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The fate of arsenic in soils adjacent to an old mine site (Bustarviejo, Spain): mobility and transfer to native flora

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Abstract

Background, aim, and scope The mobility of arsenic in soils and its transfer to other environmental components present significant environmental risks. The management of polluted land is determined by the availability, mobility, and transfer of inorganic pollutants to different ecosystem compartments. In this paper, the fate of arsenic at this mining site has been evaluated to determine future management practises to minimise such risk.

Materials and methods In a field study carried out in the area adjacent to a mining site at Bustarviejo (North Madrid, Spain), samples of soils, plants, and water were collected from areas adjacent to the core of the former mining activity. The following parameters were investigated in soil samples: pH, organic matter, pseudo-total As, P, and Fe, and labile As and P, and a sequential extraction procedure was performed to investigate As speciation in soil. Plant materials were analysed for both As and P. Arsenic concentrations in water samples (surface and soil pore water collected in the field) were also measured. Results are considered in tandem with previous data on metal concentrations in soils and plants from this site.

Results Despite high As concentrations in soils impacted by former mining activities (spoil accumulation and drainage from spoil heaps resulted in concentrations of up to 3,000 mg kg^{-1}), it was not present in a labile form. Sequential extraction revealed that arsenic was mainly retained by Al- and Fe-(oxihydr)oxides (up to 80%). Therefore, only a small proportion of the total soil pool was potentially available for plant uptake (0.3% and 7% extracted by $(NH_4)_2SO_4$ and $NH_4H_2PO_4$, respectively). There was very limited transfer of arsenic from soil to plants, and concentrations of arsenic in shoot tissues were relatively low (<8 μg g^{-1}). There was no evidence of phytotoxic effects in the flora that had colonised this site, indicating that a sustainable ecosystem had been established.

Discussion High levels of arsenic occur at this site, but arsenic mobility appears to be primarily controlled by the presence of amorphous and crystalline Fe and Al hydrous oxides. Although a low labile As fraction was extracted, concentrations of arsenic in both surface and soil pore water are of concern. The risk of arsenic remobilisation by plant uptake or transfer to the food chain via plant consumption is relatively low in these soils. Large amounts of metals and arsenic remain at the site, and potential risks need to be monitored. Some possible remediation strategies that take into account the presence of both arsenic and heavy metals will be suggested.

Recommendations Natural attenuation and phytostabilisation processes are taking place in several parts of the study area. These natural processes could be enhanced by application of both compost and a suitable Fe-based amendment. This augmentation of the re-vegetation of the affected area could act to promote both arsenic and metal stabilisation in mine tailings with additional benefits for further vegetation establishment.

Keywords Arsenic · Native plants · Sequential extraction · Soil pollution · Trace element · Phytoavailability

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1 Background, aim, and scope

Arsenic is a widely distributed trace element in nature as a constituent of some minerals, but the release of arsenic has been promoted by anthropogenic activities, such as mining and carbon combustion (Adriano 2001). Sulphide deposits are the main mineral source of As, in which the element can be present in high concentrations (Milton and Johnson 1999; Smedley and Kinniburgh 2002). These ores have been extensively exploited for some centuries, inducing a wide legacy of arsenic in many soils and mine spoils (Clemente et al. 2006; Strawn et al. 2002; Ongley et al. 2007; Ernst 2005). In Spain, there are now many historic derelict mines (Loredo et al. 1999; Clemente et al. 2003; Conesa et al. 2007; Domínguez et al. 2008; Rufo et al. 2007). At the Mónica mine (NW of Comunidad de Madrid, Spain), silver was extracted since the fifteenth century, but the mine was finally closed in 1980. A geological museum and visitor centre has now been created at the site of the former mine. There are still several areas with abundant pyritic wastes, and the presence and distribution of metals in soils and plants has been recently monitored in this area (Moreno-Jiménez et al. 2009). The arsenic-bearing mineral commonly found there is arsenopyrite (FeAsS), with matildite (AgBiS) appearing as small grains associated with it (Jiménez et al. 2004). There is a need to monitor the presence and behaviour of arsenic because the whole area is now classified as a leisure site inside an environmental reservoir proposed for the ecological network Natura 2000, following the environmental directives of the European Union (92/43/CEE Directive).

The presence of arsenic in soils may have hazardous consequences, but the mechanisms controlling As mobility in soils are not well understood. Arsenic geochemistry is complex, and many variables play a role in determining speciation and, hence, mobility (Fitz and Wenzel 2002; Gulz et al. 2005; Kumpiene et al. 2007; Madejón and Lepp 2007). As a general rule, arsenate is the predominant species present in aerobic soils (Sadiq 1997; Strawn et al. 2002). Arsenate mobility in soils is governed by the presence of iron and manganese hydroxides, organic matter, pH, and phosphate. The weakly retained fractions of As, rather than total soil concentrations, are most useful for current risk assessment procedures, mainly for crops and native flora (Fayiga and Ma 2006; Gulz et al. 2005; Anawar et al. 2008; Vázquez et al. 2008) but also for mammals (Sarkar et al. 2007). The most important factor is the rate of change in the proportion of As that moves from the total soil pool into those fractions that are most accessible by living organisms. Whilst the factors that regulate the rate of this change are poorly understood, such processes can be monitored by the use of sequential extractions (Onken and Adriano 1997; Wenzel et al. 2001; Shiowatana et al. 2001). These are useful tools in long-term management of polluted sites.

Data concerning As uptake by natural vegetation growing on As-polluted soils are not abundant, despite the fact that this can be a good way of monitoring As bioavailability and transfer into the food chain (Milton and Johnson 1999; Madejón et al. 2006). Plant responses to arsenic in the soil should always be investigated for the particular soil–plant system (Kabata-Pendias 2004). Arsenic concentrations in plants are generally found to be low in aboveground tissues (Anawar et al. 2006; Madejón and Lepp 2007; Domínguez et al. 2008), in agreement with a low translocation rate to shoots (Moreno-Jiménez et al. 2008). Many authors have used different calculations to evaluate this transfer from the soil to plant, such as transfer factor (TF) or bioaccumulation factors (BAF; Kloke et al. 1984; Huang et al. 2006). Once inside plant cells, arsenate phytotoxicity has been widely demonstrated, mainly under hydroponic conditions (Hartley-Whitaker et al. 2001; Mascher et al. 2002; Moreno-Jiménez et al. 2008), but field studies on this topic are infrequent. It is particularly well known that phosphate can inhibit arsenate uptake in plants and alleviate its toxicity (Esteban et al. 2003), and from this point of view, phosphate concentration will alter arsenate toxicology in the soil.

The objectives of the present study were (1) to characterise the fate and dispersal of arsenic in soils around a former mining area, (2) to measure arsenic levels in the wild flora, and (3) to evaluate the transfer and availability of arsenic in the soil–plant system. In summary, we address an initial strategy to manage arsenic risk in this site that also takes into account the presence of significant concentrations of heavy metals.

2 Materials and methods

2.1 Study site and plant and soil collection

The Mónica mine is within a high valley, wooded at the bottom, and with shrubby vegetation on the upper slopes (Moreno-Jiménez et al. 2009). Soils and plants were sampled in the surrounding of the mine around 200,000 m^2 between the following universal transverse Mercator coordinates: 30 T—X=0438606, Y=4524302; X=0437797, Y=4523518. Sampling was carried out between May and June 2006. Shoots of several plant species were collected, as well as representative soil samples from the soil directly adjacent to the sampled plants (0–30 cm, topsoil layer), obtaining a total of 43 soil samples and 95 shoot samples. Plant species were selected on the basis of their abundance in each vegetation unit of each plant group (ferns, herbaceous plants, shrubs, and trees). Water samples were collected in May from the La Mina and La Barranca streams that flow through the site. One of the water samples was collected upstream the spoil heaps, and the other five were collected downstream the mine. Sampling points were georeferenced by global positioning system, and sampling points for the soils were divided into three groups: (1) soils close to mining dumps (SCMD), (2) soils affected by mine drainage (SAMD), and (3) potentially unaffected soils (UAS). Further details (sampling map and plant species collected) of the site are described by Moreno-Jiménez et al. (2009). Pore water was sampled in spring 2009, using rhizon samplers sited 15 cm below the soil surface, following the methods described by Clemente et al. (2008) in five representatives places: one in a non-polluted soil, two in the soils affected by the mine drainage, and two at the mining spoils.

2.2 Analytical procedures

Bulk soil samples were air-dried for 7 days, sieved to 2 mm, and homogenised. Organic matter was determined by dichromate oxidation, and pH was measured in a soil water suspension 1:2.5 (MAPA (Ministerio de Agricultura, Pesca y Alimentación) 1994). For weakly retained As, 2 g of soils were mixed with 20 ml of $(NH_4)_2SO_4$ 0.1 M, shaken for 4 h, and the resultant suspension filtered (Vázquez et al. 2008). Soils were extracted by HNO_3:H_2O_2 digestion in autoclave prior to determination of pseudo-total element concentrations (Wenzel et al. 2001).

Arsenic fractionation was also studied in four randomised soil samples selected from each group of soils. Five sequential extraction steps were performed with 1 g of soil following Wenzel et al. (2001):

1. Twenty-five millilitre 0.05 M $(NH_4)_2SO_4$; shaken for 4 h and decanted; F1: non-specifically sorbed
2. Twenty-five millilitre 0.05 M $NH_4H_2PO_4$; shaken for 16 h and decanted; F2: specifically sorbed
3. Twenty-five millilitre 0.2 M NH_4-oxalate buffer, pH=3.25; shaken for 4 h and decanted; F3: amorphous oxides of Fe and Al
4. Twenty-five millilitre 0.2 M NH_4-oxalate buffer + ascorbic acid, pH=3.25; shaken for 30 min at 96°C and decanted; F4: crystallised oxides of Fe and Al
5. Digestion of soil with HNO_3/H_2O_2 in closed containers under 1,500 Pa and 125°C for 30 min. Mixture was filtered and diluted to 50 mL; F5: residual phase.

All extractions were performed in duplicate.

Arsenic in liquid samples was measured by atomic fluorescence spectroscopy (Millennium Excalibur System, PSAnalytical) using a matrix of HCl 25% (*v*/*v*) + ascorbic acid 0.2% (*w*/*v*) + KI 2%, and a reductant ($NaBH_4$ 0.9% + NaOH 0.4%; Vázquez et al. 2008). The detection limit of the As determination was 0.01 μg l^{-1}. P concentration in liquid extracts was measured spectrophotometrically, and Fe was analysed by atomic absorption spectrometry (MAPA (Ministerio de Agricultura, Pesca y Alimentación) 1994).

Plant samples were carefully washed initially with tap water and subsequently with distilled water, dried at 50°C for 7 days, and ground to 0.5 mm. A known weight of plant material (0.5 g) was digested with 10 mL of mili-Q H_2O, 2 mL of H_2O_2, and 3 mL of HNO_3 in an autoclave at 1,500 Pa and 125°C for 30 min. The resultant solution was filtered and made up to constant volume with 25 ml with water. Arsenic and P in plant extracts were measured in the same way as for soil extracts.

Certified reference materials (CTA-VL2, tobacco leaves, 0.97 μg As g^{-1}; CMR048-050, soil, 150 mg kg^{-1}) were also digested and analysed. These were found to contain 0.94 μg As g^{-1} and 133 mg As g^{-1}, respectively, with a coefficient of variance of <5%.

2.3 Statistical analysis and data processing

The data were analysed using Statistical Package for the Social Sciences 14.0® for Windows. Statistical differences between soil groups were determined using the non-parametric Kruskal–Wallis or Wilcoxon tests. Linear regression, simple, and bivariate correlations for the soil parameters were also performed. Sequential extractions were compared using Tukey's test. ArcGIS 9.0® was used to geoprocess As concentrations in soils. Principal component analysis (PCA) was performed on soil and plant analysis data, including data on heavy metals from Moreno-Jiménez et al. (2009, with permission). TF and BAF were calculated as follows: $\mathrm{TF} = [\mathrm{As}]_{\mathrm{shoot}}/[\mathrm{As}]_{\mathrm{pseudo-total}}$; $\mathrm{BAF} = [\mathrm{As}]_{\mathrm{shoot}}/[\mathrm{As}]_{\mathrm{ammonium\ sulphate-extractable\ As}}$.

3 Results

3.1 As distribution in soils

Three soil groups were distinguished in the study area: SCMD, soils affected by drainage water from the mine tailings, and UAS. Organic matter, pH, pseudo-total Fe, and pseudo-total and available As and P were measured in samples from each of the three soil groups (Table 1). All soils had an acid pH; no significant differences were observed despite the fact that arsenopyrite oxidation can sharply decrease soil pH (Clemente et al. 2006). No differences were found in organic matter content between soil groups, with values ranging from 0.69% to 13%. Total Fe content was high due to the presence of iron in the

Table 1 pH, organic matter (OM), iron, arsenic, and phosphorus concentrations in soils surrounding Mónica mine (Bustarviejo, Spain)

	Soils close to mining dumps			Soils affected by mine drainage			Unaffected soils			K–W
	Mean (SE)	Median	Range	Mean (SE)	Median	Range	Mean (SE)	Median	Range	
pH	4.87 (0.25)	4.17	3.89–5.99	5.27 (0.13)	5.20	4.24–6.09	5.08 (0.11)	5.16	4.01–5.90	NS
OM (%)	4.33 (1.00)	3.19	0.69–8.61	5.91 (0.94)	5.41	1.83–13.04	6.28 (0.70)	6.62	2.11–11.66	NS
Pseudo-total Fe (%)	2.02 (0.01)	1.86	1.32–3.38	2.16 (0.02)	2.03	1.01–3.45	1.36 (0.01)	1.42	0.94–1.65	**
Pseudo-total element (mg kg^{-1})										
As	1,544 (209)	1,719	686–3,003	638.2 (168.2)	561.4	26.3–1,907.0	38.1 (8.2)	27.5	3.6–107.7	***
P	283.4 (64.5)	244.0	59.3–878.0	540.1 (83.0)	561.7	216.7–1,166	283.6 (26.9)	281.4	108.8–592.4	*
$(NH_4)_2SO_4$-extractable element (mg kg^{-1})										
As	2.84 (0.53)	2.33	1.17–6.55	1.71 (0.27)	1.59	0.11–3.18	0.05 (0.02)	0.01	ND–0.24	***
P	3.72 (1.00)	3.65	ND–9.60	4.07 (1.17)	5.48	ND–12.35	3.24 (0.76)	5.03	ND–11.87	NS

Mean, standard error (SE), median, and range (n=12–16). Significant differences in chemical parameters between soil groups using Kruskal–Wallis test (K–W; 0.001 mg As kg^{-1}; 0.1 mg P kg^{-1})

NS non-significant, *ND* non-detectable

*P<0.05

**P<0.01

***P<0.001

geochemical background, but the highest concentrations were present in samples from the mining dumps (P<0.01), where arsenopyrite minerals are abundant. Total As levels were also very high in the mining dumps (>600 mg kg^{-1}) and in some of the SAMD. Significant differences between soil groups were observed for both total and $(NH_4)_2SO_4$-extractable As (P<0.001 for both). Ammonium sulphate extracted between 0.02% and 0.68% of total arsenic from the soil samples. The SAMD showed higher levels of total P (P<0.05), but no significant differences were observed for $(NH_4)_2SO_4$-extractable P.

Bivariate correlation analysis between soil variables was used to determine the best correlated variables with As distribution in the soil (Table 2). Total and extractable As were well correlated (P<0.001), with the highest Pearson's coefficient (r=0.840), suggesting that in this particular case, labile As is closely related to the total arsenic concentrations in soils. However, several other variables, such as Fe, total P, and available P were also significantly correlated with $(NH_4)_2SO_4$-extractable arsenic in these soils.

A linear regression was performed to identify important factors affecting As extractability. Some parameters (pH and organic matter) were removed from the model without significant variations. The resulting equation was $[As]_{Ext} = -0.86 + 0.001 \cdot [As]_{Tot} + 4.5 \cdot 10^{-5} \cdot [Fe]_{Tot} + 0.024 \cdot [P]_{Ext} + 0.001 \cdot [P]_{Tot}$; F=29.4 (P<0.001), $R^2_{adj} = 0.74$. The significance of the parameters was <0.001 for total As and Fe, <0.005 for available P, and <0.05 for total P.

When arsenic data was included in the PCA carried out by Moreno-Jiménez et al. (2009, with permission) with the soil parameters, As was associated with other pollutants

Table 2 Bivariate correlation analyses (Pearson's coefficient) in data from soils: pH, organic matter, pseudo-total (T), and extractable (E) concentration of arsenic and phosphorus

	pH	OM	$[Fe]_T$	$[As]_T$	$[P]_T$	$[As]_E$
OM	0.366*					
$[As]_T$			0.651***			
$[P]_T$	0.389*	0.448**	0.608***			
$[As]_E$			0.719***	0.840***	0.327*	
$[P]_E$			−0.552***	−0.556***	−0.336*	−0.500***

Only significant correlations were shown; n=41

*P<0.05

**P<0.01

***P<0.001

from past mining, such as Cd, Cu, Fe, and Zn, in factor 1 (factor loadings higher than 0.77, data not shown), which is associated with pollution. However, P, Mn, organic matter, and pH had higher loadings in factor 2 (not related to the pollution).

Figure 1 describes the concentration of arsenic in soils using geographic information system tools to clarify the dispersion of arsenic within the valley. The zones with abundant mining spoils showed the highest As levels, appearing as a hot-spot (dark grey polygon). There is also a remarkable pollution plume of arsenic associated with the runoff flow (intermediate greys), but many areas with low levels of arsenic were also present (in lightest grey colour).

Sequential extraction of soil samples was performed to clarify the fractionation of arsenic in these soils (Table 3). The percentage of arsenic extracted by the two first steps (ammonium sulphate and phosphate extractable fractions, respectively) did not show significant differences (3–7%). Steps 3 and 4 (associated to amorphous and crystalline hydrous oxides of Fe and Al) extracted most of the arsenic in the UAS (more than 80%) but also an important portion in polluted soils (41–59%). Finally, the residual step extracted higher significant percentages of arsenic in polluted soils (38–55%).

Arsenic concentrations in pore water were non-detectable (in an unpolluted soil), 0.002, 0.117, 0.062, and 2.901 mg l^{-1} (just below a spoil heap, close to the main drainage from the heap). Meanwhile, the concentrations in the stream waters were 0.054 (the furthest sampling point downstream the mine), 0.115, 0.147, 0.166, and 0.244 (the nearest) mg As l^{-1}. Upstream the main core of the mine, arsenic concentration in the stream water was 0.028 mg l^{-1}, which could be indicative of the local geochemical background.

3.2 Plants

P and As concentrations in shoots are given in Table 4, as well as TFs and BAF to evaluate arsenic transfer to plants. Means of As content in different plant groups were compared (seedless vascular, herbaceous, and woody plants). Significant differences were observed ($P<0.05$), and As and P concentrations in shoots showed similar tends: herbs > ferns > woody plants. Seedless vascular plants tended to accumulate less than 2 mg kg^{-1} in shoots, despite the three species being sampled from soils with high total As levels; and all species showed a similar behaviour. TFs for the seedless vascular plants were <0.01, meaning that plants are inefficient As accumulators. The BAF ranged between 1 and 2. Herbs showed the highest As concentration in their aboveground tissues (1.44 mg As kg^{-1}). Of all the plant species that were sampled, *Glyceria fluitans* had the highest As concentration (3 mg As kg^{-1}), followed by *Diplotaxis erucoides* (2 mg As kg^{-1}). In some species, As was not detected in shoots (i.e., *Hypericum perforatum* and *Daucus carota*). The highest BAF was found for *Digitalis thapsi* and the highest TF for *D. erucoides*. Woody plants had low As concentrations in shoots. *Salix atrocinerea* had the highest values (with a mean of 1.93 mg kg^{-1}), while *Genista cinerascens* and *Erica arborea* had the lowest (0.20). Depending on the species, shoots were divided into leaves and stems, and significant differences were observed, with As concentration clearly higher in leaves (data not shown). The TF and BAF varied considerably within this group, ranging from 0.0001 to 0.02 and 0.1 to 14, respectively. *Betula pendula* showed the lowest values of TF and BAF, *S. atrocinerea* the highest TF, and *Cytisus scoparius* the highest BAF.

Total and $(NH_4)_2SO_4$-extractable As concentrations were correlated with arsenic concentrations in the shoots of the dominant plant species (Table 5). This relationship was separately evaluated for each plant species. High and significant positive correlations were generally obtained for plant arsenic concentrations and their $(NH_4)_2SO_4$-extractable fraction in soils, while this correlation was frequently non-significant when the total As concentration was the other variable.

4 Discussion

4.1 Arsenic in soils

Table 1 shows that about 30% of the soils contained levels of arsenic 30–125 times higher than the limit of 24 mg As kg^{-1} for soils. This is the standard threshold in the Community of Madrid for human health protection that requires a toxicological assessment (BOCM 2007). Whilst arsenic exceeded the threshold value to larger extent (up to 125 times), values for Cd, Cu, and Zn were only around ten, eight, and two times higher, respectively (Moreno-Jiménez et al. 2009). Within the site boundaries, an area of more than 20,000 m^2 had soil As concentrations that exceeded 1,000 mg kg^{-1} (see Fig. 1). However, total element concentration is not a good indicator of As mobility, availability and, subsequently, risk (Sheppard 1992; Adriano 2001). Concentrations determined by chemical analysis using soft extractant agents and sequential extractions are more suitable to evaluate As transfer from soils to other ecosystem compartments (such as plants, animals, or water) than total As (Madejón et al. 2006; Menzies et al. 2007; Clemente et al. 2008; Vázquez et al. 2008). Thus, levels of easily extractable As in soil and As in plants (see Table 3) are actually very low in comparison with total amounts in the soils. Weak neutral salt solutions seem to be adequate to assess the impact of trace elements

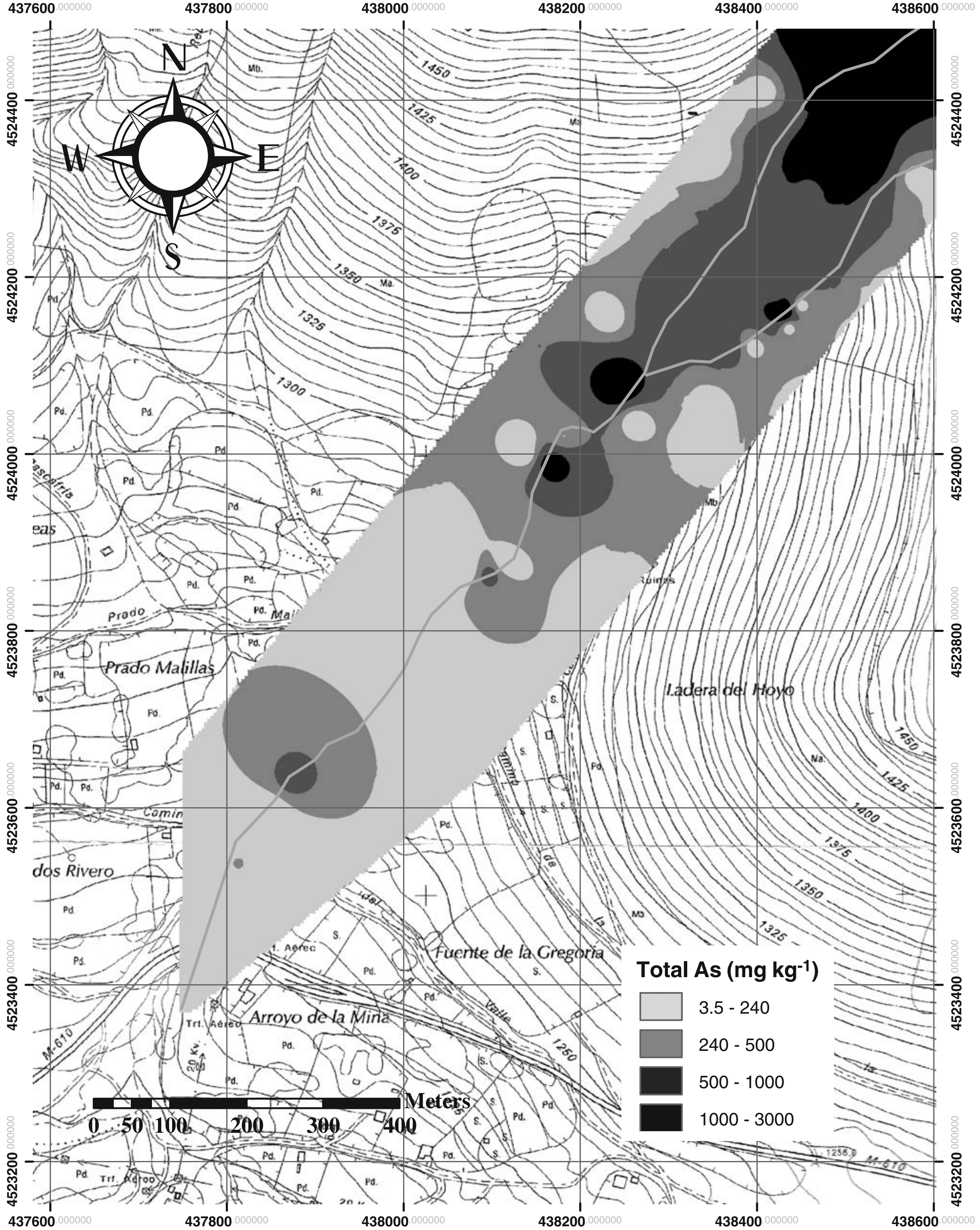

Fig. 1 Dispersion of As in surface soils across the valley. The shading categories were defined using ArcGIS 9.0® software (spatial interpolation of inverse distance weighted was applied with 12 neighbouring samples for estimation of each grid point)

Table 3 Percentage (%) of As retained in the different fractions of the sequential extraction and total concentration (mg As kg^{-1} soil)

		As fractions					Pseudo-total
		F1	F2	F3	F4	F5	
Soil groups	SCMD	0.17±0.09 a	3.68±0.68 a	18.0±3.5 a	23.7±5.2 a	54.2±7.6 b	776–2,137
	SAMD	0.27±0.07 a	3.78±0.81 a	29.3±3.9 ab	28.5±3.4 a	38.7±7.7 b	209–1,229
	UAS	0.25±0.15 a	6.78±2.09 a	39.8±1.4 b	43.9±2.2 b	6.65±3.2 a	26–105

Mean ± SE (*n*=4), different letters mean significant differences between soil groups ($P<0.05$, Tukey's test). Range for total As. Each soil sample was extracted by duplicate

on plants and soil biological activity (Kabata-Pendias 2004), and $(NH_4)_2SO_4$-extractable As has been successfully used as an indicator of As availability (Vázquez et al. 2008). This method was also successfully used with metals in field experiments (Moreno-Jiménez et al. 2009) and with multi-polluted soils in controlled experiments (Vázquez et al. 2008). In the present study, there were no significant correlations between organic matter or pH with either total or extractable As concentrations in soil. The bivariate analysis and the linear regression model showed the influence of total As on extractable arsenic in this particular case (with the highest significance in bivariate correlation and in the regression linear model), but other parameters were also shown to influence the model (P and Fe). In the same soils, total metal concentrations were also the main factor controlling the extractable metal fraction (Moreno-Jiménez et al. 2009). The influence of available P was particularly interesting but, despite its significance, it was not as important as total As or Fe. There was a negative correlation between $(NH_4)_2SO_4$-extractable P and extractable As, but the corresponding parameter is positive in the linear regression model. This means that P could mobilise As from soils but, in this case, soils with a high extractable As content also had low concentrations of labile P. The effect of phosphate on arsenate mobility in soils is well known, as they compete to be absorbed in the same soil constituents (Peryea and Kammereck 1997; Cao et al. 2003). The proportion of the total soil As pool extracted with $(NH_4)_2SO_4$ was always <0.7%. This is in agreement with other studies on As-polluted soils (Anawar et al. 2008; Vázquez et al. 2008; King et al. 2008), but contrasts with some experiments carried out with As-spiked soils where $NaNO_3$- or $(NH_4)_2SO_4$-extractable As were around 1–3% and 8–20%, respectively (Gulz et al. 2005; Carpena et al. 2008), knowing that $NaNO_3$ extracts As with a 50% less efficiency than $(NH_4)_2SO_4$ (Vázquez et al. 2008). Although many studies have used As-spiked soils (Fitz and Wenzel 2002), artificially spiked soils are unrepresentative of As geochemistry in mine tailings (Ko et al. 2008), as arsenic extractability was excessive after the spiking. Plants growing on spiked soils usually show unrealistic As uptake. The low mobility of As in the studied soils is particularly evident if we compare arsenic data with other metals in this site (Moreno-Jiménez et al. 2009). The best correlation using $(NH_4)_2SO_4$-extractable fraction than total As in soils was found with As concentrations in shoots (see Table 5), indicating that phytoavailability is better predicted by the ammonium sulphate-extractable fraction of As than by the total concentrations. When taken together with previous findings both under controlled and under field conditions (Vázquez et al. 2008; Moreno-Jiménez et al. 2009), these data support the use of $(NH_4)_2SO_4$ extraction as a robust indicator for the phytoavailable fraction of trace elements present in multi-polluted soils. Percentages of $(NH_4)_2SO_4$-extractable Cd are at least 20 times higher than As ones. Moreover, when we extracted with phosphate (see F2 in Table 3), the values were always lower than 7% of the total arsenic. The extraction of Cd using $(NH_4)_2SO_4$ is much more efficient than for As in these soils. In addition, ammonium phosphate extraction removes <7% of the total soil As pool. This fraction has also been used as an indicator of phytoavailable As (Huang et al. 2006). Arsenic mobility in soils is known to be dependent on absorption by iron, aluminium, and manganese hydroxides, clay minerals, and mineral oxyanions such as phosphates, carbonates, and sulphates (Aguilar et al. 2007; Gulz et al. 2005). The sequential extraction of these soils indicated a principal role of Fe- and Al-(oxyhydr)oxides in As geochemistry in soils (see Table 3). Arsenic in fractions 3 and 4 is mainly associated with amorphous and crystalline hydrous oxides of iron and aluminium (Wenzel et al. 2001), and up to 60–80% of As was retained in these fractions. This is in clear agreement with previous findings for different soils (Wenzel et al. 2001; Fitz and Wenzel 2002; Kumpiene et al. 2007; Clemente et al. 2008), and arsenate absorption onto Fe oxides is a frequent phenomenon after mineral oxidation in pyritic soils (Strawn et al. 2002). The residual step extracted higher significant percentages of arsenic in polluted soils (in SCMD and SAMD samples), probably due to the presence of As minerals such as arsenopyrite and matildite (Jiménez et al. 2004). Finally, arsenic in pyritic soils was associated with Fe both in pyrite minerals and in

Table 4 P and As concentrations in shoots of plants growing in the areas surrounding the Mónica mine, mean (range), n=2–12

Plant species	P (μgg^{-1})	As (μgg^{-1})	TF	BAF
Seedless vascular plants	683±196 ab	0.89±0.27 ab	–	–
Equisetum ramosissimum	870 (783–957)	1.78 (1.65–1.90)	$6.72\cdot10^{-3}$	1.45
Pteridium aquilinum	564 (98.8–1,403)	0.26 (ND–0.96)	$1.17\cdot10^{-3}$	2.00
Athyrium filix-femina	837 (129–1,940)	1.84 (1.77–1.90)	$3.61\cdot10^{-3}$	1.01
Annual and perennial herbs	870±106 b	1.44±0.32 b	–	–
Centaurea nigra	336 (268–398)	0.88 (0.09–1.78)	$5.10\cdot10^{-3}$	19.4
Hypericum perforatum	334 (238–353)	ND	–	–
Digitalis thapsi	867 (438–1,713)	1.57 (0.25–1.90)	$3.19\cdot10^{-3}$	99.8
Aira caryophyllea	1,038 (1,017–1,061)	1.81 (0.97–2.65)	$4.85\cdot10^{-3}$	1.71
Glyceria fluitans	1,047 (886–1,225)	3.52 (1.68–7.10)	$2.40\cdot10^{-3}$	0.87
Diplotaxis erucoides	1,100 (643.2–1,423)	2.06 (1.94–2.15)	$5.40\cdot10^{-3}$	2.05
Daucus carota	1,442 (1,031–1,852)	ND	–	–
Silene latifolia	1,137 (446.7–2,172)	1.47 (0.0216–1.91)	$5.01\cdot10^{-3}$	2.20
Woody plants	475.4±47.6 a	0.58±0.10 a	–	–
Cytisus scoparius	431 (129–670)	0.30 (0.06–1.05)	$5.44\cdot10^{-3}$	14.1
Cytisus oromediterraneus	302 (113–672)	0.60 (0.00–1.94)	$6.43\cdot10^{-4}$	0.17
Genista cinerascens	306 (100–598)	0.20 (ND–1.05)	$3.91\cdot10^{-4}$	0.36
Adenocarpus complicates	837 (459–1,060)	0.28 (0.16–0.36)	$8.37\cdot10^{-4}$	0.23
Thymus mastichina	279 (198–438)	0.25 (ND–0.75)	$2.15\cdot10^{-2}$	7.74
Santolina rosmarinifolia	697 (642–752)	0.52 (0.52–0.51)	$0.27\cdot10^{-3}$	0.19
Frangula alnus	590 (376–884)	0.87 (0.42–1.19)	$1.57\cdot10^{-3}$	0.47
Betula pendula	582 (507–656.6)	0.20 (0.15–0.25)	$1.79\cdot10^{-4}$	0.10
Erica arborea	536 (293–1,095)	0.22 (ND–0.71)	$2.54\cdot10^{-4}$	0.15
Salix atrocinerea	601 (171–2,148)	1.93 (0.52–2.86)	$1.93\cdot10^{-2}$	4.02

For plant groups (seedless vascular plants, woody plants, and herbs), mean ± SE are showed, and different letters mean significant differences between groups after Tukey's test ($P<0.05$). Transfer factor (TF, As in shoots divided by pseudo-total As in the soil) and bioaccumulation factor (BAF, As in shoots divided by $(NH_4)_2SO_4$-extractable As in soil) were calculated for As

(oxyhydr)oxides, resulting in a high correlation between total Fe and As. Arsenic retained in these fractions is not readily available or mobile, although it could be released if environmental conditions change. Moreover, in highly polluted soils, As is also a constituent of the least soluble fraction, i.e., as a constituent of minerals (arsenopyrite). Thus, more than 70–90% of arsenic in soils could be in the most inert fractions in mining-affected soils (Conesa et al. 2008). According to Rodríguez et al. (2003), who demonstrated that the arsenic fraction bound to Fe and Mn oxides can be dissolved in the stomach, there are arsenic risks associated with soil ingestion at this site. Soil ingestion is a major pathway of metals into livestock grazing in polluted lands (Thornton and Abrahams 1983). Cattle and horses are frequently feeding in the studied zone, and they are directly exposed to vegetation and soil particles ingestion. Regarding to humans, soil particles can be also voluntarily or involuntarily ingested, but there are also other possible pathways involving the risk assessment of trace elements in these soils such as soil particles inhalation or dermal absorption (Abrahams 2002). There are still many mine spoils uncovered by vegetation in the studied site and, subsequently, the dump particles can be easily disturbed by wind and transported to the atmosphere, enhancing the risk of mining spoils.

4.2 Transference of arsenic to plants and waters

The risk assessment was completed with data concerning plants and waters as receptors of soil pollution. All the data in our study supported the low mobility of arsenic in acidic soils. The majority of plant species preferentially store As in roots, as opposed to shoots (Moreno-Jiménez et al. 2008), with the exception of As hyperaccumulators (Zhang et al. 2002). Despite the high concentrations in soil, arsenic in plants is usually below the toxicity threshold for aboveground tissues of 3–10 mg kg^{-1} dry weight (Chaney 1989). Arsenic concentrations in shoots of many plants exceeded the value of 1–1.7 mg kg^{-1} quoted for shoots of plant growing in unpolluted soils (Kabata-Pendias and

Table 5 Pearson's coefficient (*r*) between metal concentrations in shoots and soils ($(NH_4)_2SO_4$-extractable and pseudo-total) for the dominant plant species (*n*=5–12)

	Available As	Pseudo-total As
Salix atrocinerea	0.75*	0.54
Cytisus scoparius	0.73**	0.34
Genista cinerascens	0.95***	0.79*
Erica arborea	0.68*	0.75*
Pteridium aquilinum	0.72*	0.47
Cytisus oromediterraneus	0.97**	0.67
Frangula alnus	0.79*	0.70
Mean *r*	0.79	0.61

*$P<0.05$

**$P<0.01$

***$P<0.001$

Pendias 1992), but this could also be attributed to incomplete removal of surface-borne As-containing particulates by the washing procedure. Because of the very elevated concentrations of As found in some of the mine spoils at this site, a small volume of unremoved particulates could have a significant influence on shoot As concentrations. No phytotoxic symptoms were observed in plants growing at the site even in *G. fluitans* and *S. atrocinerea*, which had shoot concentrations of 7 and 3 mg kg^{-1}, respectively. The low TFs calculated from the data suggest insignificant movement of As to plant shoots in this ecosystem. The TF values fluctuated between 0 and 0.037, all within the range of 0.0001–0.1 reported as normal (Kloke et al. 1984; Warren et al. 2003). Previous studies have commonly reported low transfer of arsenic from soil to plant shoots (Bunzl et al. 2001; Jung et al. 2002; Anawar et al. 2006; Domínguez et al. 2008), which progressively decreased when total soil As concentrations increased (Huang et al. 2006). Warren et al. (2003) found out differences between this TF depending on the source of As in soils. In the current study, we were studying a mining site with elevated As concentrations in soils and a significant proportion of the total soil As pool in non-labile fractions and, in consequence, a low TF was expected. If we compare this with other trace elements at the site, the TF for As is between 1 and 3 orders of magnitude lower than those calculated for Cd, Cu, or Zn (Moreno-Jiménez et al. 2009), following the same trend as the percentage of $(NH_4)_2SO_4$-extractable As and metals in these soils. Not only the low mobility of As in soils is determining a low transfer rate to aboveground tissues, but some physiological factors could also be affecting the low concentrations of arsenic in shoots, such as limited trans-membrane mobility of arsenate in comparison to other metals such as Hg or Cd (Esteban et al. 2003, 2008; Esteban et al. unpublished data), arsenate-phosphate interactions (Esteban et al. 2003), and low As translocation from roots (Moreno-Jiménez et al. 2008). The BAF, based on $(NH_4)_2SO_4$-extractable As, was also calculated. This coefficient reduces the contribution of soil-related differences in mobility, emphasising differences in the ability of plant species to concentrate As into target organs (Huang et al. 2006). *Pteridium aquilinum*, *D. thapsi*, and *C. scoparius* were the species with the highest BAF found in ferns, herbs, and woody plants, respectively. The main aspects of the risk to the environment through arsenic uptake by plants are (1) introduction into the food chain, (2) loss of vegetation cover induced through phytotoxicity, and (3) cycling of metals to surface soil horizons by tolerant plants to induce toxic effects on flora and fauna (Kabata-Pendias 2004). As a result, arsenic and metals at a site must be monitored over the long-term in order to monitor changes in environmental risk. Milton and Johnson (1999) found that low levels of arsenic in plants were associated with low transfer to the food chain. There is also clear evidence for sustainable As-phytostabilisation in some natural habitats (Madejón and Lepp 2007). Annual plant shoots (i.e., *G. fluitans* and *D. erucoides*) and leaves of trees showed higher As concentrations than other plants or aboveground tissues, respectively. Both are principal components of litter fall, which could play a role in remobilisation of trace elements in soils (Mertens et al. 2004). Nevertheless, its influence on arsenic biogeochemistry in soils seemed to be low in this particular case due to the low As concentrations in plant shoots in comparison with total As in soil. In agreement with previous reports (French et al. 2006; Madejón and Lepp 2007), woody plants had the lowest concentrations in shoots, supporting them as good candidates for As-phytostabilisation.

Stream waters contained elevated concentrations of As, exceeding legal limits for surface and potable waters (BOE 2000; Steinmaus et al. 2006). There is abundant literature showing high levels of As in waters impacted by sites with As geological backgrounds (Smedley and Kinniburgh 2002), arsenate being the predominant species (Williams 2001). There is a need for additional hydrogeological studies taking into account sources and discharges of surface and ground waters in this area before a suitable risk assessment can be formulated. Pore water provides a realistic picture of the mobility of trace elements in the soil (Clemente et al. 2008). Up to 3 ppm (40 μM) of As was detected in pore water from one sample site, but at other sites, concentrations of <1.6 μM confirmed the low mobility of As despite the high total concentrations present. This agrees with Fitz and Wenzel (2002), who estimated <2.3 μM arsenate in soil solution, and with Clemente et al. (2008), who found levels of up to 1.6 μM As in pore water under field conditions.

4.3 Proposals for soil management in this site

At this site, arsenic mainly affected water quality, while Cd and Zn were more labile in soils and showed significant soil-plant transfers (Moreno-Jiménez et al. 2009). The dispersal of mine wastes is the main source of arsenic and metals to soils. Subsequently, plants and surface waters are also affected. Techniques commonly used in soil remediation such as sanitary disposal and on-site or ex situ soil leaching disturb soil functions and ecological equilibrium. Here, where maintaining ecological equilibria is a priority objective, environmentally friendly alternatives should be adopted to conserve the ecosystem during any reclamation procedure. Mine wastes are difficult to treat with phytotechnologies: their properties do not allow plant establishment, and toxic elements are also leached to deeper layers (Ernst 2005). Treating multi-polluted soils with soil amendments is complex (Kumpiene et al. 2008), and many edaphic and ecological factors must be evaluated. Mine wastes should be amended to improve soil fertility and stability but also to immobilise toxic elements. One type of amendment that could fulfil these criteria is composted materials (Mench et al. 2003; Gadepalle et al. 2007; Tandy et al. 2009). The amendment would reduce metal dispersion (Gadepalle et al. 2007) that has taken place in the studied soils as well (Moreno-Jiménez et al. 2009). However, some controversial results have been reported after compost supply in As-polluted soils: some authors reported As immobilisation (Gadepalle et al. 2007), but some other authors reported an increase of the labile As fraction due to compost supply (Mench et al. 2003; Kumpiene et al. 2008; Hartley et al. 2009). Thus, compost might be carefully used in As-polluted soils because there are scientific evidences of mobilisation of arsenic from soil after compost supply, and monitoring the soil could support the safety successful of the amendment. On the other hand, compost amendment can improve the fertility on multi-polluted soils and can facilitate the establishment of a healthy vegetation cover (Bernal et al. 2006). In order to reduce As availability, there are some available alternatives such as iron amendments (Warren et al. 2003; Hartley et al. 2004), but they can release metals from soils. A combination of both compost (5%) and iron grit (1%) was successfully used as a compromise option to treat spoils from an abandoned gold mine (Mench et al. 2003) in order to assure the low mobility of As. Re-vegetation is a good and cheap solution to use in derelict areas (French et al. 2006; Robinson et al. 2006) and has been successfully used in polluted Mediterranean areas (Domínguez et al. 2008). Plant establishment in gold mine waste was also improved after compost plus iron grit supply (Mench et al. 2003). Finally, the vegetation cover would prevent soil ingestion and re-entrainment (Vamerali et al. 2009) and metalloid mobility and leaching in soils (Robinson et al. 2006). Nowadays, many authors doubt the current feasibility of phytoextraction because many questions are still open, but trace element phytostabilisation (natural or anthropogenic) could be a competitive technology (Ernst 2005; Robinson et al. 2006; Madejón and Lepp 2007). In places with low vegetation cover, *S. atrocinerea* or *C. scoparius* could be suitable candidates for reforesting, depending on the humidity of the soils. The presence of perennial plants will definitively play an important landscape and ecological role (Vamerali et al. 2009).

This site presents three potential hazards (large amounts of trace elements in mine spoils, arsenic in waters, and cadmium in plants) that need to be remediated by soil amendments and site phytostabilisation. In this context, the area should be carefully monitored in order to detect changes in the long-term risk due to the presence of elevated concentrations of trace elements in soils. Toxicological tests and risk assessment will also be carried out with highly polluted soils from this site to evaluate the actual environmental risk of trace elements and their transfer to the food chain.

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