**Trace element accumulation in woody plants of the Guadiamar Valley, SW Spain:
a large-scale phytomanagement case study.**

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Abstract

Phytomanagement employs vegetation and soil amendments to reduce the environmental risk posed by contaminated sites. We investigated the distribution of trace elements in soils and woody plants from a large phytomanaged site, the Guadiamar Valley (SW Spain), seven years after a mine spill, which contaminated the area in 1998. At spill-affected sites, topsoils (0-25 cm) had elevated concentrations of As (129 mg kg⁻¹), Bi (1.64 mg kg⁻¹), Cd (1.44 mg kg⁻¹), Cu (115 mg kg⁻¹), Pb (210 mg kg⁻¹), Sb (13.8 mg kg⁻¹), Tl (1.17 mg kg⁻¹) and Zn (457 mg kg⁻¹). Trace element concentrations in the studied species were, on average, within the normal ranges for higher plants. An exception was white poplar (*Populus alba*), which accumulated Cd and Zn in leaves up to 3 and 410 mg kg⁻¹ respectively. We discuss the results with regard to the phytomanagement of trace element contaminated sites.

27

28 *There is a low trace element transfer from contaminated soils to the aboveground parts*
29 *of afforested woody plants under a semi-arid climate.*

30

31 *Keywords:* heavy metal; bioaccumulation; phytoremediation; *Populus alba*; *Quercus*
32 *ilex*; *Olea europaea*;

33

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36

37 **1. Introduction**

38

39 Phytomanagement is the use of vegetation and soil amendments to reduce the
40 environmental risk posed by contaminated sites (Bañuelos et al, 1999; Barceló and
41 Poschenrieder, 2003). The primary aim of phytomanagement is to reduce contaminant
42 mobility and the effects of contaminants on humans and ecosystems. A successfully
43 phytomanaged site should have limited leaching and limited plant uptake of
44 contaminants. The soil surface must be stabilised so that wind and water erosion are
45 minimised and there is a reduced risk of direct soil consumption by humans and animals
46 (Robinson et al., 2003). Here we investigate a large-scale phytomanagement
47 programme, implemented after a toxic sludge spill in the Guadiamar River Valley
48 (Southwestern Spain). This programme was one of the largest soil remediation
49 operations in Europe. It included the use of soil amendments and the revegetation of
50 the affected area (about 55 km²) with native woody plants.

51

52

53 As with the phytomanagement of other trace element-contaminated sites, the success of
54 this programme depends on the combination of suitable soil amendments and well-
55 chosen plant species that tolerate the local conditions, including the elevated
56 concentrations of trace elements. Plants should not accumulate high concentrations of
57 trace elements into their aboveground parts, because this would facilitate their entry into
58 the food chain (Salomons et al., 1995) and increase the trace element concentration on
59 the soil surface upon litter fall (Johnson et al., 2003; Watmough et al., 2005). The
60 revegetated plants should limit leaching by returning rainfall to the atmosphere via
61 evapotranspiration (Tordoff et al., 2000).

62

63 Woody species constitute most of plant biomass in native Mediterranean forests and
64 shrublands. They are important primary producers in local food webs. Woody species
65 are long-lived organisms that can take up trace elements from the environment and store
66 them for a long time. In the Guadiamar Valley, potential evapotranspiration exceeds
67 rainfall (see description of study area below). Therefore, deep-rooting woody species
68 would be most effective to reduce leaching, since they can access water from a greater
69 depth in the soil profile allowing them to continue to transpire long after shallow-rooted
70 plants have shut down due to drought stress. The dry buffer zone in the soil profile so
71 created by deep-rooted plants can absorb water following a heavy rainfall event (Mills
72 and Robinson, 2003).

73

74 In previous studies, trace element accumulation was analysed in surviving trees shortly
75 after the mine-spill (Madejón et al., 2004, 2006a). High concentrations of Cd and Zn
76 were found in leaves of *Populus alba*. The leaves of *Quercus ilex* had high

concentrations of As and Pb and the fruits of *Olea europaea* had high concentrations of Cd and Pb. However, there is no previous published information on the response of afforested vegetation during the phytomanagement programme.

This paper aims to determine the environmental risk posed by trace element accumulation for the major tree and shrub species occurring in the Guadiamar Valley. These include existing trees that survived the spill as well as newly planted trees and shrubs. Specifically, we sought to determine the trace element burden under selected areas of the Guadiamar Valley, to investigate the vegetation uptake of trace elements in relation to plant species and soil characteristics, and to discuss their implications for the general phytomanagement of contaminated sites.

2. Material and Methods

2.1. Study area and studied species

The Guadiamar River Valley lies inside the Iberian Pyrite Belt, the largest massive sulphide province in Western Europe. It has a semi-arid Mediterranean climate with mild rainy winters and warm dry summers. Average annual temperature is 19 °C (min. 9 °C in January, max. 27 °C in July). Average annual rainfall is 610 mm and potential evapotranspiration is 774 mm. Soils of the Guadiamar floodplain are mostly neutral or slightly alkaline, with the exception of some terraces (in the North right bank), which have low pH. Soil texture varies from loamy sand to silty clay (Madejón et al., 2004, 2006a). Currently, the vegetation in the Guadiamar Valley includes savannah-like oak woodlands in the upper reaches, cereal and olive crops (outside the spill-affected zone)

102 in the middle, rice crops and halophytic shrubs in the saltmarshes southward to Doñana
103 National Park, where the river flows into. Fragmented riparian forests are dominated by
104 white poplar (*Populus alba*) and narrow-leaved ash (*Fraxinus angustifolia*), while in
105 terrace open woodlands the Holm oak (*Quercus ilex* subsp. *ballota*) and wild olive tree
106 (*Olea europaea* var. *sylvestris*) are most abundant.

107

108 In 1998, the failure of a large mine tailing dam at Aznalcóllar (Seville) released about 4
109 M m³ of trace element-contaminated sludge into the Guadiamar River (Garraón et al.
110 1999). The resulting flood inundated 55 km² of the basin southward towards the Doñana
111 National Park (Grimalt et al., 1999). The affected soils, mostly under agricultural
112 production, were burdened with high concentrations of As, Cd, Cu, Pb, Tl and Zn
113 (Cabrera et al. 1999).

114

115 After the accident, an emergency cleanup removed sludge and contaminated topsoil,
116 which were transported and deposited in the nearby opencast mine. Despite this
117 remediation, the underlying soils still contained elevated amounts of trace elements
118 (Moreno et al., 2001). Organic matter and Ca-rich amendments were added with the aim
119 of immobilising trace elements and improving soil fertility (CMA, 2003).

120

121 The Regional Administration (RA) purchased affected lands, which were farms with
122 some fragmented forests and savannah-like woodlands. The RA implemented the
123 Guadiamar *Green Corridor* programme, with the goal of providing a continuous
124 vegetation belt for wildlife to migrate along the Guadiamar River basin between the
125 Doñana National Park in the South and the Sierra Morena mountains in the North
126 (CMA, 2001). Revegetation on the alluvial terraces started in 1999. Depending on the

127 local habitat conditions, the target tree and shrub species to afforest were those typical
128 of Mediterranean riparian forests, such as *Populus alba*, *Tamarix africana*, *Fraxinus*
129 *angustifolia* and *Salix atrocinerea* or those typical of drier upland forests, such as
130 *Quercus ilex* subsp. *ballota*, *Olea europaea* var. *sylvestris*, *Phillyrea angustifolia*,
131 *Pistacia lentiscus*, *Rosmarinus officinalis* and *Retama sphaerocarpa*.

132

133 We focused our study on the most important trees and shrubs species, in terms of
134 abundance, used in the restoration project of the Guadiamar Valley. We also monitored
135 adult trees of the same species, from some remnants forests affected by the spill. The
136 selected tree species were: Holm oak (*Quercus ilex* subsp. *ballota* (Desf.) Samp.), wild
137 olive tree (*Olea europaea* var. *sylvestris* Brot.) and white poplar (*Populus alba* L.). For
138 shrubs, we selected narrow-leafed mock privet (*Phillyrea angustifolia* L.), mastic shrub
139 (*Pistacia lentiscus* L.), rosemary (*Rosmarinus officinalis* L.), yellow retama brush
140 (*Retama sphaerocarpa* (L.) Boiss.) and tamarisk (*Tamarix africana* Poir.). The
141 nomenclature follows that of López-González (2002).

142

143 2.2. Plant and soil sampling

144

145 Sampling occurred in the autumn of 2005. Nineteen sites along the Guadiamar
146 floodplain were selected (Fig. 1), from the non-affected areas upstream of the
147 Aznalcóllar tailings dam (37° 30' N, 6° 13' W) down to the southern limit of the Doñana
148 saltmarshes (37° 13' N, 6° 14' W). Three of these sites were unaffected by the spill.
149 Riparian species (*Populus alba* and *Tamarix africana*) were collected from ten sites and
150 the rest of the species, typical of drier upland forests, were collected from eleven sites.
151 Two sites had a mixture of both suites of species.

152 For each species, we took samples from affected and unaffected sites, with exception of
153 white poplar and tamarisk, which were only present at affected sites. The unaffected
154 sites were located either outside the riparian areas (since the flood moved forward the
155 riverbed) or upstream the mine tailing dam (Northern edge of the phytomanaged area,
156 where acid soils are predominant); in neither of those habitats we found white poplar
157 and tamarisk individuals.

158

159 At each site, we selected between three and ten individual trees for each species,
160 depending on their abundance. Around each tree, the leaf litter was removed and soil
161 samples were taken from the root-zone at 0-25 cm and 25-40 cm, using a spiral auger of
162 2.5 cm diameter. Two cores were taken at opposite sides of the trunks to make a
163 composite soil sample for each tree. Between 12 and 40 soil samples were taken at each
164 site. The total number of soil samples was 234.

165

166 For each selected tree, a composite leaf sample was taken from the outer canopy.
167 Between 15 and 25 samples per tree species and life-stage (adults and saplings) were
168 collected. For shrubs, four to six individuals of each species at each site were selected
169 and leaf samples were obtained by combining the leaves of selected individuals of the
170 same species. In the case of *Retama sphaerocarpa*, a shrub with short-lived leaves and
171 photosynthetic stems, green stems were collected. The total number of plant samples
172 was 152 (52 adult trees, 64 sapling trees and 36 shrubs) corresponding to eight species.

173

174 *2.3. Sample preparation and chemical analyses*

175

176 Soil samples were oven-dried at 40 °C until a constant weight was obtained, then sieved
177 to < 2 mm. A fraction of each sample was then ground in an agata mortar to <1 mm for
178 trace elements analysis. Soil samples were digested using concentrated HNO₃ and HCL
179 (aqua regia). The values determined with this extraction are referred to as “total”
180 concentration (Vidal et al., 1999).

181

182 The pH was determined potentiometrically in a 1:2.5 soil-water suspension. Equivalent
183 calcium carbonate was determined using a Bernard calcimeter (Hulseman, 1966)
184 Organic matter content was analysed by dichromate oxidation and titration with ferrous
185 ammonium sulphate (Walkley and Black, 1934).

186

187 Leaf samples were washed thoroughly with distilled water, dried at 70 °C for at least 48
188 h and ground using a stainless-steel mill. Leaves were digested using concentrated
189 HNO₃ (Jones and Case, 1990).

190

191 Trace element (As, Ba, Be, Bi, Cd, Co, Cu, Mn, Mo, Ni, Pb, Sb, Tl and Zn)
192 concentrations of both soils and plants samples were determined by ICP-MS
193 (inductively coupled plasma-mass spectroscopy). Quality assurance was obtained for
194 soils by analysing reference material CRM 141R (calcareous loam soil, European
195 Community Bureau of Reference). Recoveries from CRM 141R values ranged from 82
196 to 94%. The quality of the plant analyses was assessed by analysing two reference
197 materials: NCS DC 73350 (white poplar leaves, China National Analysis Center for
198 Iron and Steel) and BCR 62 (olive tree leaves, European Community Bureau of
199 Reference; Colinet et al., 1982). For all elements except Sb our experimental values

200 were 82 to 109% of the certified values. The lower recovery of Sb (60%) indicates that
201 our results may be conservative estimations of the true values for this element.

202

203 *2.4. Data analyses*

204

205 Significant differences in soil and plant trace element concentrations between spill-
206 affected and unaffected sites were analysed using the *t*-test. This test was also
207 performed to compare contamination levels between soils beneath adult trees (from
208 remnant woodlands) and beneath afforested saplings. Principal components analyses
209 (PCA) were performed to investigate trace element variability trends both in soils and in
210 plants. Data that were log-normally distributed were log-transformed for these statistical
211 analyses.

212

213 Plant-soil relationships were assessed for the tree species, from individual plant/soil
214 samples. Correlation analyses were performed between plant and soil trace element
215 concentrations. Bioaccumulation coefficients (BC), defined as the plant / soil
216 concentration quotient (Adriano, 2001), were calculated. Average trace element
217 concentrations through the sampled soil profile (0-40 cm) were considered for these
218 coefficients. Relationships between BC and soil pH and organic matter were also
219 evaluated by correlation analyses.

220

221 Significance level was fixed at the 0.05. In order to avoid the increase of type I error
222 derived from multiple testing, we controlled the ‘false discovery rate’, (FDR) at the 5%
223 level, as suggested by García (2003). We used a powerful ‘adaptive’ FDR procedure
224 (Hochberg and Benjamini, 2000) to calculate an overall threshold value ($p_t \leq 0.05$), to

225 which individual p- values were compared. After applying the adaptive-FDR procedure
226 to the overall p-value vector (including 420 p-values) we got a significance threshold
227 value (p_i) of 0.013. Therefore only p-values not exceeding this threshold value were
228 considered as significant.

229

230 All statistical analyses were performed with STATISTICA v. 6.0. (StatSoft Inc., Tulsa,
231 USA).

232

233 **3. Results**

234

235 *3.1. Trace elements in soils*

236

237 The concentrations of As, Bi, Cd, Cu, Pb, Sb, Tl and Zn were significantly higher in the
238 spill-affected soils compared to the unaffected soils, at both 0-25 cm and 25-40 cm
239 depths (Table 1). This result indicates that there was significant penetration of these
240 eight contaminants (from the mine spill) into the soil profile. In contrast, the
241 concentrations of other trace elements, namely Ba, Be, Co, Fe, Mn, Mo and Ni, were
242 not significantly different between the contaminated and uncontaminated sites.

243

244 Most affected surface soils (86 % of the samples) had As concentrations above the
245 range considered normal ($0.1 - 40 \text{ mg kg}^{-1}$) for agricultural soils (Bowen, 1979).
246 Similarly, other trace elements were above Bowen's range for normal concentrations in
247 soils. These were (in decreasing order of the percentage of samples that exceeded these
248 values) Tl (53%), Sb (52%), Pb (24%), Cd (17%), Zn (5%) and Cu (4%). Furthermore,
249 75% of affected soils exceeded the Dutch Intervention Values (DIV, see NIPHE, 2001)

250 for As (55 mg kg^{-1}), and lower percentages of samples for other elements: Sb (40%), Zn
251 (13%), Cu (8%) and Pb (4 %).

252

253 At a greater depth (25-40 cm) the concentrations of trace elements were generally lower
254 than for the surface soils. Nevertheless, the DIV were still exceeded for As (60% of
255 samples), Sb (27%), Cu and Zn (10% for both).

256

257 There were significant and positive correlations between all elements that occurred in
258 elevated concentrations in the spill-affected soils (compared to the unaffected soils).

259 This result supports the hypothesis that a single contamination event, the Aznalcóllar
260 mine accident, deposited all these trace elements. The first PCA component explained
261 47% of the total variance, and completely separated affected from unaffected soils
262 (mean scores of -0.69 and 4.03 respectively). The factor loadings of the eight trace
263 elements associated to the spill had the highest values for this first component (Table 2).

264 Given that the contaminants are mutually correlated, the first component scores for each
265 sample can be use as an index for the total contaminant burden. Detailed information
266 about the level of trace element contamination at each sample site is given in the
267 Appendix 1, along with other soil properties that affect the solubility of trace elements,
268 namely pH, carbonate and organic matter content.

269

270 There was a large degree of spatial heterogeneity in the level of contamination between
271 sites, as well as the factors affecting trace element solubility, namely carbonate, organic
272 matter and pH (Appendix 1). The Northern and Central areas were the most
273 contaminated sites; they were closest to the contamination source (tailing dam), and
274 sludge was stored mostly in these sites during the clean-up operation. The degree of

275 heterogeneity, as indicated by the coefficient of variation, increased in proportion to the
276 described index for soil contamination (data not shown).

277

278 Soil acidity was also heterogeneous in the Guadiamar floodplain. The soil pH in
279 Northern and Central regions was lower than in Southern regions, due to the different
280 nature of bedrocks in Guadiamar Valley (slate and schist in the upper reaches and
281 limestone and calcarenite in the lower reaches). In Northern and Central regions
282 strongly acid soils were observed, having pH values below 4.5 and carbonate contents
283 lower than 1% (21 % of the samples).

284

285 *3.2. Trace element concentrations in plants*

286

287 Table 3 shows the trace element concentrations in the leaves of studied species from the
288 spill-affected sites. When compared with values from unaffected sites (for the six
289 common species) there were significant differences for some elements and species (5
290 out of the 48 comparisons). Holm oak leaves accumulated significantly more As ($t =$
291 2.95, $p = 0.005$), Bi ($t = 3.26$, $p = 0.002$), Cu ($t = 2.62$, $p = 0.012$) and Zn ($t = 2.72$, $p =$
292 0.010) than in the unaffected sites (mean values of 0.10, 0.007, 6.14 and 29.9 mg kg⁻¹,
293 respectively, in unaffected sites). Wild olive tree leaves had higher concentrations of Tl
294 ($t = 2.71$, $p = 0.009$) than in unaffected sites (mean of 0.003 mg kg⁻¹). For the rest of
295 elements and species, there was no significantly higher accumulation in the spill-
296 affected sites.

297

298 Despite the higher accumulation of some trace elements in the leaves of some woody
299 plant species, in comparison to uncontaminated sites, the concentrations were within the

300 normal ranges for higher plants (as reported by Chaney, 1989, see Table 3). A notable
301 exception was white poplar, which had foliar Cd up to 3 mg kg⁻¹, and Zn up to 410 mg
302 kg⁻¹, well above normal ranges of 1 and 150 mg kg⁻¹, respectively.

303

304 The accumulation of trace elements was also influenced by the life-stage of the tree. In
305 general, adult trees that survived the spill had higher foliar concentration of many trace
306 elements than young saplings that were afforested after the spill. For example, adult
307 wild olives had higher concentration of Zn (2 x) than saplings, adult poplars had higher
308 Cd (5 x) and Zn (3.4x) concentration than saplings, and adult oaks had higher Bi (2.3x)
309 and Cu (1.8x) levels than saplings (Table 4).

310

311 In the leaves of tree species, sludge-associated trace elements were significantly and
312 positively correlated. A principal components analysis (PCA) of the trace element
313 composition of the leaf tissue revealed that the main trend (the first component
314 accounted for 26% of the total variation) was related to elements contained in the
315 sludge. Arsenic, Pb, and in a lower degree Cu, Sb, Tl (contained within the sludge) had
316 high weightings, as well as Mn, which the PCA of the soil samples did not discriminate.
317 The second component (20% of variance) was defined sharply by Cd and Zn, and in a
318 lower degree by Co (Table 2). The first PCA axis may reflect a soil contamination
319 gradient inducing a parallel gradient of leaf concentrations of trace elements, mostly As
320 and Pb. The three tree species overlap in that accumulation gradient, although olive
321 trees tend to have the lowest values (Figure 2). The second trend of variation seems to
322 have a species-specific physiological nature, clearly associated to the higher
323 accumulation of Cd, Zn, and Co in poplar leaves.

324

3.3. *Plant-soil correlations*

326

327 The correlation coefficients between the concentrations of trace elements in surface
328 soils and in leaves of trees (grouping adults and saplings) showed a low number of
329 significant relationships (Table 5). Only 5 out of 48 possible correlations, corresponding
330 to Holm oak saplings, were significant ($p < 0.013$). In other cases, marginally significant
331 correlations ($0.05 > p > 0.013$) were found. In general, there were higher correlations
332 between the trace element concentrations in plants and in the surface soils, than in
333 deeper soils (data not shown). Tree saplings had higher plant – soil trace element
334 correlations than the adult trees.

335

3.4. *Bioaccumulation coefficients*

337

338 For As, Bi, Pb, Sb and Tl, bioaccumulation coefficients (BC) were very low (< 0.03)
339 and there were no significant differences between the species (Fig. 3). In contrast, Cd,
340 Zn and Cu had the highest BCs, and there were greater differences between species. The
341 BC of Cu was similar for all species, around 0.2. There were highly significant inter-
342 specific differences in the BCs of Zn and Cd, in particular due to *Populus alba*, having
343 values close to 2.0 for Cd, and about 0.9 for Zn (Fig. 3).

344

3.5. *Effect of pH and soil organic matter on the bioaccumulation coefficients*

346

347 There were few significant correlations between pH and BCs. For white poplar, soil pH
348 was negatively correlated with BC for Zn ($r = -0.60$, $p < 0.001$). A marginally significant
349 positive correlation was found for As ($r = 0.35$, $p = 0.032$). A positive correlation

350 between soil pH and the BC for Sb was found for olive trees ($r = 0.43$, $p = 0.012$). For
351 the rest of elements and plant species, correlations pH - BC were not significant. Soil
352 organic matter did not significantly affect the BC of any element in any species.

353

354 **4. Discussion**

355

356 The soils affected by a mine spill in the Guadiamar Valley were contaminated by
357 several trace elements, namely As, Bi, Cd, Cu, Pb, Sb, Tl and Zn. These results are
358 consistent with those reported by Cabrera et al. (1999) just after the mine spill. The
359 contamination was spatially heterogeneous. Several factors may explain this non-
360 uniform contamination. Firstly, it was a function of the irregular sludge deposition
361 (Alastuey et al., 1999; López-Pamo et al., 1999). Secondly, the heterogeneous nature of
362 the alluvial sediments along the Guadiamar River (Gallart et al., 1999) may also affect
363 the distribution of residual soil contamination and may influence the different degree of
364 leaching between different sites. Cabrera et al., (1999) showed that the clay content
365 affected the degree of sludge penetration into the Guadiamar surface soils. Thirdly, the
366 residual contamination is also a function of the irregular cleanup of affected soils. The
367 contamination levels in soils around adult trees surviving the spill were significantly
368 higher than those of soils with newly afforested saplings. This is probably due to the
369 difficulty of removing sludge and topsoil from forested areas (Ayora et al., 2001).

370

371 Strong soil acidification was observed in the most contaminated sites. Soil acidification
372 may have occurred due to the leaching of acids generated by the oxidation of sulphides
373 in the remnant of sludges in the soils. Carbonates would have reduced the effects of

374 such acidification. However, the natural pH of these sites was acidic and the soil's
375 carbonate reserve was low, so there may have been less pH buffering in these sites.

376

377 Despite the relatively high soil trace element concentrations, sometimes well in excess
378 of Dutch Intervention Values, their transfer into the woody plants from the Guadiamar
379 Valley was limited. The concentrations in the aboveground parts of studied species
380 were, on average, within the normal ranges for higher plants. The notable exception was
381 the high Cd and Zn accumulation by poplar leaves in the spill-affected area. As a
382 comparison, Madejón et al. (2004) reported foliar concentrations of Cd and Zn in the
383 leaves of poplars from uncontaminated soils of the Guadiamar Valley (outside the
384 phytomanaged area) of just 0.21 mg Cd kg⁻¹ and < 82 mg Zn kg⁻¹ on a dry matter basis,
385 manifold lower than the values found in this study (3 and 410 mg kg⁻¹, respectively).
386 The average Cd concentration in poplar leaves from the affected areas of the Guadiamar
387 floodplain was greater than the concentration (0.5 mg kg⁻¹) that has been shown to
388 adversely affect livestock (Chaney, 1985).

389

390 It is well known that poplar species accumulate Cd and Zn in their leaves (Di Baccio et
391 al., 2003; Madejón et al., 2004; Robinson et al., 2005). Their high biomass production
392 combined with high Cd and Zn accumulation may make poplar suitable for the
393 phytoextraction of these elements from contaminated soils (Robinson et al., 2000,
394 Giachetti and Sebastian, 2006;). The use of poplar for phytomanagement in this site
395 may increase the risk of Cd and Zn entering the food chain. However, poplar is an
396 integral part of the native riparian ecosystem and has a high landscape value. Moreover,
397 poplar plays an important role in maintaining the stability of riparian zones. Before any
398 recommendation to change the management programme, a risk assessment is required to

399 determine the extent of Cd and Zn transfer to other organisms, as well as they
400 accumulation in the topsoil due to litter fall.

401

402 Madejón et al. (2006a) reported that oak leaves had significantly higher concentrations
403 of some trace elements than olive tree leaves, in spill-affected sites. We found similar
404 results in this study. However, for both species the average trace element concentrations
405 found in this study were lower than in the trees analysed in the first three years
406 following the Aznalcóllar accident. The higher foliar concentrations reported in surveys
407 taken immediately following the soil cleanup may have resulted from increased surface
408 deposition of elements following the generation of dust during earth moving (Madejón
409 et al., 2005).

410

411 With the exception of Holm oak saplings, very few significant soil-plant correlations
412 were found in this study. Plant-soil relationships are always complex, since a suite of
413 edaphic and climatic variables, in addition to the soil's trace element concentration,
414 determines the trace element concentration in plants. Our soil analyses revealed a high
415 variability in pH and organic matter content of the soil. Both these factors may affect
416 the phytoavailability of trace elements (Greger, 1999). The nutrient status of each site is
417 likely to have been different, further degrading leaf-soil correlations (Bargagli, 1998). In
418 addition, plant physiology influences the uptake, transport, and accumulation rates,
419 determining the foliar concentration of a trace element. Despite the correlations between
420 leaf concentrations and soil total concentrations were low, the main trend of leaf
421 chemical composition of the studied tree species reflected the gradient of soil trace
422 element contamination. This indicates that leaf analyses may indicate soil quality with
423 regard to trace element phytoavailability in contaminated sites. Since plants integrate

424 several environmental variables over an extended time, they can provide information
425 that is unobtainable from direct soil analyses (see Madejón et al. 2006b for a full
426 discussion of this topic).

427

428 There were higher plant-soil correlations for afforested saplings than for adult trees. A
429 possible explanation is that the roots of smaller plants occupy a smaller volume than
430 large trees. Therefore, the soil that was sampled is more likely to reflect closely the
431 local concentration that the roots of smaller plants were exposed to. Afforested shrubs
432 and trees have been transplanted into the contaminated and remediated soil, therefore
433 roots are growing and exposed to trace elements since the beginning. The roots of larger
434 trees explore a larger volume of soil, which a single soil analysis may not accurately
435 reflect.

436

437 Another factor may be that smaller saplings and shrubs are more affected by dust
438 deposition than larger trees. Smaller plants, and those with pubescent leaves, are more
439 likely to incorporate soil particles into the foliar tissues that even vigorous washing will
440 not remove (Jones and Case, 1990). The foliar trace element concentrations could
441 increase due to the deposition of soil, which is highly contaminated by trace elements,
442 thus producing a positive correlation between plant and soil concentrations. The highest
443 number of soil-plant correlations was observed for Holm oak saplings, low-growing
444 trees with spiny and tomentose leaves. In this case, we found significant correlations for
445 elements, such as Bi and Pb, that are relatively immobile in plant-soil systems,
446 (Adriano, 2001; Jung et al., 2002). This may indicate that, in these saplings, a higher
447 proportion of trace elements may arise from surface deposition. Foliar trace elements
448 that occur via dust deposition still pose an ecological risk, since herbivores will ingest

449 the trace elements, irrespective of their provenance. In taller trees with glabrous-leaves,
450 the influence of surface deposition may be smaller. Olive tree leaves are glabrous, so
451 they capture less aerial dust and are easier to wash before chemical analysis. White
452 poplar is a fast growing tree, and the saplings can reach several meters in height after
453 just three years, thus avoiding high surface deposition, despite their pubescent leaves.
454 The contribution of surface deposition to the total metal burden of poplars in this study
455 is likely to be low. In the case of Cd accumulation, the foliar concentration exceeded
456 that of the soil. Here the effect of surface deposition would be to decrease the observed
457 foliar Cd concentration, since the high concentration of Cd in the leaf is diluted by the
458 addition of soil that has a lower Cd concentration.

459

460 For Bi, Tl, Pb, Sb, and As, bioaccumulation coefficients were below 0.03. This is
461 consistent with previous works that report that these elements, with the exception of Tl,
462 are immobile in plant – soil systems (Kabata-Pendias and Pendias, 1992; Ross, 1994).
463 Thallium has limited mobility in the spill-affected soils in the study area (Martín et al.,
464 2004; Vidal et al., 1999), especially in dry conditions, while during wet periods plants
465 can uptake higher content of this element (Madejón et al., 2007). The BC of Cu was
466 constant in the considered species, which probably is related to the plants' regulation of
467 the uptake of this essential micronutrient. Several studies have reported a restricted
468 transport of Cu from contaminated soils to aboveground parts in different species (Ait-
469 Ali et al., 2002; Arduini et al., 1996; Kozlov et al., 2000). A single species, white
470 poplar, showed BCs higher than 1 for some elements: Cd (all sites) and Zn (some sites).
471 This indicates a high transfer of these elements from soil to leaves. Due to these high
472 rates of metal transfer, concentrations in poplar leaves can be higher than those in soils.
473 In the contaminated Guadiamar Valley, Cd and Zn accumulation by poplar represents

474 one of the greatest environmental risks regarding the entry of trace elements into the
475 food chain.

476

477 The solubility of cationic trace elements increases at low pH (Greger, 1999; Ross,
478 1994). Soil pH is the main determinant of cationic trace element solubility in the
479 Guadiamar floodplain (Burgos et al., 2006; Clemente et al., 2003). Positive correlations
480 between pH and BCs can occur for the anionic trace elements arsenate and antimonite,
481 which are more mobile at higher pHs (Adriano, 2001). In this work, despite local acidic
482 conditions, the BCs for cationic elements were not significantly correlated with soil pH,
483 with the exception of Zn BC in white poplar. The positive correlations observed for As
484 and Sb were weak. As discussed above, the physiology of these woody species may
485 limit the uptake and transport of trace elements, despite increases in soil bioavailability
486 due to pH conditions. Although low pH may be not greatly affect trace element uptake
487 by woody plants in the Guadiamar Valley, the pH of the sites requires close monitoring,
488 since acidification will result in increased trace element mobility and it may increase the
489 rates of leaching into receiving waters. In the Guadiamar system, soil contamination is
490 associated with sulphides, which will gradually oxidise and lower the soil pH. There is,
491 therefore, a risk of a chemical time bomb where significant amounts of sludge remain in
492 the soil. Maintaining neutral to basic soil pH should be an integral part of any
493 phytomanagement programme that involves cationic trace-element contaminated soils.
494 While there is little risk of plant uptake, the higher downward mobility of these
495 elements may endanger groundwater.

496

497 **5. Conclusions**

498

499 Despite the high concentrations of several trace elements in the soils from the
500 Guadiamar Valley, there was a limited transfer of these elements to the aboveground
501 parts of woody plants. With the exception of white poplar, the ecological risk of the
502 foliar trace accumulation in these plants is low. Future work could focus on the effect of
503 the trees on the downward mobility of trace elements in the Guadiamar basin. This
504 should include not just the effect of transpiration, but also the generation of preferential
505 flow pathways along root-macropores, and the solubilisation of trace elements by
506 organic acids generated from decaying leaf litter. Horizontal migration of diluted
507 elements and solid matter as surface run-off during heavy rainfall events should also be
508 taken into account.

509

510 Also warranted is a better understanding of the effect of the contaminating trace
511 elements on the vegetation dynamics in the Guadiamar Basin. While the accumulation
512 of trace elements is unlikely to pose an ecological threat, the effects of the contaminants
513 may nonetheless have ecological consequences in terms of plant growth or nutrient
514 status of plant tissue. Such information would be helpful in the selection of most
515 suitable species for the phytomanagement of trace element-contaminated areas under
516 semi-arid climate.

517

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519

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526

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694 **Figure legends**

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696 Fig. 1. Situation of the Guadamar River Valley (SW Spain) inside the Iberian Peninsula
697 and locations of the sampling sites.

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699 Fig. 2. A Principal Component Analysis (PCA) of trace element concentrations in the
700 leaves of the studied tree species. Component loadings for each trace element are shown
701 in Table 2.

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703 Fig. 3. Bioaccumulation coefficients (BC), defined as the plant / soil concentration
704 quotients, of trace elements in leaves of trees from the Guadamar Valley.

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Table 1. Mean (min.- max.) total concentration (mg kg⁻¹) of main trace element in soils in the Guadiamar Valley, from spill-affected and unaffected sites. Significance levels in the comparison (by *t*-test, controlling the overall FDR at the 5% level) between affected and unaffected sites are indicated (* *p* < 0.013, ** *p* < 0.001). Normal ranges in soils reported by Bowen (1979) and Dutch Intervention Values (DIV) are also indicated.

	Surface soils (0-25 cm)		Deep soils (25-40 cm)		Normal ranges	DIV
	Affected (<i>N</i> = 100)	Unaffected (<i>N</i> = 17)	Affected (<i>N</i> = 100)	Unaffected (<i>N</i> = 17)		
As	129 (49-339)**	17 (13-20)	95 (18-438)**	16 (14-17)	0.1-40	55
Bi	1.64 (0.57-5.40) **	0.30 (0.15-0.40)	1.35 (0.33-4.29) **	0.25 (0.18-0.35)	0.1-13	-
Cd	1.44 (0.44-3.05)**	0.23 (0.07-0.37)	1.27 (0.22-3.28)**	0.17 (0.10-0.25)	0.01-2	12
Cu	115 (66-198)**	32 (13-43)	110 (30-238)**	24 (14-31)	2-250	190
Pb	210 (73-607)**	47 (15-65)	179 (519-38)**	31 (18-38)	2-300	530
Sb	13.8 (4.5-37.7)**	3.0 (0.7-5.4)	11.1 (2.1-30.0)**	1.8 (0.9-2.8)	0.2-10	15
Tl	1.17 (4.02-0.55)**	0.29 (0.16-0.43)	0.82 (0.20-3.15)**	0.27 (0.23-0.33)	0.01-0.8	15
Zn	457 (768-183)**	109 (47-149)	376 (954-103)**	82 (53-99)	1-900	720

Table 2. Results of the Principal Component Analyses (factor loadings) of trace elements concentrations in soils (0-25 cm), and in leaves of three tree species (Holm oak, olive tree and white poplar), from the Guadamar Valley. The percentage of variance explained by each component is also indicated

	Soils		Plants	
	Comp. 1 (47 %)	Comp. 2 (29 %)	Comp.1 (26%)	Comp. 2 (20%)
As	-0.96	-0.03	-0.81	-0.20
Ba	0.06	-0.85	-0.14	0.21
Be	0.34	-0.79	-0.42	0.28
Bi	-0.95	-0.10	-0.39	0.19
Cd	-0.76	-0.10	-0.35	-0.86
Co	-0.04	-0.85	-0.21	-0.59
Cu	-0.92	-0.15	-0.62	0.12
Mn	0.20	-0.83	-0.55	0.44
Mo	-0.62	-0.04	-0.01	-0.05
Ni	0.20	-0.91	-0.26	0.29
Pb	-0.95	-0.01	-0.82	0.39
Sb	-0.94	-0.14	-0.66	0.42
Tl	-0.93	-0.01	-0.58	-0.32
Zn	-0.78	-0.12	-0.53	-0.77

782 Table 3. Trace element concentration (mg kg⁻¹) in leaves (stems in the case of *Retama sphaerocarpa*) of woody plants from spill-affected sites
783 in the study area. In the case of tree species, both adults and saplings are combined. Normal ranges for trace element in plants and maximum
784 levels tolerated by livestock are indicated (see footnotes for references).

Species	As	Bi	Cd	Cu	Pb	Sb	Tl	Zn
<i>O. europaea</i> N = 31	0.32 ± 0.04	0.025 ± 0.005	0.07 ± 0.01	6.94 ± 0.49	0.89 ± 0.06	0.031 ± 0.023	0.013 ± 0.002	42.2 ± 3.8
<i>P. angustifolia</i> N = 6	0.25 ± 0.07	0.019 ± 0.007	0.13 ± 0.03	5.67 ± 0.11	1.11 ± 0.18	0.046 ± 0.005	0.009 ± 0.004	79.9 ± 10.6
<i>P. lentiscus</i> N = 4	0.27 ± 0.12	0.026 ± 0.008	0.06 ± 0.01	4.48 ± 0.59	1.18 ± 0.39	0.028 ± 0.009	0.018 ± 0.006	14.9 ± 0.4
<i>P. alba</i> N = 40	0.50 ± 0.04	0.014 ± 0.003	3.13 ± 0.45	8.11 ± 0.35	1.21 ± 0.05	0.037 ± 0.005	0.032 ± 0.008	412 ± 43
<i>Q. ilex</i> N = 29	0.56 ± 0.08	0.026 ± 0.003	0.21 ± 0.05	10.2 ± 0.8	2.48 ± 0.26	0.070 ± 0.005	0.021 ± 0.005	80.0 ± 8.9
<i>R. sphaerocarpa</i> N = 6	0.30 ± 0.10	0.014 ± 0.002	0.31 ± 0.20	15.5 ± 3.0	1.40 ± 0.48	0.067 ± 0.016	0.006 ± 0.002	114 ± 36
<i>R. officinalis</i> N = 7	0.79 ± 0.18	0.023 ± 0.005	0.04 ± 0.01	13.2 ± 1.1	2.01 ± 0.52	0.046 ± 0.005	0.021 ± 0.007	51.2 ± 9.5
<i>T. africana</i> N = 6	0.83 ± 0.19	0.022 ± 0.009	0.46 ± 0.21	11.3 ± 1.8	1.58 ± 0.27	0.070 ± 0.051	0.213 ± 0.059	54.7 ± 19.4
Normal levels	0.01-1 ^a	0.06 ^b	0.1-1 ^a	3-20 ^a	2-5 ^a	0.005-0.1 ^c	0.05 ^c	15-150 ^a
Maximun levels for livestock ^a	50		0.5	300	30			1000

^a Chaney, 1989; ^b Bowen, 1979; ^c Adriano, 2001;

Table 4. Trace element concentrations in leaves of adult and sapling trees from affected sites. Significance levels (analysed by *t*-test) are indicated (* $p < 0.013$, ** $p < 0.001$). Values in italics are marginally significant ($0.05 > p < 0.013$), after controlling the overall FDR at the 5% level.

	<i>O. europaea</i>		<i>P. alba</i>		<i>Q. ilex</i>	
	Adult (N = 15)	Sapling (N = 16)	Adult (N = 22)	Sapling (N = 18)	Adult (N = 15)	Sapling (N = 14)
As	<i>0.42 ± 0.07</i>	0.22 ± 0.03	0.63 ± 0.06**	0.34 ± 0.05	0.59 ± 0.07	0.54 ± 0.14
Bi	0.016 ± 0.03	0.034 ± 0.010	0.018 ± 0.005	0.010 ± 0.03	0.036 ± 0.004**	0.016 ± 0.001
Cd	0.06 ± 0.01	0.08 ± 0.02	4.90 ± 0.58**	0.96 ± 0.13	0.14 ± 0.02	0.29 ± 0.09
Cu	6.55 ± 0.55	7.32 ± 0.80	8.16 ± 0.47	8.07 ± 0.54	13.1 ± 0.9**	7.18 ± 0.31
Pb	<i>1.02 ± 0.09</i>	0.77 ± 0.07	1.28 ± 0.06	1.12 ± 0.08	3.18 ± 0.40**	1.72 ± 0.21
Sb	0.037 ± 0.004**	0.025 ± 0.002	0.050 ± 0.008**	0.021 ± 0.004	0.079 ± 0.006**	0.059 ± 0.008
Tl	0.014 ± 0.003	0.013 ± 0.003	0.053 ± 0.012**	0.007 ± 0.002	0.029 ± 0.009	0.013 ± 0.003
Zn	57.0 ± 5.2**	28.2 ± 2.5	605 ± 45**	176 ± 22	83.5 ± 8.4	76.2 ± 16.4

821
822 Table 5. Correlation coefficients (r) between trace elements in soils (total
823 concentrations, 0-25 cm depth) and plants, for tree species, both for affected and
824 unaffected sites. Non significant correlations (ns) are not shown. For the rest of
825 correlations, significance levels are indicated (* $p < 0.013$, ** $p < 0.001$). Values in
826 italics are marginally significant ($0.05 > p < 0.013$), after controlling the overall FDR at
827 the 5% level.

Species		As	Bi	Cd	Cu	Pb	Sb	Tl	Zn
<i>O. europaea</i>	Adult ($N = 15$)	ns	ns	ns	ns	ns	ns	ns	ns
	Sapling ($N = 25$)	<i>0.46</i>	ns	ns	ns	ns	ns	ns	ns
<i>P. alba</i>	Adult ($N = 22$)	ns	ns	<i>0.45</i>	ns	ns	ns	ns	ns
	Sapling ($N = 18$)	ns	ns	ns	ns	ns	<i>0.54</i>	ns	ns
<i>Q. ilex</i>	Adult ($N = 15$)	ns	ns	ns	ns	ns	ns	ns	ns
	Sapling ($N = 21$)	0.79**	0.68**	0.78**	ns	<i>0.53</i>	ns	0.77**	0.78**

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Figure 1 sampling site

[Click here to download Figure: Fig.1.pdf](#)

Figure 2 Plant PCA
[Click here to download Figure: Fig. 2.pdf](#)

Figure 3 Bioaccumulation coefficients
[Click here to download Figure: Fig.3.pdf](#)

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