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1 **TITLE PAGE:**

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3 **Title: The fate of Arsenic in soils adjacent to an old-mine site (Bustarviejo, Spain): Mobility and**  
4 **transfer to native flora.**

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9 **Abstract**

10 Background & goal:

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

15 Materials & methods:

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

23 Results:

24 Despite high As concentrations in soils impacted by former mining activities (spoil accumulation and  
25 drainage from spoil heaps resulted in concentrations of up to 3000 mg kg<sup>-1</sup>), it was not present in a labile  
26 form. Sequential extraction revealed that arsenic was mainly retained by Al- and Fe-(oxihydr)oxides (up  
27 to 80%). Therefore, only a small proportion of the total soil pool was potentially available for plant  
28 uptake (0.3 and 7% extracted by (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub>, respectively). There was very limited  
29 transfer of arsenic from soil to plants and concentrations of arsenic in shoot tissues were relatively low (<  
30 8 µg g<sup>-1</sup>). There was no evidence of phytotoxic effects in the flora that had colonized this site, indicating  
31 that a sustainable ecosystem had been established.

32 Discussion:

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

40 Recommendations:

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

45

46 **Keywords:** arsenic, sequential extraction, soil pollution, trace element, phytoavailability, native plants

47

## 48 INTRODUCTION

49 Arsenic is a widely distributed trace element in nature as a constituent of some minerals, but the  
50 release of arsenic has been promoted by anthropogenic activities, such as mining and carbon combustion  
51 (Adriano 2001). Sulphide deposits are the main mineral source of As, in which the element can be present  
52 in high concentrations (Milton and Johnson 1999; Smedley and Kinniburgh 2002). These ores have been  
53 extensively exploited for some centuries, inducing a wide legacy of arsenic in many soils and mine spoils  
54 (Clemente et al. 2006; Strawn et al. 2002; Ongley et al. 2007; Ernst 2005). In Spain there are now many  
55 historic derelict mines (Loredo et al. 1999; Clemente et al. 2003; Conesa et al. 2007; Domínguez et al.  
56 2008; Rufo et al. 2008). At the Mónica mine (NW of Comunidad de Madrid, Spain), silver was extracted  
57 since 15<sup>th</sup> century but the mine was finally closed in 1980. A Geological Museum and visitor centre has  
58 now been created at the site of the former mine. There are still several areas with abundant pyritic wastes  
59 and the presence and distribution of metals in soils and plants has been recently monitored in this area  
60 (Moreno-Jiménez et al. 2009). The arsenic-bearing mineral commonly found there is arsenopyrite  
61 (FeAsS), with matildite (AgBiS) appearing as small grains associated with it (Jiménez et al. 2004). There  
62 is a need to monitor the presence and behaviour of arsenic because the whole area is now classified as a  
63 leisure site inside an environmental reservoir proposed for the ecological network Natura 2000, following  
64 the environmental directives of the European Union (92/43/CEE Directive).

65 The presence of arsenic in soils may have hazardous consequences but the mechanisms  
66 controlling As mobility in soils are not well-understood. Arsenic geochemistry is complex and many  
67 variables play a role in determining speciation and hence mobility (Fitz and Wenzel 2002; Gulz et al.  
68 2005; Kumpiene et al. 2007; Madejón and Lepp 2007). As a general rule, arsenate is the predominant  
69 species present in aerobic soils (Sadiq 1997; Strawn et al. 2002). Arsenate mobility in soils is governed  
70 by the presence of iron and manganese hydroxides, organic matter, pH and phosphate. The weakly  
71 retained fractions of As, rather than total soil concentrations, are most useful for current risk assessment  
72 procedures, mainly for crops and native flora (Fayiga et al. 2006; Gulz et al. 2005; Anawar et al. 2008;  
73 Vázquez et al. 2008) but also for mammals (Sarkar et al. 2007). The most important factor is the rate of  
74 change in the proportion of As that moves from the total soil pool into those fractions that are most  
75 accessible by living organisms. Whilst the factors that regulate the rate of this change are poorly  
76 understood, such processes can be monitored by the use of sequential extractions (Onken and Adriano

77 1997; Wenzel et al. 2001; Shiowatana et al. 2001). These are useful tools in long-term management of  
78 polluted sites.

79 Data concerning As uptake by natural vegetation growing on As-polluted soils are not abundant,  
80 despite the fact that this can be a good way of monitoring As bioavailability and transfer into the food  
81 chain (Milton and Johnson 1999; Madejón et al. 2006). Plant responses to arsenic in the soil should  
82 always be investigated for the particular soil–plant system (Kabata-Pendias 2004). Arsenic concentrations  
83 in plants are generally found to be low in above-ground tissues (Anawar et al. 2006; Madejón and Lepp  
84 2007; Domínguez et al. 2008), in agreement with a low translocation rate to shoots (Moreno-Jiménez et  
85 al. 2008). Many authors have used different calculations to evaluate this transfer from the soil to plant,  
86 such as transfer or bioaccumulation factors (Kloke 1984; Huang et al. 2006). Once inside plant cells,  
87 arsenate phytotoxicity has been widely demonstrated, mainly under hydroponic conditions (Hartley-  
88 Whitaker et al. 2000; Mascher et al. 2002; Moreno-Jiménez et al. 2008), but field studies on this topic are  
89 infrequent. It is particularly well-known that phosphate can inhibit arsenate uptake in plants and alleviate  
90 its toxicity (Esteban et al. 2003), and from this point of view phosphate concentration will alter arsenate  
91 toxicology in the soil.

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

## 97 **MATERIALS AND METHODS**

### 98 *Study site and plant and soil collection*

99 The Mónica mine is within a high valley, wooded at the bottom and with shrubby vegetation on  
100 the upper slopes (Moreno-Jiménez et al. 2009). Soils and plants were sampled in the surrounding of the  
101 mine around 200000 m<sup>2</sup> between the following UTM coordinates: 30T - X = 0438606, Y = 4524302; X =  
102 0437797, Y = 4523518. Sampling was carried out between May and June 2006. Shoots of several plant  
103 species were collected, as well as representative soil samples from the soil directly adjacent to the

104 sampled plants (0–30cm, topsoil layer), obtaining a total of 43 soil samples and 95 shoot samples. Plant  
105 species were selected on the basis of their abundance in each vegetation unit of each plant group (ferns,  
106 herbaceous plants, shrubs and trees). Water samples were collected in May from the La Mina and La  
107 Barranca streams that flow through the site. One of the water samples was collected upstream the spoil  
108 heaps and the other five were collected downstream the mine. Sampling points were georeferenced by  
109 GPS and sampling points for the soils were divided into three groups: (1) soils close to mining dumps  
110 (SCMD), (2) soils affected by mine drainage (SAMD) and (3) potentially unaffected soils (UAS). Further  
111 details (sampling map and plant species collected) of the site are described by Moreno-Jiménez et al.  
112 (2009). Pore water was sampled in spring 2009, using rhizon samplers sited 15 cm below the soil surface,  
113 following the methods described by Clemente et al. (2008) in five representatives places: one in a non-  
114 polluted soil, two in the soils affected by the mine drainage and two at the mining spoils.

#### 115 *Analytical procedures*

116 Bulk soil samples were air dried for 7 days, sieved to 2 mm. and homogenised. Organic matter  
117 was determined by dichromate oxidation and pH was measured in a soil water suspension 1:2.5 (MAPA  
118 1994). For weakly-retained As, 2 g of soils were mixed with 20 ml of  $(\text{NH}_4)_2\text{SO}_4$  0.1 M, shaken for 4  
119 hours and the resultant suspension filtered (Vazquez et al. 2008). Soils were extracted by  $\text{HNO}_3:\text{H}_2\text{O}_2$   
120 digestion in autoclave prior to determination of pseudo-total element concentrations (Wenzel et al. 2001).

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

123 1) 25 mL 0.05 M  $(\text{NH}_4)_2\text{SO}_4$ . Shaken for 4 h and decanted. F1: non-specifically sorbed.

124 2) 25 mL 0.05 M  $\text{NH}_4\text{H}_2\text{PO}_4$ . Shaken for 16 h and decanted. F2: specifically sorbed.

125 3) 25 mL 0.2 M  $\text{NH}_4$ -oxalate buffer, pH=3.25. Shaken for 4 h and decanted. F3: amorphous oxides of Fe  
126 and Al.

127 4) 25 mL 0.2 M  $\text{NH}_4$ -oxalate buffer+ascorbic acid, pH=3.25. Shaken for 30 min at 96°C and decanted.  
128 F4: crystallized oxides of Fe and Al.

129 5) Digestion of soil with HNO<sub>3</sub>/H<sub>2</sub>O<sub>2</sub> in closed containers under 1500 Pa and 125 °C for 30 min. Mixture  
130 was filtered and diluted to 50 mL. F5: residual phase.

131 All extractions were performed in duplicate.

132 Arsenic in liquid samples was measured by atomic fluorescence spectroscopy (Millennium  
133 Excalibur System, PSAAnalytical) using a matrix of HCl 25% (v/v) + ascorbic acid 0.2 % (w/v) + KI 2%,  
134 and a reductant (NaBH<sub>4</sub> 0.9% + NaOH 0.4%) (Vázquez et al. 2008). The detection limit of the As  
135 determination was 0.01 µg l<sup>-1</sup>. P concentration in liquid extracts was measured spectrophotometrically  
136 and Fe was analysed by atomic absorption spectrometry (MAPA 1994).

137 Plant samples were carefully washed initially with tap water and subsequently with distilled  
138 water, dried at 50°C for 7 days and ground to 0.5 mm. A known weight of plant material (0.5 g) was  
139 digested with 10 mL of mili-Q H<sub>2</sub>O, 2 mL of H<sub>2</sub>O<sub>2</sub> and 3 mL of HNO<sub>3</sub> in an autoclave at 1500 Pa and 125  
140 °C for 30 min. The resultant solution was filtered and made up to constant volume with 25 ml with water.  
141 Arsenic and P in plant extracts were measured in the same way as for soil extracts.

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

#### 145 *Statistical analysis and data processing*

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

## 154 **RESULTS**

155 3.1 As distribution in soils

156 Three soil groups were distinguished in the study area: soils close to the mining dumps, soils  
157 affected by drainage water from the mine tailings and unaffected soils. Organic matter, pH, pseudo-total  
158 Fe and pseudo-total and available As and P were measured in samples from each of the three soil groups  
159 (Table 1). All soils had an acid pH, no significant differences were observed despite the fact that  
160 arsenopyrite oxidation can sharply decrease soil pH (Clemente et al. 2006). No differences were found in  
161 organic matter content between soil groups, with values ranging from 0.69 to 13 %. Total Fe content was  
162 high due to the presence of iron in the geochemical background, but highest concentrations were present  
163 in samples from the mining dumps ( $P < 0.01$ ), where arsenopyrite minerals are abundant. Total As levels  
164 were also very high in the mining dumps ( $> 600 \text{ mg kg}^{-1}$ ) and in some of the soils affected by mine  
165 drainage. Significant differences between soil groups were observed for both total and  $(\text{NH}_4)_2\text{SO}_4$ -  
166 extractable As ( $P < 0.001$  for both). Ammonium sulphate extracted between 0.02 and 0.68% of total  
167 arsenic from the soil samples. The soils affected by mine drainage showed higher levels of total P  
168 ( $P < 0.05$ ), but no significant differences were observed for  $(\text{NH}_4)_2\text{SO}_4$ -extractable P.

169 Bivariate correlation analysis between soil variables was used to determine the best correlated  
170 variables with As distribution in the soil (Table 2). Total and extractable As were well correlated  
171 ( $P < 0.001$ ), with the highest Pearson's coefficient ( $r = 0.840$ ), suggesting that in this particular case labile  
172 As is closely related to the total arsenic concentrations in soils. However, several other variables, such as  
173 Fe, total P and available P were also significantly correlated with  $(\text{NH}_4)_2\text{SO}_4$ -extractable arsenic in these  
174 soils.

175 A linear regression was performed to identify important factors affecting As extractability. Some  
176 parameters (pH and organic matter) were removed from the model without significant variations. The  
177 resulting equation was:

178  $[\text{As}]_{\text{Ext}} = -0.86 + 0.001 \cdot [\text{As}]_{\text{Tot}} + 4,5 \cdot 10^{-5} \cdot [\text{Fe}]_{\text{Tot}} + 0.024 \cdot [\text{P}]_{\text{Ext}} + 0,001 \cdot [\text{P}]_{\text{Tot}}$ ;  $F = 29.4$  ( $P < 0.001$ ),  $R^2_{\text{adj}} =$   
179 0.74. The significance of the parameters was  $< 0.001$  for total As and Fe,  $< 0.005$  for available P and  $< 0.05$   
180 for total P.



181           When arsenic data was included in the PCA carried out by Moreno-Jiménez et al. (2009, with  
182 permission) with the soil parameters, As was associated with other pollutants from past mining, such as  
183 Cd, Cu, Fe and Zn, in factor 1 (factor loadings higher than 0.77, data not shown), which is associated with  
184 pollution. However, P, Mn, organic matter and pH had higher loadings in factor 2 (not related to the  
185 pollution).

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

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

197           Arsenic concentrations in pore water were non detectable (in an unpolluted soil), 0.002, 0.117,  
198 0.062 and 2.901 (just below a spoil heap, close to the main drainage from the heap) mg l<sup>-1</sup>. Meanwhile the  
199 concentrations in the stream waters were 0.054 (the furthest sampling point downstream the mine), 0.115,  
200 0.147, 0.166, 0.244 (the nearest) mg As l<sup>-1</sup>. Upstream the main core of the mine, arsenic concentration in  
201 the stream water was 0.028 mg l<sup>-1</sup>, which could be indicative of the local geochemical background.

### 202 *3.2 Plants*

203           P and As concentrations in shoots are given in Table 4, as well as transfer factors (TF) and  
204 bioaccumulation factors (BAF) to evaluate arsenic transfer to plants. Means of As content in different  
205 plant groups were compared (seedless vascular, herbaceous and woody plants). Significant differences  
206 were observed ( $P<0.05$ ) and As and P concentrations in shoots showed similar trends: herbs>ferns>woody  
207 plants. Seedless vascular plants tended to accumulate less than 2 mg kg<sup>-1</sup> in shoots, despite the three

208 species being sampled from soils with high total As levels; and all species showed a similar behaviour.  
209 Transfer factors for the seedless vascular plants were  $<0.01$ , meaning that plants are inefficient As  
210 accumulators. The BAF ranged between 1 and 2. Herbs showed the highest As concentration in their  
211 aboveground tissues ( $1.44 \text{ mg As kg}^{-1}$ ). Of all the plant species that were sampled, *Glyceria fluitans* had  
212 the highest As concentration ( $3 \text{ mg As kg}^{-1}$ ), followed by *Diplotaxis euricooides* ( $2 \text{ mg As kg}^{-1}$ ). In some  
213 species As was not detected in shoots (i.e. *Hypericum perforatum* and *Daucus carota*). The highest BAF  
214 was found for *Digitalis thapsi* and the highest TF for *D. euricooides*. Woody plants had low As  
215 concentrations in shoots. *Salix atrocinerea* had the highest values (with a mean of  $1.93 \text{ mg kg}^{-1}$ ), while  
216 *Genista cinerascens* and *Erica arborea* had the lowest (0.20). Depending on the species, shoots were  
217 divided into leaves and stems and significant differences were observed, with As concentration clearly  
218 higher in leaves (data not shown). The TF and BAF varied considerably within this group, ranging from  
219 0.0001-0.02 and 0.1-14 respectively. *Betula pendula* showed the lowest values of TF and BAF, *S.*  
220 *atrocinerea* the highest TF and *Cytisus scoparius* the highest BAF.

221 Total and  $(\text{NH}_4)_2\text{SO}_4$ -extractable As concentrations were correlated with arsenic concentrations  
222 in the shoots of the dominant plant species (Table 5). This relationship was separately evaluated for each  
223 plant species. High and significant positive correlations were generally obtained for plant arsenic  
224 concentrations and their  $(\text{NH}_4)_2\text{SO}_4$ -extractable fraction in soils, while this correlation was frequently  
225 non-significant when the total As concentration was the other variable.

226

## 227 **Discussion**

### 228 *Arsenic in soils*

229 Table 1 shows that about 30% of the soils contained levels of arsenic 30-125 times higher than  
230 the limit of  $24 \text{ mg As kg}^{-1}$  for soils. This is the standard threshold in the Community of Madrid for human  
231 health protection that requires a toxicological assessment (BOCAM 2007). Whilst arsenic exceeded the  
232 threshold value to larger extent (up to 125 times) but values for Cd, Cu and Zn were only around 10, 8  
233 and 2 times higher, respectively (Moreno-Jiménez et al. 2009). Within the site boundaries, an area of  
234 more than  $20000 \text{ m}^2$  had soil As concentrations that exceeded  $1000 \text{ mg kg}^{-1}$  (Figure 1). However, total

235 element concentration is not a good indicator of As mobility, availability and, subsequently, risk  
236 (Sheppard 1992; Adriano 2001). Concentrations determined by chemical analysis using soft extractant  
237 agents and sequential extractions are more suitable to evaluate As transfer from soils to other ecosystem  
238 compartments (such as plants, animals or water) than total As (Madejón et al. 2006; Menzies et al. 2007;  
239 Clemente et al. 2008; Vázquez et al. 2008). Thus, levels of easily extractable As in soil and As in plants  
240 (Table 3) are actually very low in comparison with total amounts in the soils. Weak neutral salt solutions  
241 seem to be adequate to assess the impact of trace elements on plants and soil biological activity (Kabata-  
242 Pendias 2004), and  $(\text{NH}_4)_2\text{SO}_4$ -extractable As has been successfully used as an indicator of As  
243 availability (Vázquez et al. 2008). This method was also successfully used with metals in field  
244 experiments (Moreno-Jiménez et al. 2009) and with multipolluted soils in controlled experiments  
245 (Vázquez et al. 2008). In the present study there were no significant correlations between organic matter  
246 or pH with either total or extractable As concentrations in soil. The bivariate analysis and the linear  
247 regression model showed the influence of total As on extractable arsenic in this particular case (with the  
248 highest significance in bivariate correlation and in the regression linear model), but also other parameters  
249 were also shown to influence the model (P and Fe). In the same soils, total metal concentrations were also  
250 the main factor controlling the extractable metal fraction (Moreno-Jiménez et al. 2009). The influence of  
251 available P was particularly interesting but, despite its significance, it was not as important as total As or  
252 Fe. There was a negative correlation between  $(\text{NH}_4)_2\text{SO}_4$ -extractable P and extractable As, but the  
253 corresponding parameter is positive in the linear regression model. This means that P could mobilise As  
254 from soils, but, in this case, soils with a high extractable As content also had low concentrations of labile  
255 P. The effect of phosphate on arsenate mobility in soils is well known, as they compete to be absorbed in  
256 the same soil constituents (Peryea and Kammereck 1997; Cao et al. 2003). The proportion of the total soil  
257 As pool extracted with  $(\text{NH}_4)_2\text{SO}_4$  was always  $<0.7\%$ . This is in agreement with other studies on As-  
258 polluted soils (Anawar 2008; Vázquez et al. 2008; King et al. 2008), but contrasts with some experiments  
259 carried out with As-spiked soils where  $\text{NaNO}_3$ - or  $(\text{NH}_4)_2\text{SO}_4$ -extractable As were around 1-3% and 8-  
260 20% respectively (Gulz et al. 2005; Carpena et al. 2008), knowing that  $\text{NaNO}_3$  extracts As with a 50%  
261 less efficiency than  $(\text{NH}_4)_2\text{SO}_4$  (Vázquez et al. 2008). Although many studies have used As-spiked soils  
262 (Fitz and Wenzel 2002), artificially spiked soils are unrepresentative of As geochemistry in mine tailings  
263 (Ko et al. 2008), as arsenic extractability was excessive after the spiking. Plants growing on spiked soils  
264 usually show unrealistic As uptake. The low mobility of As in the studied soils is particularly evident if

265 we compare arsenic data with other metals in this site (Moreno-Jiménez et al. 2009). The best correlation  
266 using  $(\text{NH}_4)_2\text{SO}_4$ -extractable fraction than total As in soils was found with As concentrations in shoots  
267 (Table 5) indicating that phytoavailability is better predicted by the ammonium sulphate-extractable  
268 fraction of As than by the total concentrations. When taken together with previous findings both under  
269 controlled and under field conditions (Vázquez et al. 2008; Moreno-Jiménez et al. 2009), these data  
270 support the use of  $(\text{NH}_4)_2\text{SO}_4$  extraction as a robust indicator for the phytoavailable fraction of trace  
271 elements present in multi-polluted soils. Percentages of  $(\text{NH}_4)_2\text{SO}_4$ -extractable Cd are at least 20 times  
272 higher than As ones. Moreover, when we extracted with phosphate (F2 in Table 3) the values were always  
273 lower than 7% of the total arsenic. The extraction of Cd using  $(\text{NH}_4)_2\text{SO}_4$  is much more efficient than  
274 for As in these soils. In addition, ammonium phosphate extraction removes <7% of the total soil As pool.  
275 This fraction has also been used as an indicator of phytoavailable As (Huang et al. 2006). Arsenic  
276 mobility in soils is known to be dependent on absorption by iron, aluminium and manganese hydroxides,  
277 clay minerals and mineral oxyanions such as phosphates, carbonates and sulphates (Aguilar et al. 2007;  
278 Gulz et al. 2005). The sequential extraction of these soils indicated a principal role of Fe- and Al-  
279 (oxyhydr)oxides in As geochemistry in soils (Table 3). Arsenic in fractions 3 and 4 is mainly associated  
280 with amorphous and crystalline hydrous oxides of iron and aluminium (Wenzel et al. 2001), and up to 60-  
281 80% of As was retained in these fractions. This is in clear agreement with previous findings for different  
282 soils (Wenzel et al. 2001; Fitz and Wenzel 2002; Kumpiene et al. 2007; Clemente et al. 2008), and  
283 arsenate absorption onto Fe oxides is a frequent phenomenon after mineral oxidation in pyritic soils  
284 (Strawn et al. 2002). The residual step extracted higher significant percentages of arsenic in polluted soils  
285 (in SCMD and SAMD samples), probably due to the presence of As-minerals such as arsenopyrite and  
286 matildite (Jiménez et al. 2004). Finally arsenic in pyritic soils was associated with Fe both in pyrite  
287 minerals and in (oxyhydr)oxides, resulting in a high correlation between total Fe and As. Arsenic retained  
288 in these fractions is not readily available or mobile, although it could be released if environmental  
289 conditions change. Moreover, in highly polluted soils, As is also a constituent of the least soluble fraction,  
290 i.e. as a constituent of minerals (arsenopyrite). Thus, more than 70-90% of arsenic in soils could be in the  
291 most inert fractions in mining-affected soils (Conesa et al. 2008). According to Rodríguez et al. (2003),  
292 who demonstrated that the arsenic fraction bound to Fe and Mn oxides can be dissolved in the stomach,  
293 there are arsenic risks associated with soil ingestion at this site. Soil ingestion is a major pathway of  
294 metals into livestock grazing in polluted lands (Thornton and Abrahams, 1983). Cattle and horses are

295 frequently feeding in the studied zone and they are directly exposed to vegetation and soil particles  
296 ingestion. Regarding to humans, soil can be also voluntarily or involuntarily ingest soil particles, but there  
297 are also other possible pathways involving the risk assessment of trace elements in these soils such as soil  
298 particles inhalation or dermal absorption (Abrahams, 2002). There are still many mine spoils uncovered  
299 of vegetation in the studied site and subsequently the dump particles can be easily disturbed by wind and  
300 transported to the atmosphere, enhancing the risk of mining spoils.

### 301 *Transference of arsenic to plants and waters*

302 The risk assessment was completed with data concerning plants and waters as receptors of soil  
303 pollution. All the data in our study supported the low mobility of arsenic in acidic soils. The majority of  
304 plant species preferentially store As in roots, as opposed to shoots (Moreno-Jiménez et al. 2008), with the  
305 exception of As-hyperaccumulators (Zhang et al. 2002). Despite the high concentrations in soil, arsenic in  
306 plants is usually below the toxicity threshold for aboveground tissues of 3-10 mg kg<sup>-1</sup> dry weight (Chaney  
307 1989). Arsenic concentrations in shoots of many plants exceeded the value of 1-1.7 mg kg<sup>-1</sup> quoted for  
308 shoots of plant growing in unpolluted soils (Kabata-Pendias and Pendias 1992), but this could also be  
309 attributed to incomplete removal of surface-borne As-containing particulates by the washing procedure.  
310 Because of the very elevated concentrations of As found in some of the mine spoils at this site, a small  
311 volume of unremoved particulates could have a significant influence on shoot As concentrations. No  
312 phytotoxic symptoms were observed in plants growing at the site even in *Glyceria fluitans* and *Salix*  
313 *atrocinerea*, which had shoot concentrations of 7 and 3 mg kg<sup>-1</sup> respectively. The low transfer factors  
314 calculated from the data suggest insignificant movement of As to plant shoots in this ecosystem. The TF  
315 values fluctuated between 0 and 0.037, all within the range of 0.0001-0.1 reported as normal (Kloke et al.  
316 1984; Warren et al. 2003). Previous studies have commonly reported low transfer of arsenic from soil to  
317 plant shoots (Bunzl et al. 2001; Jung et al. 2002; Anawar et al. 2006; Fellet et al. 2007; Domínguez et al.  
318 2008), which progressively decreased when total soil As concentrations increased (Huang et al. 2006).  
319 Warren et al. (2003) found out differences between this transfer factor depending on the source of As in  
320 soils. In the current study, we were studying a mining site with elevated As concentrations in soils and a  
321 significant proportion of the total soil As pool in non-labile fractions and, in consequence, a low TF was  
322 expected. If we compare this with other trace elements at the site, the TF for As is between 1 – 3 orders of  
323 magnitude lower than those calculated for Cd, Cu or Zn (Moreno-Jiménez et al. 2009), following the

324 same trend as the percentage of  $(\text{NH}_4)_2\text{SO}_4$ -extractable As and metals in these soils. Not only is the low  
325 mobility of As in soils determining a low transfer rate to aboveground tissues, but some physiological  
326 factors could also be affecting the low concentrations of arsenic in shoots, such as limited trans-  
327 membrane mobility of arsenate in comparison to other metals such as Hg or Cd (Esteban et al. 2003 and  
328 2008; Esteban et al. unpublished data), arsenate-phosphate interactions (Esteban et al. 2003) and low As  
329 translocation from roots (Moreno-Jiménez et al. 2008). The BAF, based on  $(\text{NH}_4)_2\text{SO}_4$ -extractable As,  
330 was also calculated. This coefficient reduces the contribution of soil-related differences in mobility,  
331 emphasizing differences in the ability of plant species to concentrate As into target organs (Huang et al.  
332 2006). *P. aquilinum*, *D. thapsi* and *C. scoparius* were the species with the highest BAF found in ferns,  
333 herbs and woody plants respectively. The main aspects of the risk to the environment through arsenic  
334 uptake by plants are: (1) introduction into the food chain, (2) loss of vegetation cover induced through  
335 phytotoxicity, (3) cycling of metals to surface soil horizons by tolerant plants to induce toxic effects on  
336 flora and fauna (Kabata-Pendias 2004). As a result, arsenic and metals at a site must be monitored over  
337 the long-term in order to monitor changes in environmental risk. Milton and Johnson (1999) found that  
338 low levels of arsenic in plants were associated with low transfer to the food chain. There is also clear  
339 evidence for sustainable As-phytostabilisation in some natural habitats (Madejón and Lepp 2007). Annual  
340 plant shoots (i. e., *G. fluitans* and *D. eurocooides*) and leaves of trees showed higher As concentrations  
341 than other plants or aboveground tissues respectively. Both are principal components of litter fall, which  
342 could play a role in remobilisation of trace elements in soils (Mertens et al. 2004). Nevertheless, its  
343 influence on arsenic biogeochemistry in soils seemed to be low in this particular case due to the low As  
344 concentrations in plant shoots in comparison with total As in soil. In agreement with previous reports  
345 (French et al. 2006; Madejón and Lepp 2007), woody plants had the lowest concentrations in shoots,  
346 supporting them as good candidates for As-phytostabilisation.

347 Stream waters contained elevated concentrations of As, exceeding legal limits for surface and  
348 potable waters (BOE 2000; Steinmaus et al. 2006). There is abundant literature showing high levels of As  
349 in waters impacted by sites with As geological backgrounds (Smedley and Kinniburgh 2002), arsenate  
350 being the predominant species (Williams 2001). There is a need for additional hydrogeological studies  
351 taking into account sources and discharges of surface and ground waters in this area before a suitable risk  
352 assessment can be formulated. Pore water provides a realistic picture of the mobility of trace elements in  
353 the soil (Clemente et al. 2008). Up to 3 ppm (40  $\mu\text{M}$ ) of As was detected in pore water from one sample

354 site, but at other sites, concentrations of < 1.6  $\mu\text{M}$  confirmed the low mobility of As despite the high total  
355 concentrations present. This agrees with Wenzel et al. (2002), who estimated < 2.3  $\mu\text{M}$  arsenate in soil  
356 solution, and with Clemente et al. (2008), who found levels of up to 1.6  $\mu\text{M}$  As in pore water under field  
357 conditions.

#### 358 *Proposals for soil management in this site*

359 At this site, arsenic mainly affected water quality, while Cd and Zn were more labile in soils and  
360 showed significant soil-plant transfers (Moreno-Jiménez et al., 2009). The dispersal of mine wastes is the  
361 main source of arsenic and metals to soils. Subsequently plants and surface waters are also affected.  
362 Techniques commonly used in soil remediation such as sanitary disposal, on-site or ex-situ soil leaching  
363 disturb soil functions and ecological equilibrium. Here, where maintaining ecological equilibria is a  
364 priority objective, environmentally-friendly alternatives should be adopted to conserve the ecosystem  
365 during any reclamation procedure. Mine wastes are difficult to treat with phytotechnologies: their  
366 properties do not allow plant establishment and toxic elements are also leached to deeper layers (Ernst  
367 2005). Treating multi-polluted soils with soil amendments is complex (Kumpiene et al. 2008) and many  
368 edaphic and ecological factors must be evaluated. Mine wastes should be amended to improve soil  
369 fertility and stability, but also to immobilise toxic elements. One type of amendment that could fulfil  
370 these criteria is composted materials (Mench et al. 2003; Gadepalle et al. 2007; Tandy et al. 2008). The  
371 amendment would reduce metal dispersion (Gadepalle et al. 2007), that is taken place in the studied soils  
372 as well (Moreno-Jiménez et al., 2009). However, some controversial results have been reported after  
373 compost supply in As-polluted soils: some authors reported As immobilisation (Gadepalle et al. 2007),  
374 but some other authors reported an increase of the labile-As fraction due to compost supply (Mench et al.  
375 2003; Kumpiene et al. 2008; Hartley and Dickinson 2009). Thus, compost might be carefully used in As-  
376 polluted soils because there are scientific evidences of mobilisation of arsenic from soil after compost  
377 supply and monitoring the soil could support the safety successful of the amendment. On the other hand,  
378 compost amendment can improve the fertility on multi-polluted soils and can facilitate the establishment  
379 of a healthy vegetation cover (Bernal et al. 2006). In order to reduce As availability there are some  
380 available alternatives such as iron amendments (Warren et al. 2003; Hartley et al. 2004), but they can  
381 release metals from soils. A combination of both, compost (5%) and iron grit (1%), was successfully used  
382 as a compromise option to treat spoils from an abandoned gold mine (Mench et al. 2003), in order to

383 assure the low mobility of As. Re-vegetation is a good and cheap solution to use in derelict areas (French  
384 et al. 2006; Robinson et al. 2006) and has been successfully used in polluted Mediterranean areas  
385 (Domínguez et al. 2008). Plant establishment in gold mine waste was also improved after compost plus  
386 iron grit supply (Mench et al. 2003). Finally, the vegetation cover would prevent soil ingestion and re-  
387 entrainment (Vamerali et al. 2009) and metalloids mobility and leaching in soils (Robinson et al. 2006).  
388 Nowadays, many authors doubt the current feasibility of phytoextraction because many questions are still  
389 open, but trace element phytostabilisation (natural or anthropogenic) could be a competitive technology  
390 (Ernst 2005; Robinson et al. 2006; Madejón and Lepp 2007). In places with low vegetation cover, *S.*  
391 *atrocinerea* or *C. scoparius* could be suitable candidates for reforestation, depending on the humidity of the  
392 soils. The presence of perennial plants will definitively play an important landscape and ecological role  
393 (Vamerali et al. 2009).

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

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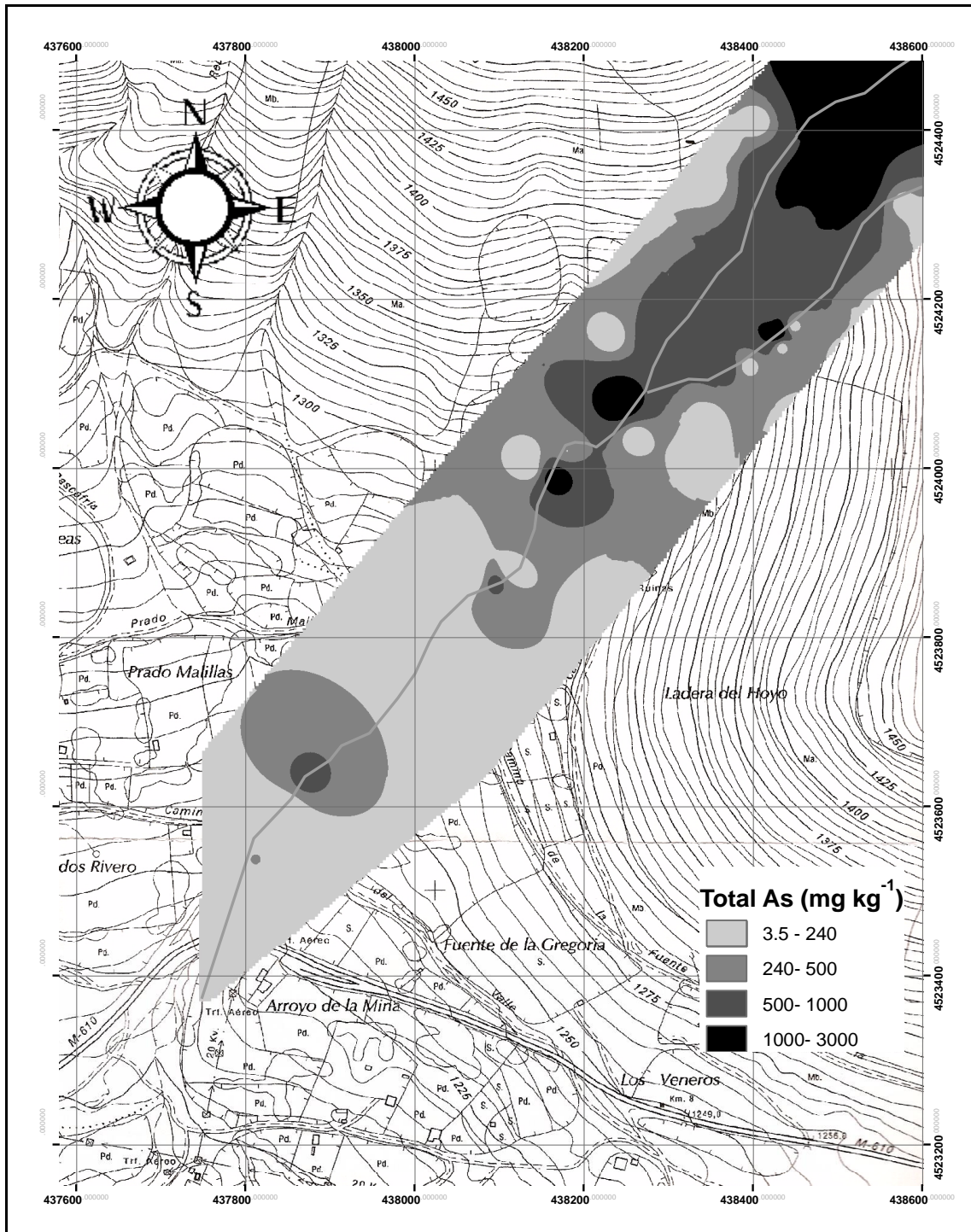
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562 Figure captions:

563 Figure 1. Dispersion of As in surface soils across the valley. The shading categories were defined using

564 ArcGIS 9.0 @ software (spatial interpolation of inverse distance weighted was applied with 12

565 neighboring samples for estimation of each grid point).



566

567



568 Table 1. pH, organic matter (OM), iron, arsenic and phosphorus concentrations in soils  
 569 surrounding Mónica mine (Bustarviejo, Spain). Mean, standard error (SE), median and  
 570 range ( $n=12-16$ ). Significant differences in chemical parameters between soil groups  
 571 using Kruskal-Wallis' test (K-W): \*\*\*= $P<0.001$ ; \*\*= $P<0.01$ ; \*= $P<0.05$ ; n.s.=non  
 572 significant. n.d.=non detectable ( $0.001 \text{ mg As kg}^{-1}$ ;  $0.1 \text{ mg P kg}^{-1}$ ).

	<b>Soils close to mining dumps</b>			<b>Soils affected by mine drainage</b>			<b>Unaffected soils</b>			<b>K- W</b>
	<b>Mean (SE)</b>	<b>Median</b>	<b>Range</b>	<b>Mean (SE)</b>	<b>Median</b>	<b>Range</b>	<b>Mean (SE)</b>	<b>Median</b>	<b>Range</b>	
<b>pH</b>	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	n.s.
<b>OM (%)</b>	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	n.s.
<b>Pseudo- total Fe (%)</b>	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	**
<b>Pseudo-total element (<math>\text{mg kg}^{-1}</math>)</b>										
<b>As</b>	1,544 (209)	1,719	686- 3003	638.2 (168.2)	561.4	26.3- 1907.0	38.1 (8.2)	27.5	3.6- 107.7	***
<b>P</b>	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	*
<b>(<math>\text{NH}_4</math>)<sub>2</sub>SO<sub>4</sub>-extractable element (<math>\text{mg kg}^{-1}</math>)</b>										
<b>As</b>	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	n.d.- 0.24	***

<b>P</b>	3.72	3.65	n.d.-	4.07	5.48	n.d.-	3.24	5.03	n.d.-	n.s.
	(1.00)		9.60	(1.17)		12.35	(0.76)		11.87	

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575 Table 2. Bivariate correlation analyses (Pearson's coefficient) in data from soils: pH, organic matter,

576 pseudo-total (T) and extractable (E) concentration of arsenic and phosphorus, ( $n=41$ ;  $*=P<0.05$ ;577  $**=P<0.01$ ;  $***=P<0.001$ ). Only significant correlations were shown.

	pH	OM	[Fe] <sub>T</sub>	[As] <sub>T</sub>	[P] <sub>T</sub>	[As] <sub>E</sub>
<b>OM</b>	0,366*					
<b>[As]<sub>T</sub></b>			0,651***			
<b>[P]<sub>T</sub></b>	0,389*	0,448**	0,608***			
<b>[As]<sub>E</sub></b>			0,719***	0,840***	0,327*	
<b>[P]<sub>E</sub></b>			-0,552***	-0,556***	-0,336*	-0,500***

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580 Table 3. Percentage (%) of As retained in the different fractions of the sequential extraction and total

581 concentration (mg As kg<sup>-1</sup> soil). Mean  $\pm$  SE ( $n=4$ ), different letters mean significant differences between582 soil groups ( $P<0.05$ , Tukey's test). Range for total As. Each soil sample was extracted by duplicate.

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

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585 Table 4. P and As concentrations in shoots of plants growing in the areas surrounding the Mónica mine,  
 586 mean (range),  $n=2-12$ . For plant groups (seedless vascular plants, woody plants and herbs) mean  $\pm$  SE are  
 587 showed and different letters mean significant differences between groups after Tukey's test ( $P<0.05$ ).  
 588 Transfer factor (TF, As in shoots divided by pseudo-total As in the soil) and bioaccumulation factor  
 589 (BAF, As in shoots divided by  $(\text{NH}_4)_2\text{SO}_4$ -extractable As in soil) were calculated for As.

Plant species	P ( $\mu\text{g g}^{-1}$ )	As ( $\mu\text{g g}^{-1}$ )	TF	BAF
<b>Seedless vascular plants</b>	683 $\pm$ 196 ab	0.89 $\pm$ 0.27 ab	-	-
<i>Equisetum ramosissimum</i>	870 (783-957)	1.78 (1.65-1.90)	6.72 $\cdot$ 10 <sup>-3</sup>	1.45
<i>Pteridium aquilinum</i>	564 (98.8-1403)	0.26 (n.d-0.96)	1.17 $\cdot$ 10 <sup>-3</sup>	2.00
<i>Athyrium filix-femina</i>	837 (129-1940)	1.84 (1.77-1.90)	3.61 $\cdot$ 10 <sup>-3</sup>	1.01
<b>Annual and perennial herbs</b>	870 $\pm$ 106 b	1.44 $\pm$ 0.32 b	-	-
<i>Centaurea nigra</i>	336 (268-398)	0.88 (0.09-1.78)	5.10 $\cdot$ 10 <sup>-3</sup>	19.4
<i>Hypericum perforatum</i>	334 (238-353)	n.d.	-	-
<i>Digitalis thapsi</i>	867 (438-1713)	1.57 (0.25-1.90)	3.19 $\cdot$ 10 <sup>-3</sup>	99.8
<i>Aira caryophylla</i>	1038 (1017-1061)	1.81 (0.97-2.65)	4.85 $\cdot$ 10 <sup>-3</sup>	1.71
<i>Glyceria fluitans</i>	1047 (886-1225)	3.52 (1.68-7.10)	2.40 $\cdot$ 10 <sup>-3</sup>	0.87
<i>Diplotaxis eruroides</i>	1100 (643.2-1423)	2.06 (1.94-2.15)	5.40 $\cdot$ 10 <sup>-3</sup>	2.05
<i>Daucus carota</i>	1442 (1031-1852)	n.d.	-	-
<i>Silene latifolia</i>	1137 (446.7-2172)	1.47 (0.0216-1.91)	5.01 $\cdot$ 10 <sup>-3</sup>	2.20
<b>Woody plants</b>	475.4 $\pm$ 47.6 a	0.58 $\pm$ 0.10 a	-	-
<i>Cytisus scoparius</i>	431 (129-670)	0.30 (0.06-1.05)	5.44 $\cdot$ 10 <sup>-3</sup>	14.1
<i>Cytisus oromediterraneus</i>	302 (113-672)	0.60 (0.00-1.94)	6.43 $\cdot$ 10 <sup>-4</sup>	0.17
<i>Genista cinerascens</i>	306 (100-598)	0.20 (n.d-1.05)	3.91 $\cdot$ 10 <sup>-4</sup>	0.36
<i>Adenocarpus complicates</i>	837 (459-1060)	0.28 (0.16-0.36)	8.37 $\cdot$ 10 <sup>-4</sup>	0.23
<i>Thymus mastichina</i>	279 (198-438)	0.25 (n.d-0.75)	2.15 $\cdot$ 10 <sup>-2</sup>	7.74
<i>Santolina rosmarinifolia</i>	697 (642-752)	0.52 (0.52-0.51)	0.27 $\cdot$ 10 <sup>-3</sup>	0.19
<i>Frangula alnus</i>	590 (376-884)	0.87 (0.42-1.19)	1.57 $\cdot$ 10 <sup>-3</sup>	0.47

<i>Betula pendula</i>	582 (507-656.6)	0.20 (0.15-0.25)	1.79·10 <sup>-4</sup>	0.10
<i>Erica arborea</i>	536 (293-1095)	0.22 (n.d-0.71)	2.54·10 <sup>-4</sup>	0.15
<i>Salix atrocinerea</i>	601 (171-2148)	1.93 (0.52-2.86)	1.93·10 <sup>-2</sup>	4.02

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591 Table 5. Pearson's coefficient (*r*) between metal concentrations in shoots and soils ((NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-  
592 extractable and pseudo-total) for the dominant plant species (*n*=5-12; \*=*P*<0.05; \*\*=*P*<0.01;  
593 \*\*\*=*P*<0.001).

	Available As	Pseudo-total As
<i>S. atrocinerea</i>	0.75*	0.54
<i>C. scoparius</i>	0.73**	0.34
<i>G. cinerascens</i>	0.95***	0.79*
<i>E. arborea</i>	0.68*	0.75*
<i>P. aquilinum</i>	0.72*	0.47
<i>C. oromediterraneus</i>	0.97**	0.67
<i>F. alnus</i>	0.79*	0.70
<b>Mean <i>r</i></b>	0.79	0.61

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