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Characterization and phytoremediation of abandoned contaminated mining area in Portugal by INAA

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Abstract This study aims to find out a vascular plant species that accumulate relatively high concentrations of arsenic (As) for its use as phytoremediator at abandoned and contaminated mining areas, such as São Domingos mines (Portugal). The assessment of As contamination levels in soils and plants of other similar sites in the north of the country (Castromil and Poço de Freitas) was also conducted; and the sample analyses were made by instrumental neutron activation analysis. *Agrostis* genera have shown higher As transfer coefficients than other studied plant species and, in particular, *Agrostis curtisii* has shown a reasonable ability to accumulate high concentration of this toxic element.

Keywords k_0-INAA · Environmental remediation · São Domingos mine · Phytoremediation · Arsenic contamination · *Agrostis curtisii*

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Introduction

Mining activity can be responsible for significant negative impacts on the surrounding environments due to contamination from mining and milling operations (such as grinding, ore concentration and disposal of tailings) [1]. Furthermore, some mine complexes have been closed without proper rehabilitation measures which led to severe toxic metals contamination of the soil and water inside and outside the mine works. Soil contamination by trace elements is a potential problem when residential and agricultural areas are allocated to the contaminated area and, as well, those elements can be dispersed due to erosion and chemical weathering of tailings [2].

Phytoremediation appears to be a good strategy to clean up soils; it consists in the ability of a plant species to accumulate relatively high concentrations of the toxic elements [3–6]. Therefore, it is possible to provide an environment-friendly technology which is cost-effective and energetically inexpensive when comparing to conventional methods of land decontamination.

São Domingos mine (located at the Lower Alentejo area of southern Portugal) is an example of an abandoned open pit copper-sulphide mine that was exploited from the Roman times up to 1960s [7] rendering the environment heavily contaminated with toxic elements, such as As [8]. A previous study investigated the São Domingos mining contaminated area [9] in order to find out the As hyperaccumulating plant; however, unfortunately no indigenous plant species with the high ability to accumulate As was found yet. Since local plant species with the ability to accumulate high As concentrations was not found at São Domingos mine [9], two other abandoned mining sites (Castromil and Poço de Freitas) similar to São Domingos mining area (with equivalent level of As contamination) were investigated with the same aim: to find out one or more native plant species with high potential of phytoremediation, which could be planted in the future at São Domingos mine for rehabilitation of the contaminated soils. Castromil mines are Au–Ag mining areas, where the mining activity was ceased in the 1940s; the site was abandoned since then. Heavy contamination of soil with As was reported before [10]. Poço de Freitas mining area is also known to be an Au mine from the Roman times. Both abandoned mining sites are located in the North of Portugal. Gold bearing ores have been one of the raw materials intensively exploited in northern Portugal since the Roman times. Arsenic is one of the toxic elements associated with this type of mines.

Instrumental neutron activation analysis (k_0-INAA) [11] was chosen to analyze the different samples due to its powerful ability to analyze elements such as As, Au, Ga, and rare earth elements, among others.

Experimental

Figure 1 shows the localization of the Portuguese mines where topsoils and plants were collected. Sampling at São Domingos mines was carried out in January 2009 and sampling at Castromil and Poço de Freitas was undertaken in November 2009.

Fig. 1 Locations of the studied Portuguese mines: (*1*) São Domingos, (*2*) Castromil, and (*3*) Poço de Freitas

The soil samples were taken out of their superficial (0–15 cm depth) layers, at four points within about 60 cm distance of each collected plant samples. Every four subsamples were subsequently combined into a single representative sample of ca. 4 kg. The soil samples were dried at room temperature up to a constant weight, mixed, homogenized, and sieved through a 2-mm mesh screen.

Plant samples were collected at the topsoil sampling sites and they were split into roots, stems, leaves, and flowers in the laboratory. Detailed field and laboratory procedures for handling, preparing—sorting, cleansing, pelletizing—and analyzing vegetation and soil samples have been previously described [9, 12–14]. Pellets of about 250 mg each were prepared for k_0-standardized neutron activation analysis.

The neutron irradiations were carried out at the Portuguese Research Reactor of the Technological and Nuclear Institute (RPI-ITN, Sacavém; maximum nominal power: 1 MW). Biological and soil samples were irradiated for 5 h and 1 h, respectively, at thermal neutron flux density of 2.25×10^{12} cm^{-2} s^{-1}, together with a comparator of Al-0.1% Au alloy (one disc of thickness: 125 μm; diameter: 5 mm). Gamma spectra were acquired using a liquid N_2-cooled, ORTEC®-calibrated, high-purity Ge detector (1.85 keV resolution at 1.33 MeV; 30 % relative efficiency). Samples were measured after 4 days. The comparator was measured after 1 week. Elemental concentrations were assessed through the k_0-IAEA program (version 3.21 [15]). Quality control was asserted by certified reference materials “IAEA-336 Lichen” and “IAEA Soil-5” concurrently with the field samples.

Results and discussion

Concentrations of relevant elements in soil samples of the three Portuguese mining sites are listed in Table 1. The concentrations of Au, Br and U in Castromil mine soil are higher than those determined at the other two mining sites. Regarding uranium, the concentrations found at Castromil mining area are higher than the average value usually found in soil [16]. Arsenic concentrations varied from 31 to 7158 mg kg^{-1} with an average value of 2518 mg kg^{-1}. Moreover, only Poço de Freitas mining site showed As concentrations lower than the maximum permissible level of 50 mg kg^{-1}for the agricultural soil [17]; the other sites exceeded the legislated levels.

Table 2 shows the Pearson correlations between the elemental contents of Castromil and Poço de Freitas soil. Correlation of La, Sm, and U evidences their common soil origin. A good correlation between As, Br, and K was

Table 1 Mean concentrations of different elements (with standard deviation and range) in soils from three mines (mg kg^{-1} d.w.)

Mines	Site	As Mean	As Range	Au Mean	Au Range	Br Mean	Br Range
Castromil	1	619 ± 148 (5)	[449–843]	9.1 ± 14.4 (6)	[0.5–38.1]	9.4 ± 4.1 (6)	[5.1–16.7]
	2	3389 ± 2477 (4)	[1123–5721]	1.32 ± 0.7 (4)	[0.6–2.1]	31.9 ± 5.4 (4)	[27.1–38.5]
Poço de Freitas	1	30.8 ± 33.1 (8)	[5.9–90.0]	0.032 ± 0.036 (7)	[0.007–0.096]	3.08 ± 1.99 (9)	[0.96–5.91]
São Domingos	1	7158 ± 1685 (3)	[6319–9098]	0.74 ± 0.19 (3)	[0.52–0.87]	1.21 ± 0.42 (3)	[0.86–1.68]
	2	2118 ± 29 (2)	[2098–2138]	0.40 ± 0.17 (2)	[0.28–0.52]	3.39 ± 2.62 (2)	[1.54–5.24]
	3	2459 ± 396 (2)	[2179–2740]	0.61 ± 0.12 (2)	[0.53–0.69]	2.49 ± 1.60 (2)	[1.36–3.62]
	4	1852 ± 2270 (4)	[529–5251]	1.45 ± 2.04 (4)	[0.35–4.51]	3.89 ± 1.03 (4)	[2.58–5.06]

Mines	Site	Ga Mean	Ga Range	K Mean	K Range	La Mean	La Range
Castromil	1	26.7 ± 3.5 (5)	[21.1–30.3]	29012 ± 4003 (6)	[22190–34690]	49.1 ± 13.9 (6)	[29.3–73.1]
	2	23.1 ± 4.4 (4)	[18.7–28.7]	20453 ± 4088 (4)	[16390–24260]	49.1 ± 3.6 (4)	[44.9–53.7]
Poço de Freitas	1	34.4 ± 6.1 (5)	[30.4–44.7]	60383 ± 4708 (8)	[51080–67290]	44.3 ± 4.1 (7)	[40.8–51.4]
São Domingos	1	–	–	4272 ± 1161 (2)	[3451–5093]	28.1 ± 1.0 (3)	[27.1–29.1]
	2	–	–	20010 ± 816 (2)	[19433–20587]	38.5 ± 5.6 (2)	[34.6–42.5]
	3	–	–	14470 ± 7891 (2)	[8890–20050]	28.6 ± 7.1 (2)	[23.6–33.6]
	4	–	–	23412 ± 2449 (4)	[21110–26023]	41.7 ± 9.3 (4)	[32.7–53.1]

Mines	Site	Na Mean	Na Range	Sm Mean	Sm Range	U Mean	U Range
Castromil	1	1171 ± 476 (6)	[384–1873]	8.8 ± 4.1 (5)	[3.2–13.2]	73 ± 40 (5)	[33–128]
	2	1285 ± 183 (4)	[1053–1471]	8.8 ± 0.4 (4)	[8.2–9.2]	20.0 ± 4.3 (4)	[14.1–24.0]
Poço de Freitas	1	4347 ± 2669 (9)	[2424–11160]	7.8 ± 1.0 (8)	[6.3–9.5]	7.8 ± 1.7 (7)	[6.1–10.1]
São Domingos	1	561 ± 139 (3)	[428–705]	2.56 ± 0.31 (3)	[2.23–2.84]	2.80 ± 0.18 (2)	[2.67–2.93]
	2	2577 ± 67 (2)	[2530–2625]	5.78 ± 1.43 (2)	[4.77–6.78]	1.96 (1)	
	3	3644 ± 1711 (2)	[2434–4854]	4.26 ± 1.62 (2)	[3.11–5.41]	–	–
	4	4962 ± 817 (4)	[3798–5561]	6.55 ± 1.35 (4)	[5.14–7.82]	1.83 (1)	–

The number of replicates for each sample is *in brackets*

found as well. The unusual high concentration of Br found in soils (especially at site 2 of Castromil) reveals that its presence is probably due to the use of potassium fertilizers, since it correlates very well with K, or soil fumigant [16]. The former use of methylbromide as a disinfectant (now forbidden in European Union countries) [18] may explain this association. Table 2 also presents the Pearson correlations found between the elemental contents of São Domingos soils. Uranium shows very good correlations with all elements but the correlation may be artificial due to the reduced number of sampling sites with U levels above the detection limit (4 sites). La and Sm have a good correlation showing their common soil origin.

Since site 2 of Castromil mines showed an As soil concentration range similar to the one determined at the São Domingos mine, the plant species collected at the site 2 of Castromil were selected to evaluate their As bioaccumulation. Table 3 presents the concentrations of elements in the aerial parts of the plant species, in dry weight (d.w.); high As concentrations at the sampling sites in Castromil and São Domingos mine were obtained. The element transfer coefficient (TC), a ratio between the element concentration in the plant and the soil, is shown in Table 3. Except for K concentrations, all the other element concentrations found in the studied plants are higher than the values of the "Reference Plant" [19].

All the plant species showed As concentrations above the normal range for plants (1–1.7 mg kg^{-1}) [20]. In fact, *A. curtisii* showed the highest As concentration with 126.1 mg kg^{-1} and the highest As transfer coefficient (2.28%).

The enrichment factor (EF) of the studied plants relatively to soil, using lanthanum (a typical soil element) as a reference element, was also calculated (see Fig. 2) according to the expression:

Table 2 Pearson correlations between chemical elements contents of soils from Castromil and Poço de Freitas mines (above) and São Domingos (below). The correlation coefficients above 0.50 are *in bold*

Pearson	As	Au	Br	Ga	K	La	Na	Sm	U
As	1	0.04	**0.88**	0.28	**0.55**	0.06	0.32	0.13	0.02
Au		1	0.03	0.02	0.22	0.09	0.30	0.29	0.04
Br			1	**0.52**	**0.73**	0.34	0.43	0.30	0.10
Ga				1	**0.79**	0.17	**0.87**	0.13	0.30
K					1	0.23	**0.65**	0.16	0.45
La						1	0.12	**0.59**	**0.74**
Na							1	0.09	0.35
Sm								1	0.19
U									1
Pearson	**As**	**Au**	**Br**	**–**	**K**	**La**	**Na**	**Sm**	**U**
As	1	0.32	**−0.72**	–	**−0.68**	−0.29	**−0.70**	**−0.61**	**0.98**
Au		1	−0.13	–	0.25	**0.61**	0.31	0.39	**0.70**
Br			1	–	**0.70**	**0.60**	**0.67**	**0.79**	**−0.64**
–					–	–	–	–	–
K					1	**0.77**	**0.89**	**0.92**	**−0.99**
La						1	**0.68**	**0.93**	**−0.94**
Na							1	**0.85**	**−0.91**
Sm								1	**−0.99**
U									1

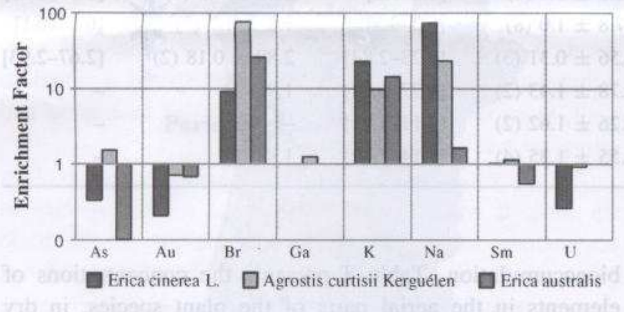

Fig. 2 Enrichment factors of different elements for three plant species

$$EF = \left([X]_{plant} / [La]_{plant}\right) / \left([X]_{soil} / [La]_{soil}\right)$$

$[X]_{plant}$ and $[X]_{soil}$ are the concentrations of element X in the plant and in the soil, respectively, and $[La]_{plant}$ and $[La]_{soil}$ are lanthanum concentration in the plant and in the soil, respectively. All plants showed higher enrichments in Br, K, and Na. Plants from Castromil mines showed an enrichment of Na with an EF value of 73 for *E. australis* and 23 for *A. curtisii*. Br is enriched relatively to soil at *A. curtisii* (EF value of 75) and at *E. australis* (EF value of 25). The results show that these plant species can grow spontaneously in these contaminated soils even if they have adverse environment, containing extremely high concentrations of As and other heavy metals and very low pH values in soils.

By definition, a plant that can uptake and accumulate more than 0.1% of an element in the tissues on a dry-weight basis may be classified as hyperaccumulator [21]. *Agrostis* genera showed higher ability to accumulate arsenic than *Erica* genera. *Agrostis curtisii* (from Castromil Mines) showed to have the highest As transfer coefficient of the studied plants (2.28%), being then considered a hyperaccumulator (Table 4).

Table 4 shows the As transfer coefficients for plant species collected in this study and the values from the literature. The maximum value of the As transfer coefficient for *A. curtisii* in this study was, however, lower than the value cited in previous literature for other species of *Agrostis* genera (with the highest value of 34.75% reported for *Agrostis truncatula*).

In general, *Agrostis* genera show a higher ability to accumulate As, as verified in the present study. Although *Agrostis curtissi* with a transfer coefficient of 2.28% can be designated as a hyperaccumulator, plant species with higher ability to accumulate As are desirable to provide a more efficient bioremediation process. However, plants with these levels of transfer coefficients can play an important role in maintaining a long-term vegetation cover at the toxic mine sites due to their tolerance to toxic

Table 3 Mean concentrations of different elements (±standard deviation) for plant species *Erica australis* from São Domingos and *Erica cinerea L.* and *Agrostis curtisii Kerguélen* from Castromil mine (mg kg^{-1} d.w.)

Species name	As	Au	K	La	Na	Sm	U
Erica australis	12.8 ± 0.1	0.0040 ± 0.0003	5782 ± 132	0.36 ± 0.02	144.1 ± 0.4	0.0457 ± 0.0004	–
	0.52	*0.66*	*39.96*	*1.27*	*3.95*	*1.07*	–
Erica cinerea L.	12.4 ± 0.4	0.0031 ± 0.0012	5160 ± 83	0.54 ± 0.02	1039 ± 11	0.100 ± 0.007	0.06 ± 0.03
	0.99	*0.42*	*30.46*	*1.06*	*90.90*	*1.12*	*0.34*
Agrostis curtisii Kerguélen	126.1 ± 2.7	0.0228 ± 0.0014	4811 ± 73	1.19 ± 0.02	725 ± 9	0.244 ± 0.017	0.45 ± 0.13
	2.28	*1.19*	*20.08*	*2.55*	*50.81*	*2.82*	*1.93*
Reference Plant [16]	*0.1*	*0.001*	*19000*	*0.2*	*150*	*0.04*	*0.01*

Soil–plant transfer coefficients (*in italic*) are shown in %

Table 4 Arsenic concentrations in soils and plants, and soil–plant transfer coefficients (%) of As obtained in this work and from published literature

Mines	Plant Species	As Transfer Coefficient (%)	[As], mg kg^{-1} Soil	[As], mg kg^{-1} Plant	Reference
São Domingos	*Agrostis castellana*	1.55	448	6.9	[9]
	Erica australis L.	0.52	2459	12.8	Present work
Castromil (site 2)	*Agrostis curtisii Kerguélen*	2.28	5528	126	Present work
	Erica cinerea L.	0.99	1250	12.4	Present work
As Ore	*Agrostis truncatula*	34.75	10000	3475	[22]
Gold	*Agrostis castellana*	4.83	12000	580	[23]
Tungsten	*Agrostis delicatula*	3.08	5200	160	[23]
Tungsten	*Erica australis L.*	12.77	148	18.9	[24]

elements such as As, with the function of stabilization of the bare ground and decreasing the fugitive dust that can contaminate the whole ecosystem [25].

Conclusion

The heavy contamination of abandoned mining sites, as São Domingos mines, with toxic elements such as arsenic recalls for an urgent strategy of cleaning up those soils. Phytoremediation appears an interesting alternative or a useful complement to the other, more expensive, soil cleaning techniques. Vascular plant species as *Agrostis curtisii*, with a moderate transfer coefficient, can be used for this purpose. However more efficient hyperaccumulators should be found for this type of soils. A study with *Agrostis trunculata* in clean and contaminated soils is in progress to assess the ability of this plant species in phytorremediation at São Domingos mines.

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