were similar to the accumulation of U in other shorter-lived species, such as grasses. Other works have reported a higher accumulation of U in long-lived shrubs species than in annual plants in abandoned U mines (Wetle et al., 2020).

4.4. Implications for the management of mine waste dumps

Restoration of open pit mines is a challenge for mining industries. Due to the large degree of land degradation provoked by metal extraction, usually affecting large surface areas, there are not many options to restore the ecosystem and the landscape without costly actions from the economic and the resource point of view. One of the most successful technologies is undoubtedly the creation of artificial soils or Technosols, which is an immediate solution for creating and managing a new ecosystem capable of supplying key ecological processes in the short-term. This is an artificial soil prepared with organic and inorganic waste materials, non-toxic or dangerous, capable of performing environmental functions and supporting plant growth. In fact, Technosols are being used at the site of study with positive results (Escolano et al., 2021), especially in areas where the total absence of soil exposes pyritic rock with the negative consequences for drainage waters. First results of this study with Technosols have shown an increase in the stabilization of soil carbon in the treated soils, which has initiated a recovery of soil functionality, increasing the biological activity and diversity, enhancing the soil buffering capacity and reducing erosion by surface runoff. The cost of the added material, however, is large, which can limit its use to entire waste dumps.

Our study has demonstrated that the implementation of assisted phytoremediation, through the addition of cheaper liming agents such as SL and the subsequent development of vegetation (spontaneous or induced) in shallow areas, can be a more economical solution, especially in countries where the restoration of mines does not have enough resources to create these artificial soils. The increase in organic matter that is naturally produced by the restoration of vegetation, together with the neutralizing power of the material, are fundamental tools to stabilize soils and to improve their physico-chemical and biological properties. It must be considered that in the present study just a single application of the liming agent was made.

There is little published information about mine rehabilitation success (Hernandez-Santin et al., 2020) and our study adds new data about the evolution of soil, plant and water quality over the rehabilitation of this U mine. As ENUSA Company did along this experimentation, we strongly encourage mine companies to collaborate with environmental scientists to promote a higher dissemination of the results of different approaches, which can help to facilitate the decision-making process in other mine rehabilitation projects.

5. Conclusions

The assisted phytoremediation had positive effects on soil conditions in an abandoned U mine, by promoting the growth of induced and spontaneous vegetation. This, in turn, increased soil organic matter and fertility. These effects resulted in a reduction of the bioavailability of potentially toxic elements, such as U and Mn, the most worrisome elements in the system. Indeed, accumulation of U, Mn and other trace elements in plants tended to decrease with time and did not pose any risk for the food web, confirming the stabilization on these elements into soil after amendment application. In addition, effects on sugar beet lime on soil physico-chemical properties lasted for at least 69 months. Given that this is a low-cost material, its use for the reclamation of similar large mining areas could be a cheap and feasible approach, although long-term monitoring of trace element dynamics is always recommended. This full-scale field study could be possible thanks to the collaboration with the mining company; this cooperation between managers and scientists is critical to promote a higher dissemination of the results of different rehabilitation approaches.

CRediT authorship contribution statement

Paula Madejón: Conceptualization, Methodology, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Project administration, Funding acquisition. **María Teresa Domínguez:** Formal analysis, Investigation, Writing – original draft, Writing – review & editing. **Ignacio Girón:** Conceptualization, Investigation. **Pilar Burgos:** Methodology, Formal analysis. **María Teresa López-Fernández:** Conceptualization, Supervision. **Óscar García Porras:** Methodology, Supervision, Resources. **Engracia Madejón:** Conceptualization, Methodology, Investigation, Writing – review & editing, Project administration, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecoleng.2022.106669.

References

Abreu, M.M., Lopes, J., Santos, E.S., Magalhães, M.C.F., 2014. Ecotoxicity evaluation of an amended soil contaminated with uranium and radium using sensitive plants. J. Geochem. Explor. 142, 112–121. https://doi.org/10.1016/j.gexplo.2014.01.029.

Alloway, B.J., 2013. Introduction. In: Alloway, B.J. (Ed.), Heavy metals in soils, 3rd ed. Springer, Dordrecht, pp. 3–10.

Antunes, S.C., Figueiredo, D.R., Marques, S.M., Castro, B.B., Pereira, R., Gonçalves, F., 2007. Evaluation of water column and sediment toxicity from an abandoned uranium mine using a battery of bioassays. Sci. Total Environ. 374, 252–259. https://doi.org/10.1016/j.scitotenv.2006.11.025.

Aran, D., Verde, J.R., Antelo, J., Macías, F., 2020. Environmental quality of soils and waters in Fé Mine: initial situation and remediation alternatives [Calidad ambiental de suelos y aguas de la Mina Fé: Situación inicial y alternativas de recuperación]. J. Soil Sci. 10, 81–100. https://doi.org/10.3232/SJSS.2020.V10.N1.06.

Bai, J., Yao, H., Fan, F., Lin, M., Zhang, L., Ding, H., Lei, F., Wu, X., Li, X., Guo, J., Qin, Z., 2010. Biosorption of uranium by chemically modified *Rhodotorula glutinis*. J. Environ. Radioact. 101, 969–973. https://doi.org/10.1016/j.jenvrad.2010.07.003.

Bastida, F., Hernández, T., García, C., 2018. Soil erosion and C losses: Strategies for building soil carbon (Book Chapter). In: The Future of Soil Carbon: Its Conservation and Formation, pp. 215–238. https://doi.org/10.1016/B978-0-12-811687-6.00008-0.

Bastida, F., García, C., Fierer, N., Eldridge, D.J., Bowker, M.A., et al., 2019. Global ecological predictors of the soil priming effect. Nat. Commun. 10 https://doi.org/10.1038/s41467-019-11472- art. no. 3481.

Bhalara, P.D., Punetha, D., Balasubramanian, K., 2014. A review of potential remediation techniques for uranium (VI) ion retrieval from contaminated aqueous environment. J. Environ. Chem. Eng. 2, 1621–1634. https://doi.org/10.1016/j.jece.2014.06.007.

Bone, S.E., Dynes, J.J., Cliff, J., Bargar, J.R., 2017. Uranium (IV) adsorption by natural organic matter in anoxic sediments. Proc. Natl. Acad. Sci. U. S. A. 114, 711–716. https://doi.org/10.1073/pnas.1611918114.

Bu, W., Zang, R., Ding, Y., 2014. Functional diversity increases with species diversity along successional gradient in a secondary tropical lowland rainforest. Trop. Ecol. 55, 393–401.

Chaney, R.L., 1989. Toxic element accumulation in soils and crops: Protecting soil fertility and agricultural food chains. In: Bar-Yosef, B., Barrow, N.J., Goldshmid, J. (Eds.), Inorganic Contaminants in the Vadose Zone. Springer, Berlin, pp. 140–158.

Chen, L., Liu, J., Zhang, W., Zhou, J., Luo, D., Li, Z., 2021. Uranium (U) source, speciation, uptake, toxicity and bioremediation strategies in soil-plant system: a review. J. Hazard. Mater. 413 https://doi.org/10.1016/j.jhazmat.2021.125319 art. no. 125319.

Clemente, R., Pardo, T., Madejón, P., Madejón, E., Bernal, M.P., 2015. Food byproducts as amendments in trace elements contaminated soils. Food Res. Int. 73, 176–189. https://doi.org/10.1016/j.foodres.2015.03.040.

Craft, E.S., Abu-Qare, A.W., Flaherty, M.M., Garofolo, M.C., Rincavage, H.L., Abou-Donia, M.B., 2004. Depleted and natural uranium: chemistry and toxicological effects. J. Toxicol. Environ. Health Part B 7, 297–317. https://doi.org/10.1080/10937400490452714.

Cumberland, S.A., Douglas, G., Grice, K., Moreau, J.W., 2016. Uranium mobility in organic matter-rich sediments: a review of geological and geochemical processes. Earth Sci. Rev. 159, 160–185. https://doi.org/10.1016/j.earscirev.2016.05.010.

Doley, D., Audet, P., 2013. Adopting novel ecosystems as suitable rehabilitation alternatives for former mine sites. Ecol. Process. 2, 1–11. https://doi.org/10.1186/2192-1709-2-22.

Domínguez, M.T., Montiel-Rozas, M.M., Madejón, P., Diaz, M.J., Madejón, E., 2017. The potential of native species as bioenergy crops on trace-element contaminated Mediterranean lands. Sci. Total Environ. 590–591, 29–39. https://doi.org/10.1016/j.scitotenv.2017.03.018.

Escolano, O., Lacal, M., Millán, R., Macías, F., Vecillas Fernández, I., Hernández González, I., Girón, J.L., 2021. Investigación y desarrollo de compuestos bioactivos a base de tecnosoles, Para evitar la generación de drenajes ácidos en minería de uranio-Proyecto TEKURA. In: Almendro Candel, M.B., Jordán Vidal, M.M. (Eds.), El Suelo: Clave Para Una gestión Ambiental Sostenible en Un Escenario de Cambio Global. Libro de resúmenes del IX Simposio Nacional sobre el Control de la Degradación y Recuperación de Suelos, SECS, Elche, pp. 535–538.

Espejo, R., 2001. El uso de la espuma de azucarería, fosfoyeso y residuos dolomíticos de convertidor como enmendantes de suelos ácidos. In: Proc I Int Congr Manage Org Waste Rural Land Grange areas, Pamplona (Spain), February 22-23, pp. 377–386.

Favas, P.J.C., Pratas, J., Mitra, S., Sarkar, S.K., Venkatachalam, P., 2016. Biogeochemistry of uranium in the soil-plant and water-plant systems in an old uranium mine. Sci. Total Environ. 568, 350–368. https://doi.org/10.1016/j.scitotenv.2016.06.024.

Fu, H., Ding, D., Sui, Y., Zhang, H., Hu, N., Li, F., Dai, Z., Li, G., Ye, Y., Wang, Y., 2019. Transport of uranium (VI) in red soil in South China: influence of initial pH and carbonate concentration. Environ. Sci. Pollut. Res. 26, 37125–37136. https://doi.org/10.1007/s11356-019-06644-3.

Fuller, A.J., Leary, P., Gray, N.D., et al., 2020. Organic complexation of U(VI) in reducing soils at a natural analogue site: implications for uranium transport. Chemosphere 254, 126859. https://doi.org/10.1016/j.chemosphere.2020.126859.

García-Navarro, F.J., Amorós Ortiz-Villajos, J.A., Sánchez-Jiménez, C.J., Bravo Martín-Consuegra, S., Márquez Cubero, E., Jiménez Ballesta, R., 2009. Application of sugar foam to red soils in a semiarid Mediterranean environment. Environ. Earth Sci. 59, 603–611. https://doi.org/10.1007/s12665-009-0058-9.

Gavrilescu, M., Pavel, L.V., Cretescu, I., 2009. Characterization and remediation of soils contaminated with uranium. J. Hazard. Mater. 163, 475–510. https://doi.org/10.1016/j.jhazm at.2008.07.103.

Gil-Pacheco, E., Suárez-Navarro, J.A., Fernández-Salegui, A.B., Sánchez-González, S.M., Suarez-Navarro, M.J., García-Sánchez, A., 2021. Factors that influence the absorption of uranium by indigenous plants on the spoil tip of an abandoned mine in western Spain. Sci. Total Environ. 759 https://doi.org/10.1016/j.scitotenv.2020.143571 art. no. 143571.

Gómez-Ros, J.M., Garcia, G., Peñas, J.M., 2013. Assessment of restoration success of former metal mining areas after 30 years in a highly polluted Mediterranean mining area: Cartagena-La Unión. Ecol. Eng. 57, 393–402. https://doi.org/10.1016/j.ecoleng.2013.04.044.

González-Fernández, P., Ordóñez-Fernández, R., Mariscal-Sancho, I., Espejo-Serrano, R., 2012. Economics of applications of sugar factory lime refuse in Mediterranean ultisols. Arch. Agron. Soil Sci. 58, S185–S189. https://doi.org/10.1080/03650340.2012.696779.

Hernandez-Santin, L, Erskine, P. D, Bartolo, R, 2020. A review of revegetation at mine sites in the Alligator Rivers Region, Northern Territory, and the development of a state and transition model for ecological restoration at Ranger uranium mine. Journal of Cleaner Production 246, 119079. https://doi.org/10.1016/j.jclepro.2019.119079.

Hesse, P.R., 1971. A Textbook of Soil Chemical Analysis. John Murray, London.

Hobb, R.J., Norton, D.A., 1996. Towards a Conceptual Framework for Restoration Ecology, vol. 4, pp. 93–110. https://doi.org/10.1111/j.1526-100X.1996.tb00112.

Houba, V.J.G., Temminghoff, E.J.M., Gaikhorst, G.A., Van Vark, W., 2000. Soil analysis procedures using 0.01 M calcium chloride as extraction reagent. Commun. Soil Sci. Plant Anal. 31, 1299–1396. https://doi.org/10.1080/00103620009370514.

Kumpiene, J., Antelo, J., Brännvall, E., Carabante, I., Ek, K., Komárek, M., Soderberg, C., Wårell, L., 2019. In situ chemical stabilization of trace element-contaminated soil – field demonstrations and barriers to transition from laboratory to the field – a

review. Appl. Geochem. 100, 335–351. https://doi.org/10.1016/j.apgeochem.2018.12.003.
Ma, D., Wei, J., Zhao, Y., et al., 2020. The removal of uranium using novel temperature sensitive urea-formaldehyde resin: adsorption and fast regeneration. Sci. Total Environ. 735, 139399 https://doi.org/10.1016/j.scitotenv.2020.139399.
Madejón, P., Murillo, J.M., Marañón, T., Cabrera, F., 2006. Bioaccumulation of trace elements in a wild grass three years after the Aznalcóllar mine spill (South Spain). Environ. Monit. Assess. 114, 169–189. https://doi.org/10.1007/s10661-006-2523-1.
Madejón, P., Pérez-de-Mora, A., Burgos, P., Cabrera, F., Lepp, N.W., Madejón, E., 2010. Do amended, polluted soils require re-treatment for sustainable risk reduction? – evidence from field experiments. Geoderma 159, 174–181. https://doi.org/10.1016/j.geoderma.2010.07.009.
Madejón, E., Madejón, P., Girón-Moreno, I., 2014. Actividades de I+D en el campo de la restauración medio ambiental. In: Jornadas de I+D Junio 2014: La Colaboración Ciencia-Tecnología-Industria Como Soporte se la Gestión Segura. Aplicación de enmiendas en suelos de Mina Saelices (Salamanca).
Madejón, P., Domínguez, M.T., Gil-Martínez, M., Navarro-Fernández, C.M., Montiel-Rozas, M.M., Madejón, E., Murillo, J.M., Cabrera, F., Marañón, T., 2018a. Evaluation of amendment addition and tree planting as measures to remediate contaminated soils: the Guadiamar case study (SW Spain). Catena 166, 34–43. https://doi.org/10.1016/j.catena.2018.03.016.
Madejón, P., Domínguez, M.T., Madejón, E., Cabrera, F., Marañón, T., Murillo, J.M., 2018b. Soil-plant relationships and contamination by trace elements: a review of twenty years of experimentation and monitoring after the Aznalcóllar (SW Spain) mine accident. Sci. Total Environ. 625, 50–63. https://doi.org/10.1016/j.scitotenv.2017.12.277.
Madejón, P., Caro-Moreno, D., Navarro-Fernández, C.M., Rossini-Oliva, S., Marañón, T., 2021. Rehabilitation of waste rock piles: impact of acid drainage on potential toxicity by trace elements in plants and soil. J. Environ. Manag. 280 https://doi.org/10.1016/j.jenvman.2020.111848 art. no. 111848.
Maltez-Mouro, S., García, L.V., Marañón, T., Freitas, H., 2005. The combined role of topography and overstorey tree composition in promoting edaphic and floristic variation in a Mediterranean forest. Ecol. Res. 20, 668–677. https://doi.org/10.1007/s11284-005-0081-6.
Mendez, M.O., Maier, R.M., 2008. Phytostabilization of mine tailings in arid and semiarid environments - an emerging remediation technology. Environ. Health Perspect. 116, 278–283. https://doi.org/10.1289/ehp.10608.
Palansooriya, K.N., Shaheen, S.M., Chen, S.S., Tsang, D.C.W., Hashimoto, Y., Hou, D., Bolan, N.S., Rinklebe, J., Ok, Y.S., 2020. Soil amendments for immobilization of potentially toxic elements in contaminated soils: a critical review. Environ. Int. 134 https://doi.org/10.1016/j.envint.2019.105046 art. no. 105046.
Papanicolaou, F., Antoniou, S., Pashalidis, I., 2010. Redox chemistry of sulphate and uranium in a phosphogypsum tailings dump. J. Environ. Radioact. 101, 601–605. https://doi.org/10.1016/j.jenvrad.2010.03.006.
Pardo, T., Clemente, R., Bernal, M.P., 2011. Effects of compost, pig slurry and lime on trace element solubility and toxicity in two soils differently affected by mining activities. Chemosphere 84, 642–650. https://doi.org/10.1016/j.jenvrad.2010.03.006.
Pérez-de-Mora, A., Burgos, P., Cabrera, F., Madejón, E., 2006. Trace elements availability and plant growth in a mine-spill-contaminated soil under assisted natural remediation, part I. soils. Sci. Total Environ. 363, 28–37. https://doi.org/10.1016/j.scitotenv.2005.10.015.
Pérez-de-Mora, A., Madejón, P., Burgos, P., Cabrera, F., Lepp, N.W., Madejón, E., 2011. Phytostabilization of semiarid soils residually contaminated with trace elements using by-products: sustainability and risks. Environ. Pollut. 159, 3018–3027. https://doi.org/10.1016/j.envpol.2011.04.015.
Rossini-Oliva, S., Mingorance, M.D., Leidi, E.O., Fernández-Espinosa, A.J., 2019. Does the polluted environment modify responses to metal pollution? A case study of two Cistus species and the excess of copper and lead. Catena 178, 244–255. https://doi.org/10.1016/j.catena.2019.02.028.
Santos-Francés, F., Gil Pacheco, E., Martínez-Graña, A., Alonso Rojo, P., Ávila Zarza, C., García Sánchez, A., 2018. Concentration of uranium in the soils of the west of Spain. Environ. Pollut. 236, 1–11. https://doi.org/10.1016/j.envpol.2018.01.038.
Schöner, A., Noubactep, C., Büchel, G., Sauter, M., 2009. Geochemistry of natural wetlands in former uranium milling sites (eastern Germany) and implications for uranium retention. Chem. Erde-Geochem. 69, 91–107.
Selvakumar, R., Ramadoss, G., Menon, Mridula P., Rajendran, K., Thavamani, P., Naidu, Ravi, Megharaj, M., 2018. Challenges and complexities in remediation of uranium contaminated soils: a review. J. Environ. Radioact. 192, 592–603. https://doi.org/10.1016/j.jenvrad.2018.02.018.
Shahandeh, H., Hossner, L.R., 2002. Role of soil properties in phytoaccumulation of uranium. Water Air Soil Pollut. 141, 165–180. https://doi.org/10.1023/A:1021346828490.
Sheppard, S.C., Evenden, W.G., Anderson, A.J., 1992. Multiple assays of uranium toxicity in soil. Environ. Toxicol. Water Qual. 7, 275–294. https://doi.org/10.1002/tox.2530070307.
Sheppard, S.C., Sheppard, M.I., Gallerand, M.-O., Sanipelli, B., 2005. Derivation of ecotoxicity thresholds for uranium. J. Environ. Radioact. 79, 55–83. https://doi.org/10.1016/j.jenvrad.2004.05.015.
Tambunan, R.P., Sukoso, S., Syekhfani, S., Priatmadi, B.J., 2017. The role of ground cover plant in soil improvement after mining activity in South Kalimantan. IOSR-JAVS 10, 92–98. https://doi.org/10.9790/2380-1011019298.
Tan, W.-F., Wang, Y.-C., Ding, L., Lv, J.-W., Fang, Q., 2019. Effects of phosphorus modified bio-char on metals in uranium-containing soil. Water Air Soil Pollut. 230 https://doi.org/10.1007/s11270-018-4074-9 art. no. 35.
Tinnacher, R.M., Nico, P.S., Davis, J.A., Honeyman, B.D., 2013. Effects of fulvic acid on uranium (VI) sorption kinetics. Environ. Sci. Technol. 47, 6214–6222. https://doi.org/10.1021/es304677c.
Vidal, M., Garzón, J.E., García, V., Villa, E., 2006. Differentiating the amending effects of calcareous materials applied to acid soils by use of optimal scaling procedures. Agrochimimica 50, 132–147. https://doi.org/10.1007/s12665-009-0058-9.
Walkley, A., Black, I.A., 1934. An examination of the Degtjareffmethod for determining soil organic matter and a proposed determination of the chromic acid titration method. Soil Sci. 37, 29–38.
Wang, Z., Qin, H., Wang, J., 2019. Accumulation of uranium and heavy metals in the soil–plant system in Xiazhuang uranium ore field, Guangdong Province, China. Environ. Geochem. Health 41, 2413–2423. https://doi.org/10.1007/s10653-019-00286-7.
Wetle, R., Bensko-Tarsitano, B., Johnson, K., Sweat, K.G., Cahill, T., 2020. Uptake of uranium into desert plants in an abandoned uranium mine and its implications for phytostabilization strategies. J. Environ. Radioact. 220–221, 106293 https://doi.org/10.1016/j.jenvrad.2020.106293.
Wong, M.H., 2003. Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere 50, 775–780. https://doi.org/10.1016/S0045-6535(02)00232-1.
Xie, S., Yang, J., Chen, C., Zhang, X., Wang, Q., Zhang, C., 2008. Study on biosorption kinetics and thermodynamics of uranium by *Citrobacter freudii*. J. Environ. Radioact. 99, 126–133. https://doi.org/10.1016/j.jenvrad.2007.07.003.
Xiong, J., Madejón, P., Madejón, E., Cabrera, F., 2015. Assisted natural remediation of a trace element-contaminated acid soil: an eight-year field study. Pedosphere 25, 250–262. https://doi.org/10.1016/S1002-0160(15)60010-8.
Yan, X., Liu, M., Zhong, J., Guo, J., Wu, W., 2018. How human activities affect heavy metal contamination of soil and sediment in a long-term reclaimed area of the Liaohe river delta, North China. Sustainability 10, 338. https://doi.org/10.3390/su10020338.
Yan, A., Wang, Y., Tan, S.N., et al., 2020. Phytoremediation: a promising approach for revegetation of heavy metal-polluted land. Front. Plant Sci. 11 https://doi.org/10.3389/fpls.2020.00359 art. no. 359.
Yasutake, A., Hirayama, K., 2002. Metal toxicology. In: Derelanko, M.J., Hollinger, M.A. (Eds.), Handbook of Toxicology. CRC Press, Boca Raton, FL, pp. 913–955.
Zhang, Z., Liu, J., Cao, X., et al., 2015. Comparison of U(VI) adsorption onto nanoscale zero-valent iron and red soil in the presence of U(VI)-CO3/Ca-U(VI)-CO3 complexes. J. Hazard. Mater. 30, 633–642. https://doi.org/10.1016/j.jhazmat.2015.07.058.