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White poplar (*Populus alba*) as a biomonitor of trace elements in contaminated riparian forests

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"Capsule:": *White poplar leaves can be used as biomonitors of soil pollution by Cd and Zn.*

Abstract

Trees can be used to monitor the level of pollution of trace elements in the soil and atmosphere. In this paper, we surveyed the content of eight trace elements (As, Cd, Cu, Fe, Mn, Ni, Pb and Zn) in leaves and stems of white poplar (*Populus alba*) trees. We selected 25 trees in the riparian forest of the Guadiamar River (S. Spain), one year after this area was contaminated by a mine spill, and 10 trees in non-affected sites. The spill-affected soils had significantly higher levels of available cadmium (mean of 1.25 mg kg^{-1}), zinc (117 mg kg^{-1}), lead (63.3 mg kg^{-1}), copper (58.0 mg kg^{-1}) and arsenic (1.70 mg kg^{-1}), than non-affected sites. The concentration of trace element in poplar leaves was positively and significantly correlated with the soil availability for cadmium and zinc, and to a lesser extent for arsenic (log–log relationship). Thus, poplar leaves could be used as biomonitors for soil pollution of Cd and Zn, and moderately for As.

Keywords: Arsenic; Bioindicator; Cadmium; Guadiamar River; Zinc

1. Introduction

Trace elements are defined as those chemical elements with low concentrations in plant tissues (lower than 0.1%), independent of their toxicity or nutritional value (Bargagli, 1998). The responses of plants to a concentration gradient of trace elements in the soil solution can follow three main patterns (Baker, 1981; Baker et al., 2000). (1) "Excluders" have a low uptake of trace elements, by active exclusion in the roots, even at high external concentrations in the soil solution. (2) "Accumulators" are able to tolerate high concentrations of trace elements in their tissues, and this accumulation can be produced even at low external concentrations in the soil solution. (3) "Indicators" have a relatively constant root uptake over a wide gradient of trace elements in the soil. As a consequence they show a linear relationship between the concentrations in the plant tissues and in the soil.

Plants having the indicator or accumulator types of response could potentially be used as "biomonitors", defined as organisms that contain information on the quantitative aspects of the quality of the environment (Markert et al., 2003). There are several advantages of using the concentration of trace elements in plant leaves or stems, to monitor the level of soil pollution where they grow. The accumulation of a trace element in plants confirms its availability in the soil; as distinct from having a high total metal concentration, which can be immobile in the soil complex. Many plants concentrate trace elements in their aerial portions to levels many times that in soil solution (Baker et al., 2000; Ma et al., 2001). Thus, biomonitors can be used to detect low concentrations that are not always easy to measure directly using chemical extraction techniques; even if they are measurable as total levels, their ecological

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relevance is often difficult to determine from soil concentrations. Plants capture and uptake trace elements from a large soil volume (via roots and mycorrhizae) that can be monitored by analysing the leaves.

Iron and trace elements such as Cu, Mn and Zn are essential for plant nutrition, and required for the activity of various types of enzymes. On the other hand, trace elements such as As, Cd and Pb do not have any known physiological function in plants, and can be toxic.

Some trace elements, such as Cd and Zn, are rather mobile in soils and thus readily available for plants, although the uptake mechanisms are not well known. For example, some part of the soil Cd can be taken up passively while other part is taken up actively, coupled to H^{+}-ATPase. Different ion-channels have been suggested as important pathways for the uptake of trace elements (Greger, 1999; Demidchik et al., 2002). On the contrary, other trace elements, such as Cu, As and Pb tend to accumulate in the roots and are scarcely translocated into aboveground organs (Greger, 1999; Siedlecka et al., 2001).

Trace elements can also penetrate into the leaves; the amount would depend upon the metal and plant species. For example, Cd, Zn and Cu can penetrate into the leaf, while Pb is mostly adsorbed to the epicuticular lipids at the surface (Greger, 1999). Extensive information about uptake and translocation of trace elements by plants can be found in Rengel (1997), Prasad and Hagemeyer (1999), and Kabata-Pendias and Pendias (2001).

Trees, as long-lived organisms, reflect the cumulative effects of environmental pollution from the soil and the atmosphere. There are several examples of trees, used as biomonitors for air and soil pollution, e.g. the palm *Phoenix dactylifera* in Turkey (Askoy and Öztürk, 1996) and in Saudi Arabia (Al-Shayeb et al., 1995), the Scotch pine *Pinus sylvestris* in Poland (Dmuchoswski and Bytnerowicz, 1995), the Lombardy poplar *Populus nigra* in Bulgaria (Djingova et al., 1995, 1996, 1999, 2001), a wild tree *Tibouchina pulchra* in Brazil forests (Moraes et al., 2003), as well as several tree species of urban environments in Greece (Sawidis et al., 1995, 2001) and in Hong Kong (Lau and Luk, 2001).

The objectives of this paper were to (1) survey the leaves and twigs of white poplar (*Populus alba*) trees growing on the banks of the contaminated Guadiamar River (south Spain), to investigate the accumulation of eight trace elements, namely As, Cd, Cu, Fe, Mn, Ni, Pb and Zn; (2) study the total (extracted by *aqua regia*) and soluble (extracted by EDTA) concentration of the same eight trace elements in the soil, where the sampled trees were growing, at two depths (0–25 and 25–40 cm); (3) identify those elements having a significant and positive correlation between their concentration in poplar leaves and their bioavailability in the soil; (4) evaluate the degree of biomonitoring sensitivity for those elements; (5) discuss the plant–soil relationships for the eight trace elements.

2. Material and methods

2.1. Tree species

The white poplar (*P. alba* L.) trees, studied in the Guadiamar riparian forests, had a height of 5–19 m with a broad crown, and a trunk diameter of 10–35 cm (Madejón, 2004). In general, the bark is pale greenish white. Deciduous leaves are silvery-white underneath and green on the top. Leaves are 6–12 cm long with ovate shape and have 3–5 coarsely toothed lobes. White poplar has rapid growth, and is naturally found on a wide range of soil types, being relatively tolerant to pollution. They grow on the banks of the rivers, in south and central Europe, western Asia and North Africa; besides, they are widely used as ornamental urban trees (see general botanical description in Tutin et al., 1964; Ruiz de la Torre, 2001).

2.2. Study area

The upper part of the Guadiamar Basin, in south Spain, is located in the pyritic mining belt, which has been exploited for copper and other ores since Roman times (ca. 2000 years ago). In April 1998, a mine spill affected about 55 km^2 of land that was flooded with a volume of ca. 5×10^6 m^3 slurry (for a review, see the special issue in Grimalt et al., 1999). This large-scale pollution event had a major ecological impact because the Guadiamar River discharges into the Guadalquivir marshes of the Doñana National Park, which is a wintering area for many European water birds. To mitigate the mine accident a large-scale restoration plan was launched, including the compulsory purchase of the land (formerly devoted to crops and pastures) and the design of a public nature reserve. This so-called "Green Corridor" will connect the lowlands (Doñana National Park) and the mountains (Sierra Morena Natural Park, CMA, 2001).

An emergency soil clean-up procedure quickly started after the mine spill. The toxic sludge covering the ground and a major portion of the contaminated soil surface were mechanically removed and disposed off in a mine open-pit. In the more accessible areas (e.g., former croplands), soil remediation was carried out adding organic matter and calcium-rich amendments. However, despite these cleaning up and remediation measures, the affected zone still continued to have a consistent pollution of trace metals with a fairly irregular distribution (Moreno et al., 2001). Soil and sediment pollution was particularly high around tree trunks, where cleaning machinery could not work, and especially near the river channel. Therefore, most of the soil around the studied poplar trees were relatively more polluted than the average soil in the Guadiamar River floodplain.

Soils of the Guadiamar floodplain are mostly neutral or slightly alkaline, with the exception of some terraces (in the right bank), which are rich in gravel deposits and have low pH. Soil texture is varied, from loamy sand to silty clay (Table 1). See review of soils in the area in Simón et al. (1999) and Madejón (2004).

The riparian forest growing on the banks of the Guadiamar River is dominated by white poplar (*P. alba*); other tree species are ash (*Fraxinus angustifolius*), elm (*Ulmus minor*) and willows (*Salix* spp.). In many places, riparian natural forests have been replaced by *Eucalyptus* plantations, used in the pulp industry.

2.3. Sampling and analysis

We sampled poplar trees along a river stretch of about 40 km. We started in *Gerena* (**GE**) (37° 31′ N, 6° 11′ W) upstream of the tailing dam, and ended in *Vado del Quema* (**QU**) (37° 14′ N, 6° 15′ W), about 31 km downstream the spill source, and close to the Guadalquivir marshes. The remaining sampling sites were *Soberbina* (**SO**), *Doblas* (**DO**), *Lagares* (**LA**) and *Aznalcázar* (**AZ**) at approximately 4.5 km, 12 km, 15 km and 25 km from the dam, respectively (Fig. 1). Thirty poplar trees were selected, and permanently marked with metal tags. The five trees in **GE** site were not affected by the spill, while the other 25 trees were in sites flooded by the spill. In October 1999 (18 months after the flooding) we collected samples of leaves and twigs (stems) at about 5 m height, from the outer canopy. In general, leaves of the outer canopy tend to accumulate more mineral elements than those of the inner canopy, due to their higher transpiration rates (Bargagli, 1998). Five additional trees were located in another river, *Rivera de Huelva* (**RH**) (37° 29′ N, 6° 01′ W), as an external control (outside the Guadiamar Basin), and were sampled in October 2000.

Soil samples were taken from the root-zone of each tree, about 2 m from the trunk, and at two depths (0–25 and 25–40 cm), by using a spiral auger of 2.5 cm diameter. Three subsamples around the trunk were taken to make a composite soil sample per tree.

Plant material was partitioned for analysis into leaves and stems. Stems were between 0.5 and 3 cm in diameter. Samples were washed for at least 15 s approximately with a solution of 0.1 g L^{-1} phosphate-free detergent, then with a 0.1 N HCl solution, and finally with distilled water. Next, they were dried at 70 °C for at least 48 h, and ground by using a stainless-steel mill.

Eight trace elements (As, Cd, Cu, Fe, Mn, Ni, Pb and Zn) were determined in plant tissues by wet oxidation with concentrated HNO_3 under pressure in a microwave digester (see method in Jones and Case, 1990). Analyses of the extracts were performed by ICP-OES (inductively coupled plasma spectrophotometry) for Fe and Mn, and by ICP-MS (inductively coupled plasma-mass spectroscopy) for As, Cd, Cu, Ni, Pb and Zn.

The accuracy of the analytical method was assessed by carrying out analyses of two BCR (Community Bureau of Reference) reference samples: CRM 279 (sea lettuce) and CRM 281 (ryegrass) (Griepink and Muntau, 1987, 1988). The values obtained for this reference material, by ICP-MS, were concordant with the certified values (Table 2).

Table 1
Texture, acidity and fertility of soils in Guadiamar riparian forests (mean values ± standard error, $N = 5$) (Madejón, 2004)

Site	Longitude/latitude	Depth (cm)	pH	$CaCO_3$ (%)	N (%)	P (mg kg^{-1})	Texture
Non-affected sites							
RH	6° 01′ 34.0″ W	0–25	8.0 ± 0.50	2 ± 0.7	0.10 ± 0.01	12.5 ± 1.8	Clay
	37° 29′ 4.8″ N	25–40	7.9 ± 0.54	1.2 ± 0.5	0.06 ± 0.01	8.7 ± 1.6	Clay
GE	6° 11′ 19″ W	0–25	7.7 ± 0.10	2 ± 1	0.08 ± 0.02	12 ± 2	Loamy sand
	37° 31′ 39″ N	25–40	7.7 ± 0.09	2 ± 1	0.06 ± 0.01	7.3 ± 2.0	Loamy sand
Spill-affected sites							
SO	6° 13′ 1″ W	0–25	5.4 ± 0.30	<0.5	0.14 ± 0.02	5.8 ± 0.6	Sandy loam
	37° 27′ 9″ N	25–40	5.0 ± 0.20	<0.5	0.08 ± 0.01	4.7 ± 1.1	Sandy loam
DO	6° 13′ 35″ W	0–25	7.3 ± 0.20	19 ± 4	0.16 ± 0.02	13 ± 2	Clay loam
	37° 23′ 45″ N	25–40	7.4 ± 0.10	18 ± 2	0.13 ± 0.03	10 ± 1	Clay loam
LA	6° 13′ 39″ W	0–25	7.5 ± 0.07	12 ± 2	0.13 ± 0.01	13 ± 2	Loam
	37° 22′ 25″ N	25–40	7.5 ± 0.02	10 ± 3	0.11 ± 0.01	10 ± 3	Loam
AZ	6° 15′ 38″ W	0–25	7.1 ± 0.20	8 ± 1	0.17 ± 0.03	20 ± 11	Loam
	37° 18′ 12″ N	25–40	7.3 ± 0.15	7 ± 2	0.14 ± 0.01	11 ± 4	Loam
QU	6° 15′ 51″ W	0–25	7.2 ± 0.16	9 ± 0.5	0.09 ± 0.02	20 ± 11	Sandy loam
	37° 14′ 46″ N	25–40	7.4 ± 0.09	12 ± 1	0.09 ± 0.01	15 ± 2	Loam

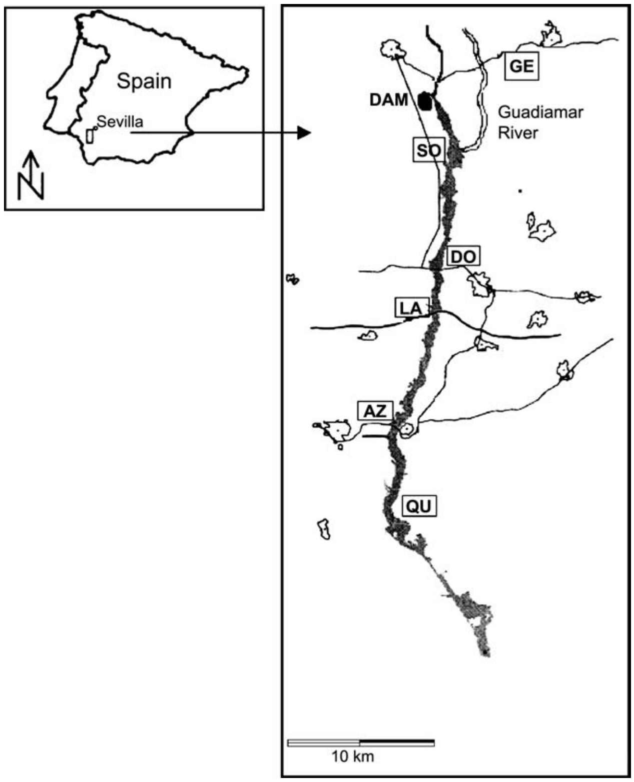

Fig. 1. Map of the spill-affected zone (in grey), with location of the tailing dam (in a tributary of the main river) and the sampling sites along the Guadiamar River (modified from Simón et al., 2001). **GE** site (control) was located upstream of the pollution source, and Rivera de Huelva site (external control, not shown in this map) was in another river basin.

Soil samples were oven-dried at 40 °C, for at least 48 h, and crushed to pass a 2 mm sieve, and then ground to <60 μm for trace element analysis. Total content of trace elements was determined (in the soil fraction <60 μm) by ICP-OES, after digesting the samples with a mixture of concentrated HNO_3 and HCl (*aqua regia*). Bioavailability of the same trace elements was estimated (in the soil fraction <2 mm) by extraction with a 0.05 M EDTA solution (Ure et al., 1993), and analysis by ICP-OES. Concentration of trace elements in the soil solution is expressed on a dry weight basis.

Table 2
Analysis of BCR reference samples (mean values ± 95% confidence interval, mg kg^{-1} dry matter) (values in parentheses are indicative; Fe value for ryegrass (CRM 281) was not provided by the BCR; experimental values were calculated from $N = 6$ (sea lettuce) and $N = 5$ (ryegrass))

Element	CRM 279 (sea lettuce)		CRM 281 (ryegrass)	
	Certified	Experimental	Certified	Experimental
As	3.09 ± 0.20	2.69 ± 0.11	0.057 ± 0.004	0.080 ± 0.07
Cd	0.274 ± 0.022	0.202 ± 0.007	0.120 ± 0.003	0.104 ± 0.02
Cu	13.14 ± 0.37	11.63 ± 0.73	9.65 ± 0.38	9.66 ± 2.24
Fe	(2300 ± 100)	2113 ± 72.3	—	150 ± 27.6
Mn	(2030 ± 31.5)	1758 ± 64.8	81.6 ± 2.6	79.2 ± 5.7
Ni	(15.9 ± 0.4)	13.1 ± 0.53	3.0 ± 0.17	2.58 ± 0.31
Pb	13.48 ± 0.36	12.47 ± 1.09	2.38 ± 0.11	2.31 ± 0.68
Zn	51.3 ± 1.2	52.18 ± 3.29	31.5 ± 1.4	34.0 ± 8.04

2.4. Statistical analyses

The concentration of the eight elements in poplar trees and in soil was compared between spill-affected sites ($n = 25$) and non-affected ones ($n = 10$), by means of one way ANOVA. When significant differences relative to control sites (both upstream and external references) were found, the inference that the mine spill induced a pollution of the soil and/or the tree for that particular element was suggested. In addition, the concentration values were compared with the normal range for soils (according to Bowen, 1979), to evaluate the pollution level. Data normality and homocedasticity were tested prior to analysis; and when necessary, variables were transformed logarithmically.

To investigate the global pollution trends, principal component analyses (PCA) were performed, separately, for the concentration of eight trace elements in 35 poplar trees (leaf and stem) and in the 35 corresponding soil samples (for both, total and EDTA-extracted values). Previously, variables were transformed by square root to obtain a normal distribution (McCune and Mefford, 1999). The variation trend in soil pollution (according to PCA_{SOIL} axis 1) was then compared with the trend in chemical composition of poplar leaves (PCA_{PLANT} axis 1).

The poplar biomonitoring capacity was evaluated by means of correlation (Pearson r) and linear regression analyses, between the concentration of trace elements in white poplar tissues (leaves and stems) and their bioavailability (after EDTA extraction) in the soil solution.

The programs PC-ORD (McCune and Mefford, 1999) and STATISTICA (StatSoft, 1997) were used for the analyses mentioned above.

3. Results and discussion

3.1. Trace elements in soils

3.1.1. Total concentration

The concentration of trace elements obtained by extraction with *aqua regia* provided an estimate of their total (also called *quasi*-total) content in soil (Vidal et al., 1999). Total concentration values for As, Cd, Cu, Pb and Zn were significantly greater in the spill-affected sites than in the control sites, for both soil depths (Table 3). However, only As, Cd and Pb reached values above the background range. The differences between the concentration in spill-affected sites (surface soil) versus controls amounted 15 times greater for Pb, 12 times for As, and 3 times for Cd. Maximum values of these trace elements

Table 3
Total and available (EDTA-extracted) concentration (mg kg^{-1}) of iron and trace elements in soils (at two depth layers) of 25 spill-affected sites and 10 control sites (mean ± S.E.) in the riparian forest of the Guadiamar River (background (total) values according to Bowen (1979) are indicated; significance levels in the comparison (by ANOVA) between spill-affected and non-affected sites are indicated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$)

Element	Surface soil (0–25 cm)		Deep soil (25–40 cm)		Background (range)
	Spill-affected (N = 25)	Non-affected (N = 10)	Spill-affected (N = 25)	Non-affected (N = 10)	
Total					
As	111 ± 19**	9 ± 0.6	49 ± 4***	10 ± 0.6	0.1–40
Cd	4.29 ± 0.53***	1.56 ± 0.15	3.64 ± 0.41***	1.57 ± 0.16	0.01–2
Cu	179 ± 21***	19 ± 1.7	188 ± 25***	18 ± 1.9	2–250
Fe	35,508 ± 2772*	25,960 ± 2315	32,872 ± 2095	26,320 ± 2405	2000–550,000
Mn	468 ± 31	500 ± 24	531 ± 40	498 ± 27	20–10,000
Ni	17.3 ± 0.9	18 ± 1.7	19 ± 0.9*	15 ± 2.4	2–750
Pb	305 ± 53**	21 ± 2.2	140 ± 20***	16 ± 1.7	2–30
Zn	583 ± 66***	58 ± 5	488 ± 75***	13 ± 4.0	1–900
Available					
As	1.70 ± 0.25***	0.27 ± 0.10	0.84 ± 0.05***	0.22 ± 0.08	
Cd	1.25 ± 0.19***	0.03 ± 0.01	1.17 ± 0.24**	0.02 ± 0.01	
Cu	58.0 ± 6.9***	5.66 ± 1.01	62.7 ± 8.5***	4.5 ± 0.8	
Fe	184 ± 33	113 ± 22	121 ± 16	103 ± 21	
Mn	51 ± 7*	78 ± 10	37.3 ± 6.1***	95.6 ± 15.5	
Ni	0.64 ± 0.08	0.70 ± 0.09	0.50 ± 0.06*	0.76 ± 0.10	
Pb	63.3 ± 14.7***	5.45 ± 0.89	38.2 ± 5.9**	4.3 ± 0.6	
Zn	117 ± 15***	4.56 ± 0.97	84.6 ± 14.0***	2.7 ± 0.4	

in polluted soil reached up to 1110 mg kg^{-1} for Pb, 440 mg kg^{-1} for As (both at **AZ** site), and 13.9 mg kg^{-1} for Cd (at **DO** site).

The multivariate analysis (PCA) of the soil samples reflects the differential content and distribution of the trace elements. First PCA axis extracted 65.7% of variance for total values of trace elements in surface soil, Cu, Cd, As, Zn and Pb being the elements with higher (negative) contribution to that axis (figure not shown). The second PCA axis had lower significance (22.2% of variance) and was determined by the (negative) contribution of Ni and Mn.

3.1.2. Available concentration

For a given trace element, only a fraction of its total content in the soil is available for the plants. The pool of available elements will be composed of those ions present in the soil solution, as well as those that are readily solubilised and are therefore able to move into the plant roots (Bargagli, 1998). Mild soil extractants, such as EDTA, are currently used to estimate this available fraction (although this method can overestimate the availability of elements such as Cu and Pb; Vidal et al., 1999).

The spill-affected soils had a greater availability of As, Cd, Cu, Pb and Zn compared with controls. The rank of spill polluting elements were Cd (42 times greater than control), Zn (× 26), Pb (× 12), Cu (× 10) and As (× 6). In contrast, the availability of Mn and Ni was lower in spill-affected soil, whereas Fe did not show a significant difference (Table 3).

There was a high variation among sites in the availability of trace elements. For example, in the topsoil of spill-affected sites, Pb ranged from 7.7 to 279 mg kg^{-1} (with coefficient of variation of 116%); As from 0.6 to 5.1 mg kg^{-1} (CV of 75%); Cd from 0.1 to 3.7 mg kg^{-1} (CV of 74%), and Cu from 6.1 to 140 mg kg^{-1} (CV of 60%). The observed high variation in As and metal concentrations might reflect the heterogeneous spatial pattern of soil pollution. In some sites, for example close to tree trunks, the accessibility to clean-up machinery was limited and some sludge could not be removed (Ayora et al., 2001).

The first PCA axis for the surface (0–25 cm) soil samples explained 55.9% of the variance, and was determined by the differential availability of four elements (having higher negative coefficients): Cu, Zn, Pb and Cd. The distribution of soil samples along this axis seems to reflect a main gradient of spill-induced pollution (Fig. 2). These four chalcophilic elements were abundant in the sludge and their relative abundance values in the polluted soils were inter-correlated.

The second axis reflected a secondary, less explicative gradient (28.6%), and was associated with the availability of three siderophilic elements: Mn, Ni and Fe. This secondary chemical gradient was probably related to the geological nature of the parent material, and not to the spill pollution.

The PCA results for the deeper soil layer (25–40 cm) were similar (figure not shown). The first axis explained 53.4% of variance, and was associated with the variation in Cd, Zn, Cu and Pb, whereas the second axis explained 27.6% of variance and was determined by the availability of Mn and Ni.

The soil pollution gradient in the Guadiamar riparian forest was reflected by the PCA analyses of both total

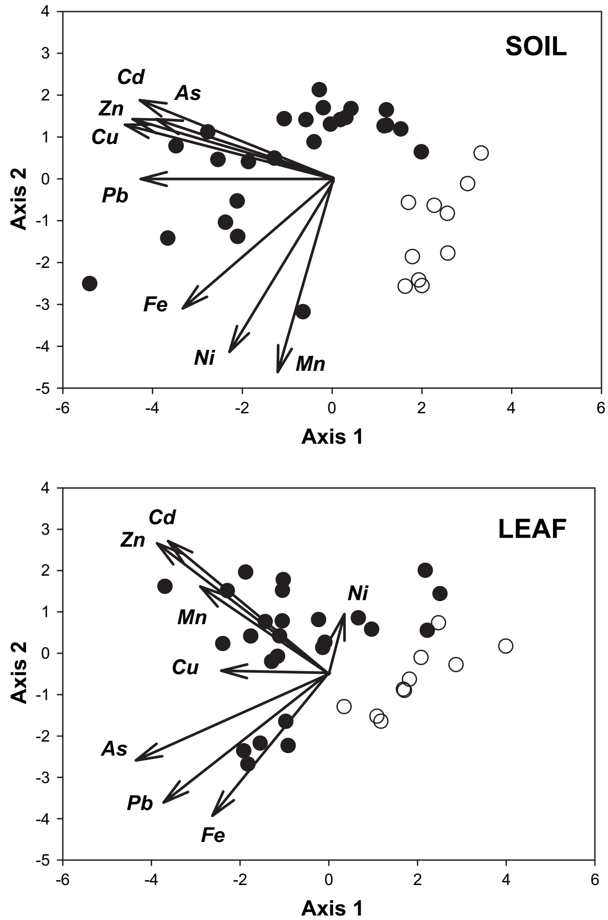

Fig. 2. Ordination of soil (above) and poplar leaf (below) samples by the two first axes of principal component (PCA) analysis, according to the concentration of eight trace elements. Black symbols are spill-affected samples and white symbols are non-affected samples. Vectors represent the eigenvector coefficients (multiplied by five, for clarity) of the trace elements.

and EDTA-extracted values, and the patterns were similar. For example, the scores of (surface) soil samples along the PCA first axis for EDTA-extracted values were highly correlated ($r = 0.90$ and $p < 0.001$) with their scores in the PCA for total values. In this paper, available (EDTA-extracted) values of trace elements in soil will be used to compare with their bioaccumulation in plants.

3.2. Trace elements in poplar trees

The spill-polluted poplar trees had a significantly higher concentration of As, Cd and Zn, in both leaves and stems, compared to control areas (Table 4).

The accumulation of As was greater (for 15 out of the 25 trees) than the normal range reported for leaf tissues (according to Bowen, 1979). However, As values were always below the lower limit of phytotoxicity (5 mg kg^{-1}; Kabata-Pendias and Pendias, 2001). Arsenic has a comparatively low soil–plant transfer coefficient (Kloke et al., 1984), and the accumulation measured here in poplar trees was rather moderate, compared for example with the maximum value of 20.8 mg kg^{-1} documented for *P. nigra* (Djingova et al., 1995).

The concentration of Cd in polluted poplar tissues showed the highest increase, compared to control trees; 18 times for leaves and 10 times for stems (Table 4). Cadmium values in polluted poplar trees exceeded the normal values in plants. The highest Cd value recorded (15.4 mg kg^{-1} at **DO** site) was into the phytotoxic range for plants (5–700 mg kg^{-1}; reported by Chaney, 1989); although they did not reach the extreme values (200 mg kg^{-1}) reported for poplar in soils severely polluted by Cd (Robinson et al., 2000). In general, Cd is effectively absorbed by plant roots and accumulated in aboveground organs; having thus a comparatively high transfer soil–plant coefficient (Kloke et al., 1984).

Mean concentration of Zn in polluted poplars was 7 times in leaves and 2 times in stems, higher than in control trees (Table 4). Zinc in leaves was above normal values. The extreme recorded value (1312 mg kg^{-1} at **DO** site) was into the phytotoxic range (500–1500 mg kg^{-1}; Chaney, 1989). Zinc has a relatively high transfer coefficient from soil to plant, and most plants can tolerate high Zn levels (see Kloke et al., 1984). Other studies have found high Zn concentrations in the leaves of *Populus* species; for example 300 mg kg^{-1} in Bulgaria (Djingova et al., 1995), and up to 2000 mg kg^{-1} under extreme conditions (Ernst, 1998).

The accumulation of Cu in stems of spill-affected poplars was higher than in controls, whereas the difference was not significant in leaves (Table 4). In any case, the measured Cu values were always within the normal range, and below the phytotoxic range for plants (25–40 mg kg^{-1}; Chaney, 1989).

Fe also showed a differential accumulation in stems, but not in leaves, of polluted poplars, compared to controls (Table 4). The maximum Fe concentration recorded for leaves (814 mg kg^{-1} at **LA** site) was above the normal range. However, a possible external contamination by dust (and despite the careful washing of the plant material) probably contributed to these high values.

Apparently, there were no significant differences between spill-affected and (overall) control trees for Pb (Table 4). However, leaves from the external control (**RH** site) had significantly lower Pb concentration (mean of 0.38 mg kg^{-1}) than in the spill-affected sites (mean of 5.00 mg kg^{-1}). On the other hand, leaves from the upstream control (**GE** site) had a relatively high Pb concentration (mean of 6.27 mg kg^{-1}), similar to the spill-affected sites, indicating another source of Pb pollution and obscuring the spill effects. In any case, the highly significant increase of Pb (both total and available) in polluted soils (Table 3) is not proportionately

Table 4
Concentration of eight trace elements (mg kg^{-1} dry matter) in leaves and stems of white poplar trees from spill-affected and non-affected sites in the riparian forest of the Guadiamar River (when polluted trees were significantly different (by ANOVA test) from control trees, the significance level was indicated: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; normal values (range) for trace elements in plants, according to Kabata-Pendias and Pendias (2001), and for Fe according to Bowen (1979), are also indicated)

Element	Poplar leaf		Poplar stem		Normal
	Spill-affected ($N = 25$)	Non-affected ($N = 10$)	Spill-affected ($N = 25$)	Non-affected ($N = 10$)	
As	2.70 ± 0.24***	0.99 ± 0.23	0.77 ± 0.07***	0.25 ± 0.07	1.0–1.7
Cd	3.82 ± 0.64**	0.21 ± 0.03	3.18 ± 0.30***	0.31 ± 0.04	0.05–0.2
Cu	7.63 ± 0.17	7.44 ± 0.26	6.62 ± 0.33***	3.96 ± 0.26	5–30
Fe	336.6 ± 30.7	251.0 ± 28.0	88.0 ± 6.9***	44.2 ± 8.0	2–250
Mn	93.6 ± 15.8	51.0 ± 7.3	16.7 ± 2.6	12.5 ± 2.2	30–300
Ni	1.33 ± 0.09	1.05 ± 0.11	0.72 ± 0.05	0.78 ± 0.10	0.1–5
Pb	5.00 ± 0.44	3.93 ± 0.84	2.06 ± 0.30	1.54 ± 0.55	5–10
Zn	542.1 ± 58.0***	81.6 ± 14.8	139.0 ± 6.1***	59.2 ± 4.4	27–150

reflected in the leaves (Table 4). In general, Pb is rather immobile in soil and easily forms organic complexes with fulvic acids (Greger, 1999). In addition, it concentrates primarily in the roots and is poorly translocated to the vegetative parts, resulting in a comparatively low transfer coefficient from soil to plant (Kloke et al., 1984).

Comparing the plant organs, leaves accumulated a higher concentration of trace elements than stems (Table 4).

The pollution trend in poplar leaves, defined by the first PCA axis with 41% of the variance, was associated with the increasing concentration of As, Zn, Pb and Cd (with that rank of negative coefficients). On the other hand, Fe and Pb (with negative coefficients) as opposed to Cd and Zn (with positive coefficients) defined the second axis (21% of variance) (Fig. 2).

Thus, the distribution of the poplar samples, according to their values for the PCA axis 1, reflects a main pollution gradient: five poplar trees from the external control site and five trees from the upstream sites are grouped to the right of the graph; mixed with three affected trees (from the least polluted, **QU** site). In contrast, all the trees in spill-affected soils are towards the left of the graph; having the extreme scores of those leaves taken from the highest polluted **DO** site (Fig. 2).

The trend of variation in the concentration of the eight trace elements in poplar leaves, as shown by PCA analysis, had similarities and differences with that in the correspondent soils (Fig. 2). The sample scores in the PCA_{SOIL} axis 1 were positive and significantly correlated with those in the PCA_{PLANT} axis 1 ($r = 0.65$, $p < 0.001$). Therefore, the main patterns of soil bioavailability of trace elements, in particular of Zn and Cd, explained to a great extent, the poplar tissue composition.

Four of the elements (Zn, Cd, Pb and As) contributing the most to the soil pollution trend (with highest negative coefficients for PCA_{SOIL} axis 1) also contributed to the leaf chemical trend (with highest negative coefficients for PCA_{PLANT} axis 1). However, there was a divergence with regard to the secondary gradient. Thus, Zn and Cd had a common behaviour in both soil and plant analyses, but As and Pb differed between soil (associated to Zn and Cd) and plant (opposed to Zn and Cd) chemical patterns (Fig. 2). Cu was the element contributing most to the soil pollution gradient (PCA_{SOIL} axis 1) but had low significance to the leaf chemical gradient (PCA_{PLANT} axis 1) (Fig. 2).

The pollution trends in poplar stems (small branches and twigs), as detected by the PCA analysis, were similar to those in the leaves (figure not shown). The first axis explained 51% of variance, and was associated mainly with As, Fe, Zn, Cd and Pb. The second axis explained 20% of variance and was determined by the opposing accumulation of Cu (positive coefficient) *versus* Ni (negative coefficient).

Different patterns in the concentrations of trace elements between plants and soils have also been found in other studies (e.g., Markert, 1987). Interactions between elements may arise from antagonistic and synergistic processes, which occasionally can involve the metabolism of more than two elements. These interactions may affect the uptake and translocation of a particular element, independent of its availability in the soil. Moreover, factors besides soil pollution, such as atmospheric deposition onto leaf surfaces, seasonal physiology, the tissues under study, and the species-specific capacities for uptake, translocation and compartmentalisation of trace elements, may contribute to the observed differential bio-accumulation (Bargagli, 1998).

3.3. White poplar as a biomonitor of trace elements

The correlation coefficients between the concentration of eight trace elements in poplar tissues (leaves and stems) and their availability (EDTA extractable) in the soil solution of the tree rooting volume, are shown in Table 5. In total, 16 out of the 32 correlations were significant. Poplar leaves were correlated with surface soil for four elements (Cd, Zn, Mn and Cu) and with

Table 5
Correlation coefficients between the availability (EDTA-extracted) of eight trace elements in the soil (at 0–25 cm and 25–40 cm depth) and their concentration in the leaves and stems of white poplar (significance levels are *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$)

Element	Leaf		Stem	
	Surface soil (0–25 cm)	Deep soil (25–40 cm)	Surface soil (0–25 cm)	Deep soil (25–40 cm)
As	0.33	0.47**	0.44**	0.57***
Cd	0.63***	0.84***	0.70***	0.78***
Cu	0.34*	0.38*	0.28	0.29
Fe	0.03	0.10	0.35*	0.09
Mn	0.42*	−0.08	0.41*	0.11
Ni	−0.21	−0.19	0.19	0.01
Pb	0.19	0.20	0.09	0.23
Zn	0.51**	0.68***	0.58***	0.56***

deep soil also for four elements (Cd, Zn, As and Cu). Similarly, poplar stems were correlated with surface soil for five elements (Cd, Zn, As, Mn and Fe) and with deep soil for three elements (Cd, As and Zn). In general, leaf concentration of trace elements was better correlated (higher correlation coefficients) with the pollution level in the deep soil, than with surface soil (with the exception of Mn).

The best correlation was obtained for Cd, between its accumulation in leaves and its availability in deep soil ($r = 0.84$, $p < 0.001$, Fig. 3). The Cd accumulation in leaves was also significantly correlated with its availability in surface soil; and the accumulation in stems with the availability in both soil layers (Table 5).

Zn concentrations in poplar leaves and stems were significantly correlated with Zn availability in surface and deep soil. The best correlation was between leaves and deep soil ($r = 0.68$, $p < 0.001$, Fig. 3).

As concentration in poplar leaves was correlated to its availability in deep soil, but only marginally ($p = 0.052$) with surface soil (Table 5). The concentration of As in stems was correlated to the availability in both surface and deep soil. When log values were plotted, the correlation leaf–soil became significant ($r = 0.65$, $p < 0.001$ for deep soil, Fig. 3), and improved in the case of stem–soil ($r = 0.69$, $p < 0.001$ for deep soil). These results indicated that the relationship between plant and soil for As is of log–log type, rather than linear.

Mn, Cu and Fe correlation coefficients between plant and soil were significant (with low values) only in 5 out of 12 possible pairs (Table 5). Mn concentrations in poplar leaves and stems were correlated with Mn availability in surface soil, but not in deep soil. Cu concentration in leaves, but not in stems, was correlated with Cu availability in surface and deep soil. The only significant correlation for Fe was between its concentration in stems and the availability in surface soil.

Pb did not show any significant correlation between its concentration in poplar tissues (either leaves or stems) and their availability in the rooting soil. However, after logarithmic transformation, the leaf–soil correlation became significant ($r = 0.36$, $p = 0.031$), as did the stem–soil correlation ($r = 0.44$, $p = 0.008$), for surface soil in both cases.

Nickel was the element with lowest correlation coefficients between plant and soil, even showing negative values (Table 5).

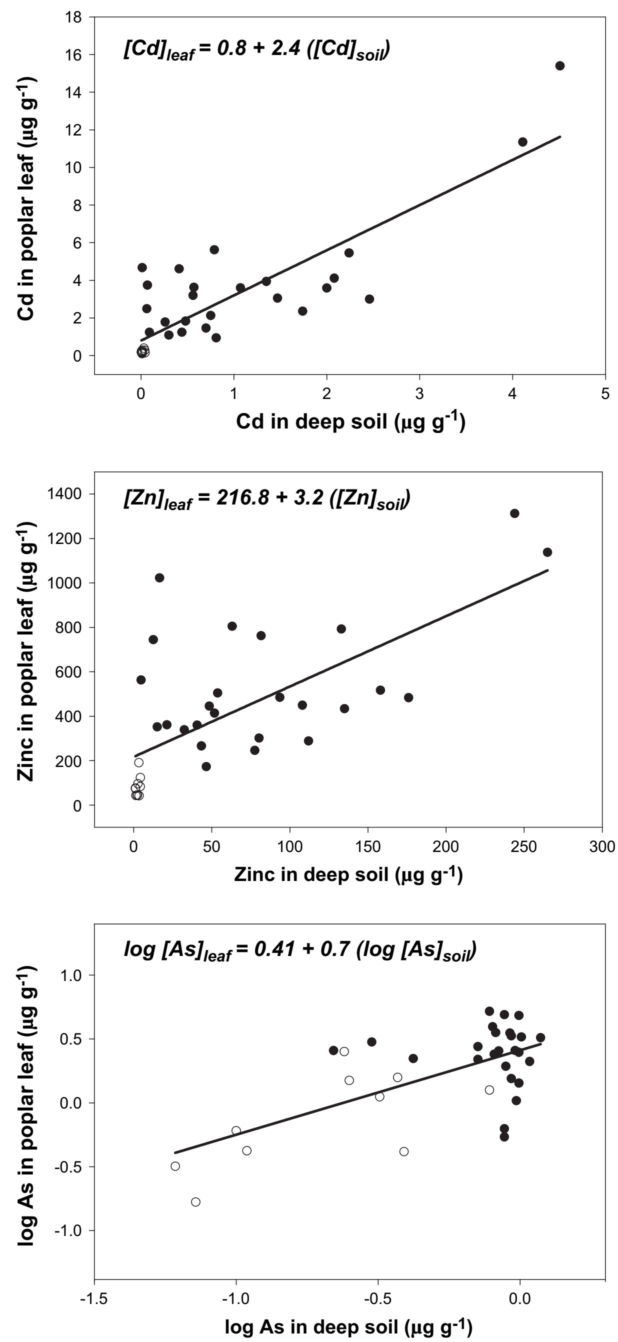

Fig. 3. Correlation between the availability (after EDTA extraction) of Cd (top graph), Zn (middle) and As (bottom) in soil, and their concentration in poplar leaves. In the case of As, the scale for both axes was logarithmic.

Leaves of trees have been widely used for biomonitoring of trace element pollution. In particular, leaves of poplar (several species) have been selected in a number of studies (Djingova et al., 1995, 1996, 1999, 2001; Sawidis et al., 1995; Stratis et al., 1996; Bargagli, 1998; Robinson et al., 2000). Bark of trees has been also used for passive biomonitoring (Böhn et al., 1998). However, as a rule, the axial transport of elements from roots (or other assimilation organs) to the bark is assumed to be negligible. In fact, leaves are the main sink for many pollutants, and therefore they are more sensitive to their effects than flowers, fruits or other plant organs (Bargagli, 1998).

In the Guadiamar riparian forests, the best soil–plant correlation was obtained for Cd, especially when leaves and deep soil (25–40 cm) were considered (Fig. 3). Good correlation between poplar leaves and soil has also been obtained by Robinson et al. (2000). Cadmium is a good bioindicator for soil pollution because its accumulation in higher plants is principally from soil (except in conditions of high atmospheric Cd fallout, see Wagner, 1993). Cadmium is absorbed passively through the roots and freely translocated to the leaves and other organs (Das et al., 1997).

A reasonably good soil–plant correlation was also obtained for Zn (Fig. 3), using both leaves and stems, and for both soil depths. Soluble forms of Zn are readily available to plants; the uptake is linearly correlated with its concentration in the soil solution (Kabata-Pendias and Pendias, 2001). Zinc and Cd have similar geochemical and environmental properties, and they can interact during plant uptake, transport and accumulation in leaves (Das et al., 1997).

A significant and positive (log–log) correlation was obtained for As between the concentration in stems and leaves, and the availability in soil (Fig. 3). These log–log patterns, rather than linear, seem to indicate that small amounts of As in soil are readily accumulated in poplar leaves, but after a certain pollution level (saturation point) the accumulation in leaves does not vary (Fig. 3). Despite some authors arguing that As mobility in the spill-affected soils was rather low (Vidal et al., 1999), there were actual evidences of significant plant–soil relationships, in As content, for poplar trees (as mentioned above), and also for Bermuda grass (*Cynodon dactylon*) growing in remediated soils (Madejón et al., 2002). Several reports have also documented positive linear relationships between As content in plants and its concentration in soil, for both total and available fractions (Bech et al., 1997; Kabata–Pendias and Pendias, 2001). The pattern of As accumulation in leaves, and thus the potential for biomonitoring, will depend on several factors: the plant species' particular capacities, the accumulation in roots *versus* shoots (Smith et al., 1998; Ma et al., 2001), and the soil properties.

The correlation coefficient between plant and soil obtained for Cu was rather low (Table 5), compared to that for Cd and Zn, and despite the high Cu content in the sludge (up to 2000 mg kg^{-1}, Cabrera et al., 1999). Bioavailability of Cu is highly plant-specific, and depends on soil properties that determine its chemical mobility. Copper absorption may be competitively inhibited by Zn, interacting at root level (Kabata-Pendias and Pendias, 2001) and that interaction will be expected here, given the relatively high Zn concentration in spill-polluted soils. In this study, Cu and Zn were highly correlated in their soil availability ($r = 0.87$, $p < 0.001$), but there was no significant correlation between their concentration in leaves ($r = 0.25$, $p = 0.14$). In contaminated soils, Cu can substantially accumulate in the plant roots without any significant increase in Cu content of aerial part. This means that the Cu content in the aboveground portion of the plants would underestimate the Cu bioavailability of soil.

The concentration of Fe in the sludge was very high (up to 43%, López-Pamo et al., 1999), and also was high in poplar organs (the second, after Zn; Table 4). The accumulation of Fe in stems of spill-affected trees was double than in control, and was significantly correlated with its availability in surface soil (Table 5). Iron availability depends to a large extent on soil pH and redox potential, and it is affected by several plant and environmental conditions, including concentration of macronutrients and ratios between heavy metals (Chaney et al., 1972; Bienfait, 1988; Bruggemann et al., 1990; Kabata-Pendias and Pendias, 2001). Thus, poplar stems (but not leaves) could be used as bioindicators for Fe accumulation in surface soil.

Many plants accumulate Pb in their roots, but translocation to shoots tends to be greatly limited (about 3% in most cases; Kabata-Pendias and Pendias, 2001). Spill-affected soils had a higher Pb concentration than control (×12 times for EDTA-extracted in surface soil; Table 3). However, there was no significant difference in Pb concentration in polluted poplar trees, compared to the upstream controls. On the other hand, trees from the external control (outside the Guadiamar Basin) had significantly lower concentration of Pb in leaves and stems. The pollution gradient in leaf tissues (as reflected in the PCA axis 1) included Pb as a major contributing element although was opposed to Zn and Cd in the secondary gradient (Fig. 2). There were significant log–log relationships, although with low coefficients, between Pb in plant and soil. Based on the weak relationship found in the Guadiamar riparian forest, poplar leaves cannot be recommended as a biomonitor of Pb accumulation in soil, and despite its proven utility in atmospheric pollution monitoring.

Trees have been widely used for low cost, environmental clean-up and remediation of polluted sites. In particular, they are very useful as "ecological engineers" for controlling hydrology, degrading organic contaminants in groundwater plumes, stabilising eroded sediments, removing heavy metals from contaminated soil, and as biological filters for sewage sludge disposal (Punshon, 2001; Robinson et al., 2003). White poplar (*P. alba*) is an abundant, native tree in the riparian forest of the Guadiamar River; it has also been planted in the "Green Corridor" afforestation program, after the mine spill. This ecological corridor will connect the lowland protected areas (Doñana National Park) with the forested mountains (Sierra Norte Natural Park; CMA, 2001). Poplar trees will contribute to stabilise the soil and river sediments, and to immobilise the trace elements. Additionally, poplar leaves can be used to biomonitor the evolution of pollution levels of Cd and Zn, and to a lesser extent of As, in the spill-affected soils.

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