# 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.

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

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

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## 1. Introduction

Phytomanagement is the use of vegetation and soil amendments to reduce the environmental risk posed by contaminated sites (Bañuelos et al, 1999; Barceló and Poschenrieder, 2003). The primary aim of phytomanagement is to reduce contaminant mobility and the effects of contaminants on humans and ecosystems. A successfully phytomanaged site should have limited leaching and limited plant uptake of contaminants. The soil surface must be stabilised so that wind and water erosion are minimised and there is a reduced risk of direct soil consumption by humans and animals (Robinson et al., 2003). Here we investigate a large-scale phytomanagement programme, implemented after a toxic sludge spill in the Guadiamar River Valley (Southwestern Spain). This programme was one of the largest soil remediation operations in Europe. It included the use of soil amendments and the revegetation of the affected area (about 55 km$^2$) with native woody plants.

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

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

In previous studies, trace element accumulation was analysed in surviving trees shortly after the mine-spill (Madejón et al., 2004, 2006a). High concentrations of Cd and Zn 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 ºC (min. 9 ºC in January, max. 27 ºC 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) in the middle, rice crops and halophytic shrubs in the saltmarshes southward to Doñana National Park, where the river flows into. Fragmented riparian forests are dominated by white poplar (*Populus alba)* and narrow-leafed ash (*Fraxinus angustifolia*), while in terrace open woodlands the Holm oak (*Quercus ilex* subsp. *ballota*) and wild olive tree (*Olea europaea* var. *sylvestris*) are most abundant.

In 1998, the failure of a large mine tailing dam at Aznalcóllar (Seville) released about 4 M $m^3$ of trace element-contaminated sludge into the Guadiamar River (Garralón et al. 1999). The resulting flood inundated 55 $km^2$ of the basin southward towards the Doñana National Park (Grimalt et al., 1999). The affected soils, mostly under agricultural production, were burdened with high concentrations of As, Cd, Cu, Pb, Tl and Zn (Cabrera et al. 1999).

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

The Regional Administration (RA) purchased affected lands, which were farms with some fragmented forests and savannah-like woodlands. The RA implemented the Guadiamar *Green Corridor* programme, with the goal of providing a continuous vegetation belt for wildlife to migrate along the Guadiamar River basin between the Doñana National Park in the South and the Sierra Morena mountains in the North (CMA, 2001). Revegetation on the alluvial terraces started in 1999. Depending on the local habitat conditions, the target tree and shrub species to afforest were those typical of Mediterranean riparian forests, such as *Populus alba*, *Tamarix africana, Fraxinus angustifolia* and *Salix atrocinerea* or those typical of drier upland forests, such as *Quercus ilex* subsp. *ballota, Olea europaea* var. *sylvestris, Phillyrea angustifolia, Pistacia lentiscus, Rosmarinus officinalis* and *Retama sphaerocarpa*.

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

### *2.2. Plant and soil sampling*

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

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

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

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

### *2.3. Sample preparation and chemical analyses*

Soil samples were oven-dried at 40 ºC until a constant weight was obtained, then sieved to < 2 mm. A fraction of each sample was then ground in an agata morter to <1 mm for trace elements analysis. Soil samples were digested using concentrated $HNO_3$ and HCL (aqua regia). The values determined with this extraction are referred to as "total" concentration (Vidal et al., 1999).

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

Leaf samples were washed thoroughly with distilled water, dried at 70 ºC for at least 48 h and ground using a stainless-steel mill. Leaves were digested using concentrated $HNO_3$ (Jones and Case, 1990).

Trace element (As, Ba, Be, Bi, Cd, Co, Cu, Mn, Mo, Ni, Pb, Sb, Tl and Zn) concentrations of both soils and plants samples were determined by ICP-MS (inductively coupled plasma-mass spectroscopy). Quality assurance was obtained for soils by analysing reference material CRM 141R (calcareus loam soil, European Community Bureau of Reference). Recoveries from CRM 141R values ranged from 82 to 94%. The quality of the plant analyses was assessed by analysing two reference materials: NCS DC 73350 (white poplar leaves, China National Analysis Center for Iron and Steel) and BCR 62 (olive tree leaves, European Community Bureau of Reference; Colinet et al., 1982). For all elements except Sb our experimental values were 82 to 109% of the certified values. The lower recovery of Sb (60%) indicates that our results may be conservative estimations of the true values for this element.

### *2.4. Data analyses*

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

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

Significance level was fixed at the 0.05. In order to avoid the increase of type I error derived from multiple testing, we controlled the 'false discovery rate', (FDR) at the 5% level, as suggested by García (2003). We used a powerful 'adaptive' FDR procedure (Hochberg and Benjamini, 2000) to calculate an overall threshold value ($p_t \leq 0.05$), to which individual p- values were compared. After applying the adaptive-FDR procedure to the overall p-value vector (including 420 p-values) we got a significance threshold value ($p_t$) of 0.013. Therefore only p-values not exceeding this threshold value were considered as significant.

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

## 3. Results

### *3.1. Trace elements in soils*

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

Most affected surface soils (86 % of the samples) had As concentrations above the range considered normal (0.1 – 40 mg $kg^{-1}$) for agricultural soils (Bowen, 1979). Similarly, other trace elements were above Bowen's range for normal concentrations in soils. These were (in decreasing order of the percentage of samples that exceeded these values) Tl (53%), Sb (52%), Pb (24%), Cd (17%), Zn (5%) and Cu (4%). Furthermore, 75% of affected soils exceeded the Dutch Intervention Values (DIV, see NIPHE, 2001) for As (55 mg kg$^{-1}$), and lower percentages of samples for other elements: Sb (40%), Zn (13%), Cu (8%) and Pb (4 %).

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

There were significant and positive correlations between all elements that occurred in elevated concentrations in the spill-affected soils (compared to the unaffected soils). This result supports the hypothesis that a single contamination event, the Aznalcóllar mine accident, deposited all these trace elements. The first PCA component explained 47% of the total variance, and completely separated affected from unaffected soils (mean scores of -0.69 and 4.03 respectively). The factor loadings of the eight trace elements associated to the spill had the highest values for this first component (Table 2). Given that the contaminants are mutually correlated, the first component scores for each sample can be use as an index for the total contaminant burden. Detailed information about the level of trace element contamination at each sample site is given in the Appendix 1, along with other soil properties that affect the solubility of trace elements, namely pH, carbonate and organic matter content.

There was a large degree of spatial heterogeneity in the level of contamination between sites, as well as the factors affecting trace element solubility, namely carbonate, organic matter and pH (Appendix 1). The Northern and Central areas were the most contaminated sites; they were closest to the contamination source (tailing dam), and sludge was stored mostly in these sites during the clean-up operation. The degree of heterogeneity, as indicated by the coefficient of variation, increased in proportion to the described index for soil contamination (data not shown).

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

### *3.2. Trace element concentrations in plants*

Table 3 shows the trace element concentrations in the leaves of studied species from the spill-affected sites. When compared with values from unaffected sites (for the six common species) there were significant differences for some elements and species (5 out of the 48 comparisons). Holm oak leaves accumulated significantly more As ($t$ = 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 = 0.010) than in the unaffected sites (mean values of 0.10, 0.007, 6.14 and 29.9 mg kg$^{-1}$, respectively, in unaffected sites). Wild olive tree leaves had higher concentrations of Tl ($t$ = 2.71, p = 0.009) than in unaffected sites (mean of 0.003 mg kg$^{-1}$). For the rest of elements and species, there was no significantly higher accumulation in the spill-affected sites.

Despite the higher accumulation of some trace elements in the leaves of some woody plant species, in comparison to uncontaminated sites, the concentrations were within the normal ranges for higher plants (as reported by Chaney, 1989, see Table 3). A notable exception was white poplar, which had foliar Cd up to 3 mg kg$^{-1}$,and Zn up to 410 mg kg$^{-1}$, well above normal ranges of 1 and 150 mg kg$^{-1}$, respectively.

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

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

### *3.3. Plant-soil correlations*

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

### *3.4. Bioaccumulation coefficients*

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

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

There were few significant correlations between pH and BCs. For white poplar, soil pH was negatively correlated with BC for Zn ($r = -0.60$, $p < 0.001$). A marginally significant positive correlation was found for As ($r = 0.35$, $p = 0.032$). A positive correlation between soil pH and the BC for Sb was found for olive trees (r = 0.43, p = 0.012). For the rest of elements and plant species, correlations pH - BC were not significant. Soil organic matter did not significantly affect the BC of any element in any species.

## 4. Discussion

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

Strong soil acidification was observed in the most contaminated sites. Soil acidification may have occurred due to the leaching of acids generated by the oxidation of sulphides in the remnant of sludges in the soils. Carbonates would have reduced the effects of such acidification. However, the natural pH of these sites was acidic and the soil's carbonate reserve was low, so there may have been less pH buffering in these sites.

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

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

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

With the exception of Holm oak saplings, very few significant soil-plant correlations were found in this study. Plant-soil relationships are always complex, since a suite of edaphic and climatic variables, in addition to the soil's trace element concentration, determines the trace element concentration in plants. Our soil analyses revealed a high variability in pH and organic matter content of the soil. Both these factors may affect the phytoavailability of trace elements (Greger, 1999). The nutrient status of each site is likely to have been different, further degrading leaf-soil correlations (Bargagli, 1998). In addition, plant physiology influences the uptake, transport, and accumulation rates, determining the foliar concentration of a trace element. Despite the correlations between leaf concentrations and soil total concentrations were low, the main trend of leaf chemical composition of the studied tree species reflected the gradient of soil trace element contamination. This indicates that leaf analyses may indicate soil quality with regard to trace element phytoavailability in contaminated sites. Since plants integrate several environmental variables over an extended time, they can provide information that is unobtainable from direct soil analyses (see Madejón et al. 2006b for a full discussion of this topic).

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

Another factor may be that smaller saplings and shrubs are more affected by dust deposition than larger trees. Smaller plants, and those with pubescent leaves, are more likely to incorporate soil particles into the foliar tissues that even vigorous washing will not remove (Jones and Case, 1990). The foliar trace element concentrations could increase due to the deposition of soil, which is highly contaminated by trace elements, thus producing a positive correlation between plant and soil concentrations. The highest number of soil-plant correlations was observed for Holm oak saplings, low-growing trees with spiny and tomentose leaves. In this case, we found significant correlations for elements, such as Bi and Pb, that are relatively immobile in plant-soil systems, (Adriano, 2001; Jung et al., 2002). This may indicate that, in these saplings, a higher proportion of trace elements may arise from surface deposition. Foliar trace elements that occur via dust deposition still pose an ecological risk, since herbivores will ingest the trace elements, irrespective of their provenance. In taller trees with glabrous-leaves, the influence of surface deposition may be smaller. Olive tree leaves are glabrous, so they capture less aerial dust and are easier to wash before chemical analysis. White poplar is a fast growing tree, and the saplings can reach several meters in height after just three years, thus avoiding high surface deposition, despite their pubescent leaves. The contribution of surface deposition to the total metal burden of poplars in this study is likely to be low. In the case of Cd accumulation, the foliar concentration exceeded that of the soil. Here the effect of surface deposition would be to decrease the observed foliar Cd concentration, since the high concentration of Cd in the leaf is diluted by the addition of soil that has a lower Cd concentration.

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

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

## 5. Conclusions

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

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

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## Figure legends

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

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

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

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 (*N* = 100) | Unaffected (*N* = 17) | Affected (*N* = 100) | Unaffected (*N* = 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 Guadiamar 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 |

Table 3. Trace element concentration (mg kg$^{-1}$) in leaves (stems in the case of *Retama sphaerocarpa*) of woody plants from spill-affected sites 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 levels tolerated by livestock are indicated (see footnotes for references).

| Species | As | Bi | Cd | Cu | Pb | Sb | Tl | Zn |
|---|---|---|---|---|---|---|---|---|
| *O. europaea*<br>*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 |
| *P. angustifolia*<br>*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 |
| *P. lenstiscus*<br>*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 |
| *P. alba*<br>*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 |
| *Q. ilex*<br>*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 |
| *R. sphaerocarpa*<br>*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 |
| *R. officinalis*<br>*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 |
| *T. africana*<br>*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.

| | *O. europaea* | | *P. alba* | | *Q. ilex* | |
|---|---|---|---|---|---|---|
| | Adult (*N* = 15) | Sapling (N = 16) | Adult (N = 22) | Sapling (N = 18) | Adult (N = 15) | Sapling (N = 14) |
| As | *0.42 ± 0.07* | 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 | *1.02 ± 0.09* | 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 |

Table 5. Correlation coefficients ($r$) between trace elements in soils (total concentrations, 0-25 cm depth) and plants, for tree species, both for affected and unaffected sites. Non significant correlations (ns) are not shown. For the rest of correlations, significance levels 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.

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

Figure 1 sampling site

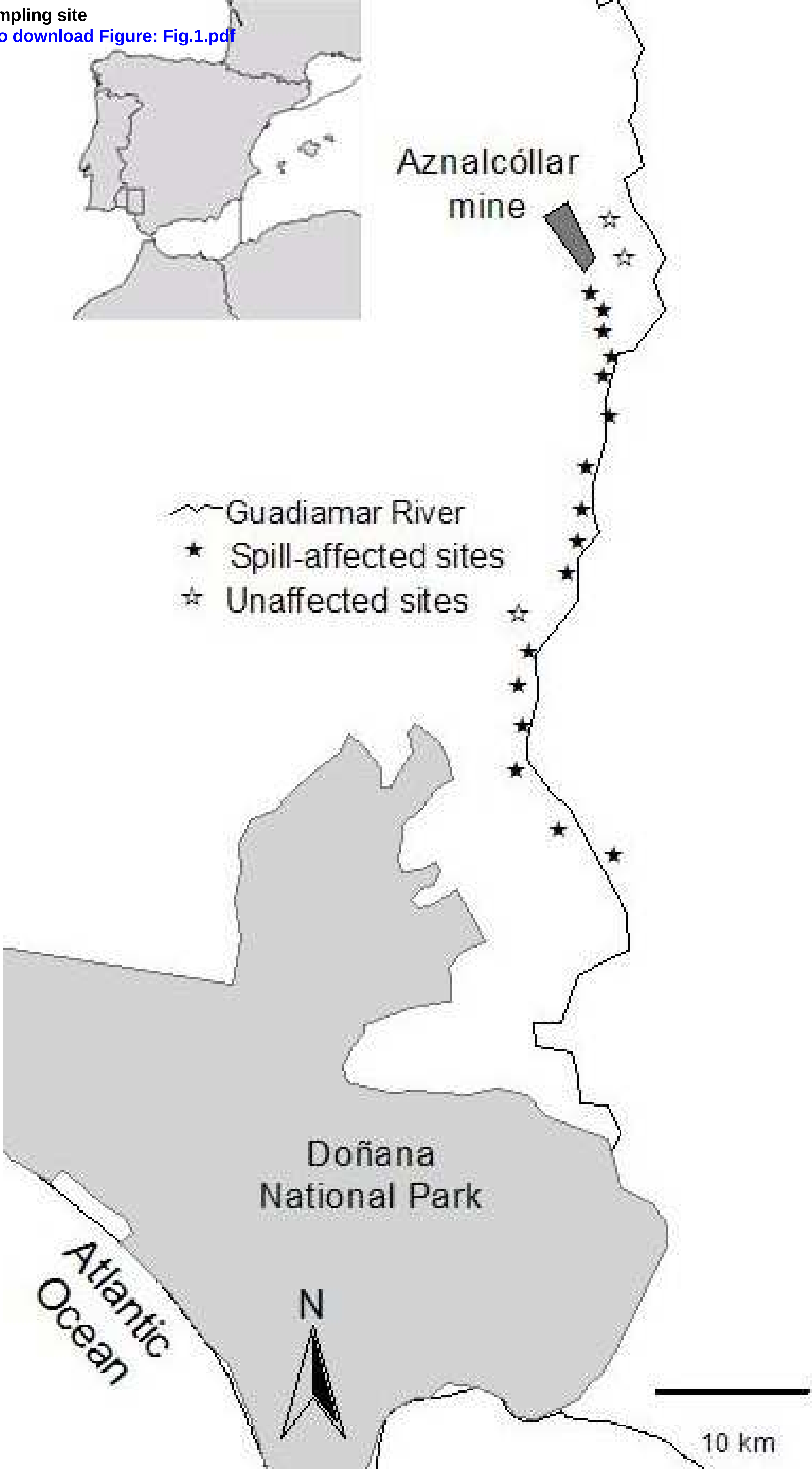

**Figure 2 Plant PCA**

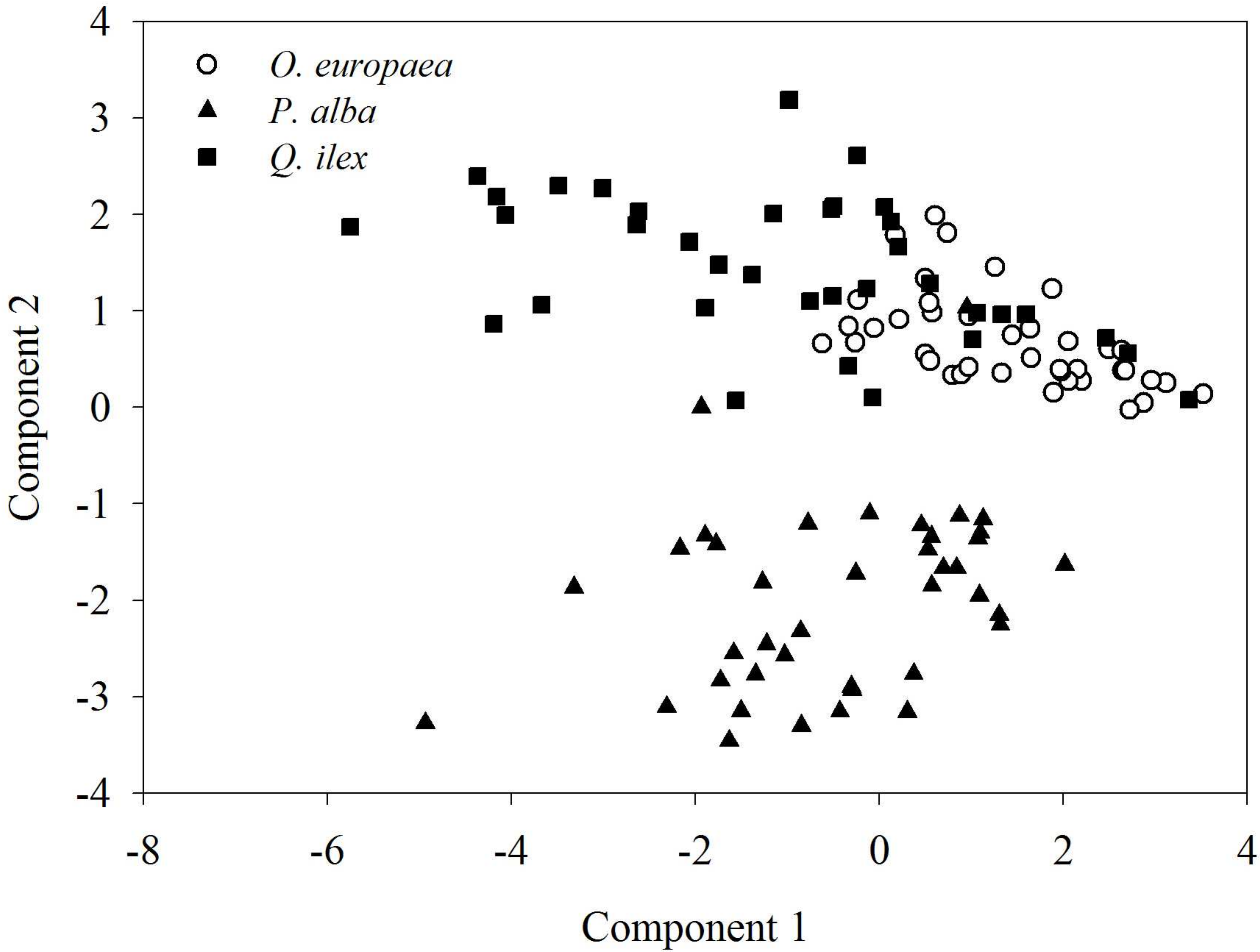

**Figure 3 Bioaccumulation coefficients**

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