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Determination of phytoextraction potential of plant species for toxic elements in soils of abandoned sulphide-mining areas

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Abstract This study has determined contamination levels in soils and plants from the São Domingos mining area, Portugal, by k_0-INAA. Total concentrations of As, Sb, Cr, Hg, Cu, Zn and Fe in soils were very high, exceeding the maximum limits in Portuguese legislation. Concentrations of toxic elements like As, Sb and Zn were highest in roots of *Erica andevalensis*, *Juncus acutus*, *Agrostis castellana* and *Nicotiana glauca*. Additionally, As, Br, Cr, Fe, Sb and Zn in all organs of most plants were above toxicity levels. Those species that accumulated relatively high concentrations of toxic elements in roots (and tops) may be cultivated for phytostabilisation of similar areas.

Keywords k_0-INAA · Environmental remediation · Iberian pyrite belt · Mainland Portugal · Phytostabilisation potential · São Domingos mine · Sulphide-mining areas · Toxic elements · Vascular plants · *Agrostis castellana* · *Erica andevalensis* · *Juncus acutus* · *Nicotiana glauca*

Introduction

The former mining complex of São Domingos is located at the Lower Alentejo area of southern Portugal, in the heart of the Iberian pyrite belt (northern sector; 37°38′00–37°40′30 N, 7°19′05–7°20′05 W). The mine site is thought to have been excavated since the Chalcolithic Age, and continuously—and most intensively—from 1868 through 1966, when the whole operation was shut down for depletion of the sulphide-ore reserves [1]. The mine has never been properly decommissioned, and its effective abandon has caused a serious impact in local ecosystems, regional watersheds and the environment at large. Through its heyday, more than 25 Mt of ore were extracted, leaving some 750000 t of mining waste and metallurgical debris dispersed around the former mine works [2, 3], with an apparent landscape impact within no less than 30 km^2 [4].

Mining activities are important vectors of land contamination throughout the world [5], a serious problem to the extent that about 1.4 million sites have been referenced as contaminated with potentially toxic metals in Western Europe alone [6–8]. Therefore, different remediation strategies are currently—and increasingly—being used to clean up soils. The problem is, there are invariably high costs associated with reclamation of land disturbed by mining—financial and, sometimes, environmental costs as well. Phytoremediation or, better yet, the specific variants that the original concept has evolved into [9–16], may thus

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provide environment-friendly, cost-effective, low-impact alternatives to conventional methods of land decontamination. Of course, this requires an understanding of the basic processes of elemental uptake, translocation, (hyper)-accumulation and tolerance of potential plant candidates, prior to engineering and/or maximizing their ability for phytoremediation [17, 18].

The present study used k_0-standardized, instrumental neutron activation analysis (k_0-INAA) [19], to determine levels of toxic elements in superficial soils and native plants from the São Domingos mining area. The main objective was to evaluate the potential of such plants for phytoremediation purposes, especially in what concerns the phytostabilisation of similarly affected soils.

Experimental

The following species were sampled in representative locations of the São Domingos area (botanical family and common name in brackets): *Agrostis castellana* Boiss. & Reuter (Poaceae; bent grass); *Corrigiola litoralis* L. (Caryophyllaceae; strapwort), *Erica andevalensis* Cabezudo & Rivera (Ericaceae; Andévalo heather); *Erica australis* L. (Ericaceae; Spanish heath); *Eucalyptus camaldulensis* Dehnh. (Myrtaceae; red gum); *Genista polyanthos* R. Roem. ex Willk. (Leguminosae (Fabaceae); wild gorse); *Juncus acutus* L. (Juncaceae; spiny rush); *Nerium oleander* L. (Apocynaceae; oleander); *Nicotiana glauca* R.C. Graham (Solanaceae; wild tobacco); *Piptatherum miliaceum* (L.) Coss. (Poaceae; smilo grass); *Rumex scutatus* L. subsp. *induratus* (Boiss. & Reuter) Maire & Weiller (Polygonaceae; garden sorrel).

After being cleared from major debris (soil particles, extraneous biological materials) and cleansed under deionised water, plant samples were split into constituent parts—roots, stems, leaves, flowers—freeze-dried, ground in Teflon^{TM} (balls and capsule) mills, thoroughly homogenized, and made into 250-mg pellets for further analysis.

The locations' soils were sampled out of their superficial (0–15 cm depth) layers, at four points within about 60 cm radii of each collected plant. Every four subsamples were subsequently combined into a single, site-representative sample of ca. 4 kg. Topsoil samples were allowed to dry at room temperature, mixed, homogenized, and sieved through a 2-mm mesh screen. Detailed field and laboratory procedures for handling, preparing—sorting, cleansing, pelletizing—and analyzing vegetation and soil samples have been previously described [20–22].

All elemental determinations were carried out at the Portuguese Research Reactor of the Technological and Nuclear Institute (RPI-ITN, Sacavém; maximum nominal power: 1 MW), by k_0-INAA. Typically, samples were irradiated for 30 s and 7 h at thermal neutron fluxes of 2.60×10^{12} n cm^{-2} s^{-1} and 2.25×10^{12} n cm^{-2} s^{-1} for short and long irradiations, respectively, together with one disc (thickness: 125 μm; diameter: 5 mm) of an Al–0.1%Au alloy as comparator. Gamma spectra were acquired on 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 10 min (short irradiations), and after 4 days and 4 weeks (long irradiations). The comparator was measured after one day (short irradiations) and one week (long irradiations).

Elemental concentrations were assessed through the k_0-IAEA program (version 3.21). Quality control was asserted by analyzing certified reference materials (IAEA-336 "Trace and Minor Elements in Lichen"; IAEA-SL-1 "Lake Sediment") concurrently with the field samples. For the elements discussed here, deviations from IAEA-336 certified values were under 12% at a 95% confidence level, except for Hg and Se (both within 20%, due to partial losses during irradiation); and from IAEA-SL-1 they were under 20% at the same level (Hg, Sb and Se were below the detection limit of the method). Additional details on irradiation conditions and reactor parameters for the current implementation of k_0-INAA at ITN can be found elsewhere [23, 24].

Results and discussion

Total concentrations of relevant trace elements in the São Domingos soil samples are listed in Table 1. For reference purposes, maximum allowed levels by Portuguese and international (Canadian) regulations are given as well [25, 26]. The first thing to notice is the high variability between collection sites, reflecting the complex mixture of soil and heterogeneous waste—ancient and modern—that resulted from centuries-long mining of the São Domingos ore body [3]. The As, Hg, Sb, Se and Zn concentrations reached 448–3565 mg kg^{-1}, 4.5–26.3 mg kg^{-1}, 98–1099 mg kg^{-1}, 3.1–61.1 mg kg^{-1} and 178–4035 mg kg^{-1}, respectively, which are significantly higher than the regulated values or, at least, the more conservative guidelines (Hg). Generally speaking, the concentration data herein concur with recent results from the same area [3, 4].

Trace-element concentrations in roots, stems, leaves and flowers of sampled plants are shown in Table 2. Silver was low in all plant species; among them, though, the roots of *J. acutus* and *E. andevalensis* accumulated much more Ag than other plant species and/or parts. The concentrations of two highly toxic elements, As (range: 87.3–752.3 mg kg^{-1}) and Sb (range: 15.6–237.1 mg kg^{-1}) [27, 28], were higher in roots of *J. acutus*, *P. miliaceum*, *A. castellana* and *E. andevalensis*—with the highest content in roots of *J. acutus*—

Table 1 Mean elemental contents and their counting statistics' uncertainties (below, in *italics*) in topsoils of São Domingos' sampling locations, in mg kg^{-1} dry weight. Values in the *last row* are soil quality guidelines (SQG) according to the Portuguese[a] or Canadian[b] regulations [25, 26], whenever and whichever available

Site (#)	Ag	As	Br	Co	Cr	Fe	Hg	La	Sb	Sc	Se	Zn
1	5.7	2459	1.6	16.3	84.4	151400	13.0	34.4	1099	14.3	19.7	1379
	31	*3.1*	*34*	*3.6*	*22.1*	*1.5*	*17.7*	*2.2*	*4.8*	*1.4*	*11.1*	*10.4*
1	8.0	2788	2.1	9.8	81.2	142900	26.3	27.2	1093	12.2	18.8	592
	35.8	*3.3*	*38.3*	*7.1*	*6.8*	*1.6*	*12.7*	*2.7*	*4.8*	*1.9*	*28.3*	*3.8*
3	2.6	448	1.2	15.0	128.1	79280	<LD	58.1	98	22.6	3.1	178
	48.6	*3.5*	*32.9*	*2.2*	*3.8*	*1.6*	–	*1.9*	*5.4*	*1.9*	*49.3*	*6.4*
5	8.0	3565	1.0	4.9	77.2	186400	4.5	31.6	711	11.7	61.1	247
	16.3	*3.0*	*34.7*	*8.8*	*13*	*1.4*	*27*	*2.0*	*4.8*	*1.8*	*5.0*	*16.3*
6	4.0	1014	9.7	79.9	139.4	157800	<LD	31.2	156	15.4	10.6	4035
	25.3	*3.0*	*8.5*	*1.8*	*4.2*	*1.4*	–	*2.0*	*4.9*	*1.6*	*12.9*	*1.9*
SQG	40[b]	12[b]	NA	300[b]	87[b]	NA	1[a]; 50[b]	NA	40[b]	NA	2.9[b]	150[a]; 360[b]

LD Limit of detection, *NA* Not available

[b] Values for As (inorganic), Cr (total), Hg (inorganic), Se and Zn are actual Canadian soil quality guidelines; values for Ag, Co and Sb are Canadian interim remediation criteria for soil that have not yet been replaced by actual soil quality guidelines. All values refer to the worst-case scenario of an industrial land use; corresponding limits for agricultural, residential/parkland and commercial uses may differ, and, in some cases, be substantially lower

indicating that As and Sb are passively absorbed and stay in the root system [29]. However, their concentrations were lower in stems, leaves and flowers for all species: only *E. andevalensis* and *N. glauca* showed more than 10 mg kg^{-1} of As in leaves and flowers. Overall, the As concentrations in most samples were above the normal range for plants (1–1.7 mg kg^{-1}) [30].

The flowers of *A. castellana* and *N. glauca* accumulated Br in excess of 150 mg kg^{-1}, while other plant species/parts scored lower than 50 mg kg^{-1}, except for leaves of *C. litoralis* (91.5 mg kg^{-1}) and stems of *N. glauca* (70.9 mg kg^{-1}). Though detectable, Co concentrations were very low in all plants' parts for the majority of species; only the root samples of *A. castellana*, *E. andevalensis* and *J. acutus* showed concentrations above 5.0 mg kg^{-1}. Chromium was present in excess of 25 mg kg^{-1} in root samples of *A. castellana* and *J. acutus*, and also in flower samples of *N. glauca*, thus well above the common levels found in plant material [30].

Iron concentrations were very high in all plants' parts, most especially in roots, which may be viewed not only as an indication of an enhanced Fe-tolerance, but also that Fe is not easily transported in plant tissues [30]. Such high levels of Fe in roots may otherwise hinder the uptake process of other trace elements, inasmuch as a similar effect has been reported for high levels of Fe compounds in soil [30]. As a matter of fact, Mo concentrations were extremely low and undetectable in most vegetable samples, despite significant Mo levels in some soils (up to 33.4 mg kg^{-1} at site #5).

Mercury concentrations were also very low (<1 mg kg^{-1}) and undetectable in many samples, except for the roots of *E. andevalensis* and *J. acutus*. The stem, leaf and flower samples of *N. glauca* contained elevated concentrations of Se compared to other plant species/parts; of these, only the root samples of *A. castellana* and *J. acutus* showed concentrations around 8.0 mg kg^{-1}. Zinc concentrations were relatively high in all parts of most plant species, with the highest levels found in leaves of *N. glauca* and *C. litoralis*—1169 mg kg^{-1} and 1392 mg kg^{-1}, respectively. Some authors regard Zn as highly mobile in the phloem while others consider Zn to have intermediate mobility [31–33], thus, overall, likely to be concentrated in mature leaves [30]. The uptake of this essential micronutrient seems rather efficient for all the present species and, compared to other trace elements, so does the transport of Zn to stems, leaves and flowers in most of them.

The results of this study point out that a few plant species growing in contaminated soils of the São Domingos mining area can indeed survive—sometimes, even thrive—in an environment containing extremely high concentrations of As and other toxic elements, even if they fail to show a definite ability for hyperaccumulating them. It seems that such species are just hypertolerant, rather than true hyperaccumulators. It should be recalled that only plants that can take up and concentrate more than 0.1% of an element in their tissues on a dry-weight basis may be classified as hyperaccumulators, and provided that other factors (bioaccumulation, translocation) are accounted for as well [34–36]. Phytoextraction potential notwithstanding, all above elemental mass fractions should be viewed as true plant-tissue concentrations, and not as reflecting a mere

Table 2 Mean elemental contents and their counting statistics' uncertainties (unc.) in vascular plants' parts from São Domingos' sampling locations, in mg kg^{-1} dry weight

Site	Plant species	Plant parts	Ag	unc.	As	unc.	Br	unc.	Co	unc.	Cr	unc.
1	*E. andevalensis*	Roots	2.3	14.9	87.3	2.9	2.6	4.2	6.0	2.2	8.1	11.5
		Stems	0.2	30.3	6.6	2.9	0.8	3.8	2.1	1.8	4.9	4.6
		Leaves	0.2	28.6	13.1	3.1	1.7	4.4	0.4	8.5	7.7	12.3
		Flowers	0.2	28.6	13.6	3.3	1.5	5.6	0.4	10.5	6.2	9.4
1	*J. acutus*	Roots	3.2	20.7	752.3	3	41.0	3.5	5.2	3.5	31.0	8
		Stems	0.1	19.1	3.6	5	15.9	3.4	0.2	6.7	5.7	4.5
		Flowers	<LD	–	4.0	4.8	1.7	4.5	0.4	7.8	3.5	11
2	*E. camaldulensis*	Stems	0.1	39.9	0.6	8.4	7.9	3.5	0.8	3.7	6.2	3.4
		Leaves	<LD	–	1.0	9.8	18.2	3.4	1.7	2	4.4	4.6
3	*A. castellana*	Roots	<LD	–	100.9	3	19.1	3.5	6.6	3.2	25.6	3.1
		Stems	<LD	–	0.4	9	41.2	3.4	0.3	4.8	4.5	10.2
		Leaves	<LD	–	6.9	3.3	34.5	3.4	0.3	9.3	6.7	5.6
		Flowers	0.2	47	8.7	6	156.8	3.4	0.7	8.3	7.3	4.3
3	*C. litoralis*	Roots	0.2	29.9	7.6	3.3	13.0	3.4	1.2	3.1	7.3	5.5
		Stems	0.1	47.9	1.2	11.5	50.3	3.4	1.2	2.8	6.3	7.7
		Leaves	0.3	32.8	1.9	12	91.5	3.4	1.6	2.5	7.0	8.4
3	*R. scutatus*	Roots	0.3	20.1	6.5	3	7.0	3.4	1.1	2.9	6.6	4.2
		Stems	<LD	–	3.3	3	0.6	4	0.6	3.2	3.5	4.5
		Leaves	<LD	–	1.5	4.7	25.5	3.4	2.7	1.9	4.8	5.1
3	*N. oleander*	Stems	<LD	–	2.8	3.4	6.8	3.4	0.2	6.7	6.4	4
		Leaves	<LD	–	3.1	3.4	32.7	3.4	0.1	11	6.7	3.8
		Flowers	<LD	–	1.0	11.2	30.0	3.4	0.1	19.3	5.9	4
3	*G. polyanthos*	Stems	<LD	–	1.9	4.2	9.5	3.4	0.1	12.4	6.3	3.5
		Leaves	<LD	–	0.4	10.1	29.6	3.4	0.2	10.9	5.6	4
4	*C. litoralis*	Roots	0.8	20.8	49.3	2.8	12.1	3.4	4.6	2.7	12.9	4.4
		Stems	0.1	38	1.9	4.9	15.0	3.5	1.1	3	5.3	5.5
		Leaves	0.5	23.9	3.3	4.5	19.1	3.4	1.7	3.5	4.6	7.3
5	*E. australis*	Roots	0.3	28.7	53.3	2.8	0.9	5	2.1	2.3	10.0	5.9
		Stems	<LD	–	0.4	15.6	14.3	3.4	0.3	5.9	4.0	4.6
		Leaves	0.1	36.8	9.6	2.8	0.7	4.5	0.5	4.8	5.1	5
6	*P. miliaceum*	Roots	0.5	17.1	148.3	2.8	17.0	3.4	1.6	4.8	8.2	8.1
		Stems	<LD	–	1.0	11.9	18.3	3.4	<LD	–	7.7	7.7
		Leaves	<LD	–	3.7	3.3	15.7	3.4	0.1	17.1	12.3	4.2
		Flowers	<LD	–	1.0	6.4	7.9	3.5	0.0	26.7	4.3	4.1
6	*N. glauca*	Stems	0.2	42.9	3.0	10.2	70.9	3.4	0.9	3.8	6.7	8.3
		Leaves	0.3	18.7	14.5	2.9	14.0	3.4	2.3	1.8	4.1	12.1
		Flowers	<LD	–	8.6	7.2	157.3	3.4	2.3	4.5	32.0	4.4

Site	Plant species	Plant parts	Fe	unc.	Hg	unc.	Sb	unc.	Se	unc.	Zn	unc.
1	*E. andevalensis*	Roots	5834	1.8	4.50	6	40.7	4.8	2.09	11.7	368	3.1
		Stems	468	3.4	0.14	24.2	3.5	5	0.17	24.3	211	1.5
		Leaves	1031	3.3	0.13	34.5	6.6	5.1	<LD	–	57	4.2
		Flowers	883	3.8	<LD	–	6.9	5.1	<LD	–	47	4.4
1	*J. acutus*	Roots	55300	1.2	11.84	7.1	237.1	4.7	7.96	8.2	234	9.2
		Stems	194	4.8	0.10	33.7	1.6	5.9	0.20	19	19	5.9
		Flowers	200	10.5	0.16	46.7	1.5	6.3	0.38	42.6	38	4.1
2	*E. camaldulensis*	Stems	87	12.8	<LD	–	0.2	11.9	<LD	–	99	2
		Leaves	150	8.2	<LD	–	0.2	10	<LD	–	110	2.5

Table 2 continued

Site	Plant species	Plant parts	Fe	unc.	Hg	unc.	Sb	unc.	Se	unc.	Zn	unc.
3	*A. castellana*	Roots	16090	1.5	0.74	31.2	15.6	5.1	1.07	32.7	202	2.6
		Stems	79	16.7	0.08	43.2	0.1	13.7	0.09	43.8	129	1.9
		Leaves	304	5	<LD	–	1.0	7	0.16	47.1	138	3.4
		Flowers	1097	3.8	<LD	–	1.2	7.8	<LD	–	133	29.9
3	*C. litoralis*	Roots	1345	2.2	<LD	–	1.7	5.6	0.21	36.2	141	3
		Stems	154	10.8	<LD	–	0.2	8.1	<LD	–	160	3.3
		Leaves	308	8.7	0.39	15.5	0.4	8.5	0.12	47.7	229	1.8
3	*R. scutatus*	Roots	1079	2.3	0.08	43.4	1.3	5.4	<LD	–	82	3.4
		Stems	183	5.2	0.03	49.6	0.4	5.7	0.28	13.1	48	2.7
		Leaves	232	5.6	<LD	–	0.2	10.7	0.12	40.9	272	2.9
3	*N. oleander*	Stems	521	3.1	0.09	19.9	0.6	5.4	0.12	32.2	124	2.9
		Leaves	489	3.6	0.37	12.6	0.5	5.9	0.21	26.4	123	2.3
		Flowers	182	4.7	0.06	43.5	0.2	16.3	<LD	–	30	5.4
3	*G. polyanthos*	Stems	351	4.9	0.13	24.1	0.4	6.2	<LD	–	57	5
		Leaves	70	21	0.11	15.1	0.1	11.6	<LD	–	53	2.7
4	*C. litoralis*	Roots	3823	2	<LD	–	6.7	5.2	2.74	9.7	546	2.8
		Stems	169	9.8	0.10	24.8	0.2	7.7	1.27	7	532	2.1
		Leaves	446	4	0.16	48.5	0.3	8.4	3.86	5.9	1392	1.6
5	*E. australis*	Roots	2444	1.7	0.18	26.7	7.8	4.8	2.63	6.7	181	2.5
		Stems	46	11.1	0.06	39.8	0.1	19.9	<LD	–	47	2.9
		Leaves	527	3.3	0.04	41.2	1.2	5.3	0.46	12.7	57	6.1
6	*P. miliaceum*	Roots	6832	1.4	0.29	26.2	23.8	4.8	2.34	7.5	326	1.9
		Stems	65	47.8	0.06	39.7	0.2	10.9	<LD	–	50	5.5
		Leaves	270	7	0.24	18.5	0.5	6.3	<LD	–	49	4.8
		Flowers	79	16.1	0.14	39.4	0.1	10.1	0.13	41.2	38	3.2
6	*N. glauca*	Stems	112	8.7	<LD	–	0.1	13.7	4.40	3.9	367	2.2
		Leaves	134	9.6	0.11	30.8	0.2	11.2	6.01	3.5	1169	2.7
		Flowers	883	7.5	0.30	31.8	1.3	9.2	7.77	6.1	352	2.4

LD Limit of detection

contamination of the biological material with resuspended soil, given the strong divergence between the local levels of an intrinsic soil tracer—Sc (11.7–22.6 mg kg^{-1}; Table 1)—and its biological levels in all aerial parts of all sampled plants, pooled together (<0.2 mg kg^{-1}; much lesser values in most cases).

Still, there is much more to phytoremediation than just hyperaccumulation proper. Plants that became tolerant to noxious elements and are resilient enough to colonise contaminated lands can have an important role in maintaining a long-term (sustainable) vegetation cover on toxic mine sites, thus contributing to stabilise the bare ground and abate the impact of fugitive dusts onto the whole ecosystem [37]. Considering the sheer size of the affected area, an exclusive use of high-cost remediation technologies has been deemed unrealistic [4]. The present results suggest that, among the local species, *E. andevalensis*, *J. acutus*, *A. castellana* and *N. glauca* could be extensively grown for land stabilisation in the São Domingos area—and similar (climate, soil, waste) areas, for that matter—within an integrated strategy of clean-up and rehabilitation that would also benefit from their floristic (ornamental) value (Fig. 1). Such a strategy might as well work both ways in the particular case of *E. andevalensis*, an endemic, endangered species that can only be found here and in the Andévalo sector of the Huelva province (SW Spain).

Conclusions

After centuries of mining activity, followed by decades of effective abandon, there is a much-needed, long-overdue, major clean-up to be done in the area adjoining the former São Domingos mining and metallurgical works. Considering the magnitude of such an effort, phytostabilisation of the affected lands may provide an interesting alternative or,

Erica andevalensis *Juncus acutus* [spiny rush]

Agrostis castellana [bent grass] *Nicotiana glauca* [wild tobacco]

Fig. 1 Vascular plants from the São Domingos mining area, selected for their phytostabilisation potential

at least, a useful complement to costly soil-amendment techniques. Four vascular species—*E. andevalensis*, *J. acutus*, *A. castellana* and *N. glauca*—are suggested for that purpose, based on habitat, toxitolerance and decorative aspects.

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