



Biomonitoring of trace elements in the leaves and fruits of wild olive and holm oak trees

Paula Madejón¹, Teodoro Marañón*, José M. Murillo

IRNAS, CSIC, P.O. Box 1052, 41080 Sevilla, Spain

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Abstract

Biomonitoring of trace elements is essential to assess ecosystem health, in particular in landscapes influenced by human activity. The Guadiamar Valley (SW Spain) was polluted in 1998 by a spill from an open-pit pyrite mine affecting about 55 km². In this paper, we used two common species of tree, namely wild olive and holm oak, to biomonitor the concentration of nine trace elements—As, Cd, Cu, Fe, Mn, Ni, Pb, Tl and Zn—in this spill-affected area over the 3-year period 1999–2001. We analysed the leaves and fruits of trees growing in the spill-affected terraces, and compared them with adjacent trees in the non-affected upper terraces. The main trace elements polluting the soil were Zn, As, Pb and Cu. In general, the oak leaves were richer in trace elements than the olive leaves, reaching phytotoxic levels for As and Pb, while the olive fruits (pulp) were more polluted than the oak seeds (protected inside a hard pericarp), reaching toxic values for Cd and Pb. The concentration of trace elements in the leaves and fruits decreased with time and, in consequence, the toxicity risk to the food web diminished.

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1. Introduction

There is a general decline in forest ecosystem health throughout Europe. About 20% of the 311,726 trees monitored in 2003, distributed over 39 countries

of the European network system, showed symptoms of damage. The most important causes included extreme weather conditions, insect and fungi attack, and air pollution (Lorenz et al., 2004). Besides this general trend, on a continental scale, there are other detrimental factors for forest ecosystem health such as fire, urbanisation, pollution from chemical plants and mine tailings acting on a local scale.

A severe pollution event, the Aznalcóllar spill from an open-pit pyrite mine, occurred in 1998 in the Guadiamar Valley (SW Spain). About 55 km² of land, including riparian forests and oak woodlands, were

* Corresponding author. Tel.: +34 95 4624711; fax: +34 95 4624002.

E-mail address: teodoro@irnase.csic.es (T. Marañón).

¹ Present address: School of Biological and Earth Sciences, Liverpool John Moores University, Byrom Street, Liverpool L3 3AF, UK.

affected. In previous studies, we documented the utility of the white poplar (*Populus alba* L.), a dominant riparian tree, to biomonitor trace elements, in particular Cd and Zn, in the spill-affected area (Madejón et al., 2004). We also analysed the pollution levels in a common grass, bermudagrass (*Cynodon dactylon* (L.) Pers.), to monitor remediated soils (Madejón et al., 2002). In this paper, we analyse the temporal change in trace element levels, in the leaves and fruits of two common species of tree in the Mediterranean forest namely the wild olive (*Olea europaea* L., var. *sylvestris* (Mill.) Lehr) and holm oak (*Quercus ilex* L. subsp. *ballota* (Desf.) Samp. (Amaral, 1990)).

Trees are long-living organisms, which can take up trace elements from the soil, water or air, and retain them for a long time. Otherwise, these elements are sooner or later released to the ecosystem. They can enter the food web, through animals feeding on the fruits, leaves and roots. For example, the leaves and twigs of olive may constitute complementary feed for cattle and also for wild animals, especially during the dry summer. The leaves of oak may be heavily eaten by insects (such as caterpillars of *Lymantria dispar*), which are in turn prey for birds and reptiles. The leaves of both tree species are at the bottom of the food web in Mediterranean forests. The fruits of wild olives are the main source of food for wintering birds, for example *Turdus* spp. and *Sturnus* spp. in the Mediterranean forest. The high concentration of trace elements in the tissues of these fruits (fleshy drupes) in addition to the dust deposited on the surface may have detrimental effects on birds feeding upon them. Trace elements can also be recycled to the soil through shedding of the leaves and fruits. There is a need for long-term studies on the fate of trace elements in forest ecosystems (Bargagli, 1998).

The aims of this study were: (1) To monitor over the 3-year period 1999–2001 the concentration of nine trace elements (As, Cd, Cu, Fe, Mn, Ni, Pb, Tl and Zn) in the leaves and fruits of wild olive and holm oak trees affected by the 1998 mine spill. (2) To determine whether the main mine sludge pollutants are being taken up and accumulated in the leaves and fruits of local trees. (3) To evaluate the potential risk for the food web, by analysing the trace element concentrations in unwashed samples of the leaves and fruits,

and comparing the values obtained with the established toxic thresholds.

2. Materials and methods

2.1. Study area and species of trees

The study area is located in the Guadiamar Valley (SW Spain). The area has a semi-arid Mediterranean climate with mild rainy winters and warm dry summers. The average annual rainfall (for the period 1976–2001, in ‘La Hampa’ Station) was 484 mm and, for each year of the study, it was as follows: 485 mm for 1999, 595 mm for 2000 and 710 mm (47% more than average) for 2001.

The headwaters of the Guadiamar Basin are underlain by schists, sandstones and volcanic rocks, from the Carboniferous period. Within this basin the Aznalcóllar mine, which mines sulphide ore, is located and forms part of the “Iberian pyrite belt”. The lower course of the river is underlain by Miocene blue marls, and the floodplain is composed of Pleistocene alluvial terraces and Holocene deposits (Gallart et al., 1999).

A mine tailing dam, located at the lower course, about 40 m above sea level, collapsed on April 1998, releasing ca. 5×10^6 m³ of slurry and affecting about 55 km² of the valley (for a review, see the special issue in Grimalt and Macpherson, 1999). This large-scale pollution event had a major environmental impact because the Guadiamar River discharges into the Guadalquivir marshes of Doñana National Park, a well-known wintering place for European waterbirds.

An emergency soil clean-up operation 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 disused open-pit mine. In the more accessible areas (e.g., former croplands), soil remediation was carried out, adding organic matter and calcium-rich amendments (CMA, 2003).

A sequence of terraces, located 4.5 km downstream from the tailing dam, were chosen as the study site (37°27'N, 6°13'W). They were formed of gravel and sand deposits, and covered by a savanna-like woodland, dominated by holm oak and wild olive trees.

Woodlands in the lower river terrace were flooded by the mine spill.

Two species of tree: the wild olive and the holm oak, which are typical of Mediterranean forests, were selected for the biomonitoring study (they will be hereafter referred to as olive and oak for simplicity). The fruits of oak are used to feed free-range hogs and livestock, and in the past they were also used for human consumption; thus, holm oak in the savanna-like woodlands can be considered as a semi-domesticated fruit tree. The fleshy fruits of olive are rich in lipids and are a staple diet for many wintering birds in the Mediterranean region. Leaves and twigs of olive are palatable and frequently browsed by goats and wild game. Oak and olive trees also provide shelter for livestock, firewood from pruning, and refuge and breeding sites for a large number of vertebrates (Marañón, 1988; Terradas, 1999).

2.2. Plant and soil sampling

Ten olive and 10 oak individuals, growing in the terraces of the Guadiamar River, were chosen and marked for periodic monitoring. Half of the trees were in a lower river terrace flooded by the spill, while the other half were about 40 m away in a nearby upper terrace, which had not been flooded (the latter will be hereafter referred to as “control” trees).

Samples of the leaves and fruits were taken from the south-exposed tree canopy, at about 3–5 m height, during the early autumn of each of the 3 consecutive years 1999 to 2001. Plant materials were washed (for 10 s approximately) with a solution of phosphate-free detergent, then with a 0.1 N HCl solution and finally with distilled water.

Oak seeds (discarding the hard pericarp of the acorn) and olive fruits (only the fleshy pulp) were also used to monitor chemical pollution. These were dried at 70 °C, ground and passed through a 500 µm stainless-steel sieve.

To investigate the possible toxic impact of the trace elements in the leaves and fruits, through their consumption as food, we needed to know the total content, including the adhered soil on their surfaces (e.g., Brekken and Steinnes, 2004). A sub-sample of plant material was directly ground, without previous decontamination by washing. The values obtained in the leaves were compared with the published thresh-

old limits for toxicity in livestock (Chaney, 1989), and those obtained for the olive fruits (mostly eaten by birds) were compared with tolerance levels for chicken (Chaney, 1989).

Soil samples were taken from the root zone of each tree, about 2 m from the trunk, and at two depths (0–25 cm and 25–40 cm), by using a spiral auger of 2.5 cm diameter. Three sub-samples around the trunk were taken to make a composite soil sample per tree. They were transported to the laboratory, oven-dried at 40 °C and crushed to pass through a 2 mm sieve, and then ground to <60 µm for S and total trace element determinations.

2.3. Chemical analysis

Nine trace elements (As, Cd, Cu, Fe, Mn, Ni, Pb, Tl and Zn) were extracted by wet oxidation with concentrated HNO₃ under pressure in a microwave digester, for washed and unwashed plant samples. Analysis of Fe and Mn in the extracts thus obtained was performed by ICP-OES (inductively coupled plasma spectrophotometry). For the washed samples, the analysis of As, Cd, Cu, Ni, Pb, Tl and Zn was performed by ICP-MS (inductively coupled plasma-mass spectroscopy). While for the unwashed plant samples, all the elements were analysed by ICP-OES.

The accuracy of the analytical method was assessed by carrying out analyses of two BCR reference samples: BCR 62 (olive leaves) and CRM 279 (sea lettuce) (see Colinet et al., 1982; Griepink and Muntau, 1988, respectively). The experimental values and the certified values for the reference material are shown in Table 1.

Soil samples (<2 mm) were analysed for pH potentiometrically in a 1:2.5 soil–water suspension. Particle size distribution was measured by the hydrometer method (Gee and Bauder, 1986). Sulphur, As, Cu, Pb and Zn were determined by ICP-OES, after digesting the samples (<60 µm) with a mixture of conc. HNO₃ and HCl (‘aqua regia’); these values are referred to as ‘total’ concentration (Vidal et al., 1999). The potentially available concentrations of trace elements were determined by ICP-OES after extracting the samples (<2 mm) with a 0.05 M EDTA solution. The total and available concentrations of trace elements are given on a dry weight basis.

Table 1

Results of the analyses of reference materials (mean values \pm 95% tolerance interval, mg kg⁻¹ dry matter)

Element	CRM 279 (sea lettuce)		BCR 62 (olive leaves)	
	Certified	Experimental	Certified	Experimental
As	3.09 \pm 0.20	2.69 \pm 0.11	(0.20)	0.16 \pm 0.016
Cd	0.274 \pm 0.022	0.202 \pm 0.007	0.10 \pm 0.02	0.074 \pm 0.009
Cu	13.14 \pm 0.37	11.63 \pm 0.73	46.6 \pm 1.8	44.4 \pm 2.5
Fe	(2300 \pm 100)	2113 \pm 72.3	–	271.8 \pm 0.55
Mn	(2030 \pm 31.5)	1758 \pm 64.8	57.0 \pm 2.4	54.9 \pm 0.46
Ni	(15.9 \pm 0.4)	13.1 \pm 0.53	–	1.91 \pm 0.33
Pb	13.48 \pm 0.36	12.47 \pm 1.09	25.0 \pm 1.5	27.4 \pm 1.32
Tl	(0.038 \pm 0.005)	0.027 \pm 0.005	(0.03)	0.028 \pm 0.002
Zn	51.3 \pm 1.2	52.18 \pm 3.29	16.0 \pm 0.7	19.1 \pm 0.65

Values in parentheses are indicative (not certified); Fe and Ni values for olive leaves were not provided by BCR. Experimental values were calculated from $N=6$ (sea lettuce) and $N=5$ (olive leaves).

To evaluate the soil pollution severity, we used the pollution load index (PLI as defined by Tomlinson et al., 1980). This index is based on the values of the concentration factors (CF) of each metal in the soil. The CF is the quotient obtained by dividing the concentration of each metal in the soil ($C_{\text{heavy metal}}$) by the base line or background value (concentration in unpolluted soil, $C_{\text{background}}$).

$$CF_i = \frac{C_{\text{heavy metal}}}{C_{\text{background}}}$$

Background values were estimated from the mean concentrations of As, Cu, Pb and Zn for unaffected soils in the area (Cabrera et al., 1999). For each sampling site, PLI may be calculated as the n th root of the product of the n CF:

$$PLI = \sqrt[n]{(CF_1 \times CF_2 \times \dots \times CF_n)}$$

Values of PLI close to 1 indicate heavy metal loads near the background level, while values above 1 indicate soil pollution (Cabrera et al., 1999).

2.4. Statistical analyses

The differences between the concentrations of trace elements in the spill-affected terrace soils, leaves and fruits and those in the control site were assessed by the Student's t -test.

The differences among the 3 years in the chemical composition of the plant organs were analysed by ANOVA. When temporal variation

was found to be significant, post hoc multiple comparison of mean values by Tukey's test was used; in the case that there was not homogeneity of this variance, an alternative, Games-Howell's test, was applied.

Data normality was tested prior to analysis and, when necessary, variables were transformed logarithmically. If, after transformation, the data did not have a normal distribution, we used non-parametric tests: Mann-Whitney U -test for comparison of mean values and the Kruskal-Wallis ANOVA by ranks test for analysis of variance. All statistical analyses were carried out with the program SPSS 10.0 for Windows.

3. Results and discussion

3.1. Soils

In general, the soils had a low pH, a very low CaCO₃ content and a light texture (Table 2). The spill-affected terrace had polluted soils with a high S concentration (six times more than that of the control) and consequently high PLI values, especially in the surface soil (Table 2). Sulphur was very abundant in the pyritic-derived sludge, up to 40% (Alastuey et al., 1999), and through a partial S oxidation could have decreased soil pH values. The spill-affected soils had a significantly lower pH than the control (Table 2). Trace element availability increases at lower pH and lower CaCO₃ content in soils (Greger, 1999). Also, light textures (loam) can

Table 2

Soil characteristics, concentration of S and pollution load index (PLI) values for spill-affected soils and their comparison with control soils ($N=10$, mean \pm S.E.)

	Depth (cm)	pH	CaCO ₃ (%)	S (mg kg ⁻¹)	PLI	Texture
<i>Spill-affected soils</i>						
	0–25	4.1 \pm 0.2***	<0.5	2574 \pm 333***	4.26	Loam
	25–40	4.5 \pm 0.3**	<0.5	1231 \pm 229***	2.84	Clay loam
<i>Control</i>						
	0–25	5.6 \pm 0.1	<0.5	422 \pm 47	1.11	Loam
	25–40	5.7 \pm 0.2	<0.5	239 \pm 25	0.96	Loam

Values marked with asterisks are significantly different from the control, according to Student's *t*-test (** $P < 0.01$, *** $P < 0.001$).

increase the availability of metalloids, such as As (Smith et al., 1998).

Spill-affected soil samples had a much higher total content of trace elements than the control: e.g., 7-fold for Zn, 5-fold for As and Pb, and 3-fold for Cu (Table 3). Six trace elements—As, Cd, Cu, Fe, Pb and Zn—which were abundant in the mine sludge (Cabrera et al., 1999) had a significantly higher 'total' concentration in the soils of the spill-affected terrace, compared to the control (Table 3). The sludge was rich in Fe (up to 37%, Alastuey et al., 1999) and, in consequence, the spill-affected soils had an even higher Fe concentration (3.6%) than the control. Manganese was also abundant in control soils (0.97%), but the concentration was similar in spill-affected soils, because the mine sludge was poor in Mn. The total Ni concentration was slightly higher in the surface layer of the spill-affected

soils, but not statistically significant in the deeper layer, compared to the control; the mine sludge was also relatively poor in Ni.

The available portion of the trace elements was much higher in the spill-affected soils than in the control (Table 3). The rank of elements in the spill-induced soil pollution was (for the 0–25 cm depth): Cd (26 times that of the control), Zn (25 times), As (5 times), Cu, Fe and Pb (4 times). The availability of Mn and Ni was also greater in the spill-affected soils, despite not being a major polluting element in the mine sludge; this increase could be provoked by the lower pH in these soils. Lowered soil pH has been shown to increase the availability of some elements to local plants.

The patterns of the available concentration of trace elements in the spill-affected soils are more mean-

Table 3

Total and available concentrations of trace elements (mg kg⁻¹) in spill-affected soils, and the comparison with control soils ($N=10$, mean \pm S.E.)

	Depth (cm)	As	Pb	Cd	Zn	Fe	Mn	Cu	Ni
<i>Total</i>									
Spill-affected soils	0–25	82.0 \pm 24*	201 \pm 62*	3.46 \pm 1.24*	618 \pm 119*	35,930 \pm 2301*	1079 \pm 130	146 \pm 23*	26.2 \pm 1.6*
	25–40	33.1 \pm 4.0*	126 \pm 17*	2.25 \pm 0.43*	371 \pm 91*	35,427 \pm 1436*	982 \pm 95	119 \pm 25*	27.9 \pm 1.5
Control	0–25	14.8 \pm 1.0	41.9 \pm 3.4	1.19 \pm 0.03	83.8 \pm 3.3	26,970 \pm 506	968 \pm 46	43.8 \pm 9.5	20.6 \pm 1.6
	25–40	13.2 \pm 0.7	33.3 \pm 3.1	1.36 \pm 0.05	76.4 \pm 3.4	16,297 \pm 5456	891 \pm 72	29.3 \pm 2.4	23.4 \pm 0.7
<i>Available (EDTA)</i>									
Spill-affected soils	0–25	3.50 \pm 1.4*	44.3 \pm 5.5*	1.05 \pm 0.40*	174 \pm 26*	908 \pm 233*	359 \pm 24*	56.5 \pm 10*	3.32 \pm 0.23*
	25–40	0.95 \pm 0.23*	32.3 \pm 6.2*	0.36 \pm 0.16*	70.5 \pm 24*	546 \pm 228*	254 \pm 16*	33.7 \pm 7.0*	2.5 \pm 0.25*
Control	0–25	0.68 \pm 0.25	12.4 \pm 1.9	0.04 \pm 0.01	7.0 \pm 1.2	219 \pm 23	244 \pm 19	15.2 \pm 5.0	1.63 \pm 0.17
	25–40	0.37 \pm 0.09	4.6 \pm 0.63	0.01 \pm 0.01	2.20 \pm 0.35	107 \pm 7.2	187 \pm 22	5.12 \pm 0.7	1.1 \pm 0.13
<i>Sludge</i>		2878	7888	25.1	7096	359,350	647	1552	15.9

Values marked with asterisks are significantly different from the control ($P < 0.05$ of Student's *t*-test). Chemical composition of the mine sludge (according to Cabrera et al., 1999; except Fe as in Alastuey et al., 1999) is shown as reference of the pollution source.

ingful for the plant uptake processes than the total values. The pool of available elements is composed of those ions present in the soil solution, as well as those that are readily solubilised, and therefore able to move into the plant roots (Bargagli, 1998). Mild soil extractants, such as EDTA, are commonly used to estimate this available fraction; however, some authors (e.g., Vidal et al., 1999) argue that this method can overestimate the availability of elements such as Cu and Pb.

As expected, trace element concentrations were greater in the 0–25 depth than in the 25–40 depth, as the sludge was confined to the first 25 cm in most soils after the mine accident (Cabrera et al., 1999). However, trace element concentrations at 25–40 cm depth were, in general, significantly greater in the affected soils than in control soils (Table 3), indicating that the whole root systems of the trees were affected by the soil pollution.

3.2. Tree leaves and fruits

There were differences in the concentration of trace elements between leaves and fruits, between tree species, over the 3 years examined, and between the spill-polluted trees compared with the control; for simplicity, we have drawn separately figures for element concentration in olive leaves (Fig. 1), oak leaves (Fig. 2), olive fruits (Fig. 3) and oak seeds (Fig. 4). In general, oak leaves accumulated higher amounts of trace elements than olive leaves (compare Figs. 1 and 2). The elements As, Fe, Pb and Tl tended to decrease with time, while Cd and Zn temporal trend was rather uniform; and lastly Mn, Cu and Ni had a less defined temporal pattern. Spill-polluted trees tended to accumulate higher contents of trace elements in their leaves respect to control trees. With regards to fruits and seeds, there is scarce information on the trace element accumulation in them for Mediterranean wild trees. In contrast with the patterns found in the leaves (Figs. 1 and 2), the concentration of most elements was higher in olive fruits (pulp) than in oak seeds (compare Figs. 3 and 4). A more detailed account of the bioaccumulation patterns of the different elements grouped according to their pattern type, in washed samples, is given below.

3.2.1. Elements As, Fe, Pb and Tl

The concentration of As, Fe, Pb and Tl in (washed) olive leaves from the spill-affected sites decreased consistently from the first to the third year studied (Fig. 1), the maximum values being reached during 1999 (1 year after the spill): 1.9 mg kg⁻¹ As, 160 mg kg⁻¹ Fe, 8 mg kg⁻¹ Pb and 0.02 mg kg⁻¹ Tl. In oak leaves, there was a decreasing pattern with time for As, Fe, Pb and Tl (as with olive), and also Cu (Fig. 2). The maximum values, reached in 1999, were: 24 mg kg⁻¹ As, 2760 mg kg⁻¹ Fe, 61 mg kg⁻¹ Pb and 0.3 mg kg⁻¹ Tl (always much higher than in olive). These high concentration levels were above the limits of phytotoxicity (as reported by Kabata-Pendias and Pendias, 1992) for As (5 mg kg⁻¹) and Pb (30 mg kg⁻¹), but not for Tl (20 mg kg⁻¹).

The concentration of As in the olive leaves was significantly higher in the spill-affected sites than in the control, for the 3 years monitored (Fig. 1). However, Fe, Pb and Tl did not show significant differences, for 1999 and 2000. Interestingly, the trend was reversed in 2001, and spill-polluted olive leaves had lower concentrations of Fe, Pb and Tl than the control (Fig. 1). On the other hand, there were no differences in the concentrations of As, Fe, Pb and Tl in oak leaves, between those trees from the lower terrace (flooded by the spill) and those from control (Fig. 2).

The accumulation of As, Fe, Pb and Tl in olive fruits was relatively high in 1999 (1 year after the mine spill), but decreased significantly in both 2000 and 2001 (Fig. 3). In comparison, the accumulation of As, Pb and Tl in oak seeds were much lower than in olive fruits (e.g., 85-fold for As in 1999), and there were no significant changes among years in the spill-flooded trees. In the case of Fe, its accumulation in the oak seeds was also lower than in the olive fruits (62-fold), but there was a significant decrease with time (Fig. 4).

Although the soils in the upper terrace (control), not flooded by the spill, had a significantly much lower concentration of trace elements (Tables 2 and 3), that was not the case for the trees. The frequent clouds of dust produced during sludge removal and soil clean-up operations in 1999 (Querol et al., 1999) polluted the leaves and fruits of the trees in the lower terraces, and also in the nearest upper terraces (control), not directly flooded by the spill. Thus,

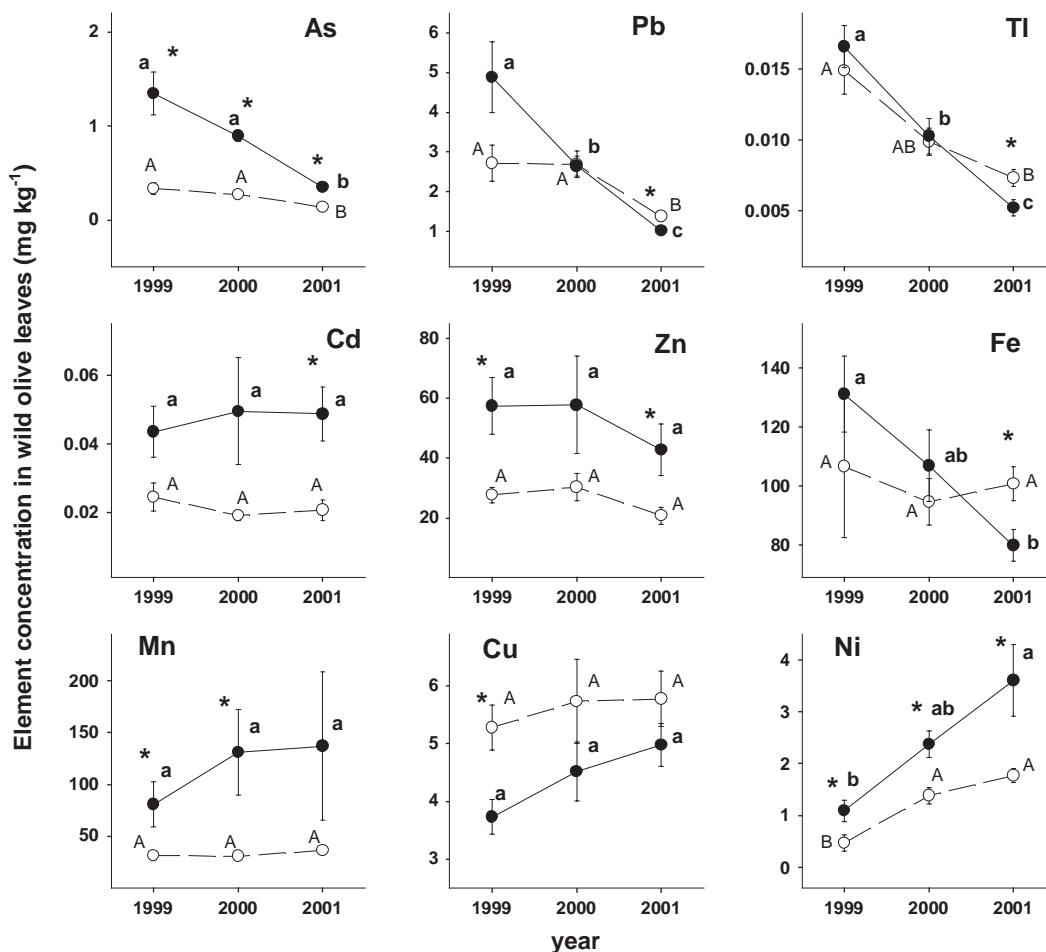


Fig. 1. Temporal variation in the concentration of nine trace elements, in the (washed) leaves of *Olea europaea* (mean value and S.E., $N=5$). Significant differences among years are marked with different letters. Trees from spill-affected soils (in black) are compared with trees from control soils (in white), and significant differences are marked with asterisks.

atmospheric pollution might have been responsible for the high Fe concentration in oak leaves and olive fruits during 1999 in both sites, i.e., flooded and non-flooded (see other examples in Reimann et al., 2001a).

The high concentrations of Fe and Pb in the leaves of oak found in this study are greater than other values reported for *Q. ilex* (e.g., Santamaría and Martín, 1998; Monaci et al., 2000). These results combine the effects of soil pollution (rich in Fe and Pb, Table 3) and heavy aerial pollution in 1999, affecting also trees near to the spill-flooded area.

There is an important effect of plant morphology on the differential pollution features for oak and olive.

On one hand, the different leaf morphology (spiny and tomentose underneath in oak, versus glabrous and smoother in olive) influences the capture of aerial dust, as well as their response to the external decontamination (by previous washing) to the chemical analysis (Jones and Case, 1990). Thus, the As concentration in oak leaves was much higher (10 times more) than in olive leaves. Also control olive trees suffered significantly lower aerial contamination by As than spill-flooded trees (Fig. 1), unlike oak trees having heavy As pollution during 1999 at both sites (Fig. 2). On the other hand, differences in fruit morphology (fleshy fruit in olive, and seeds protected

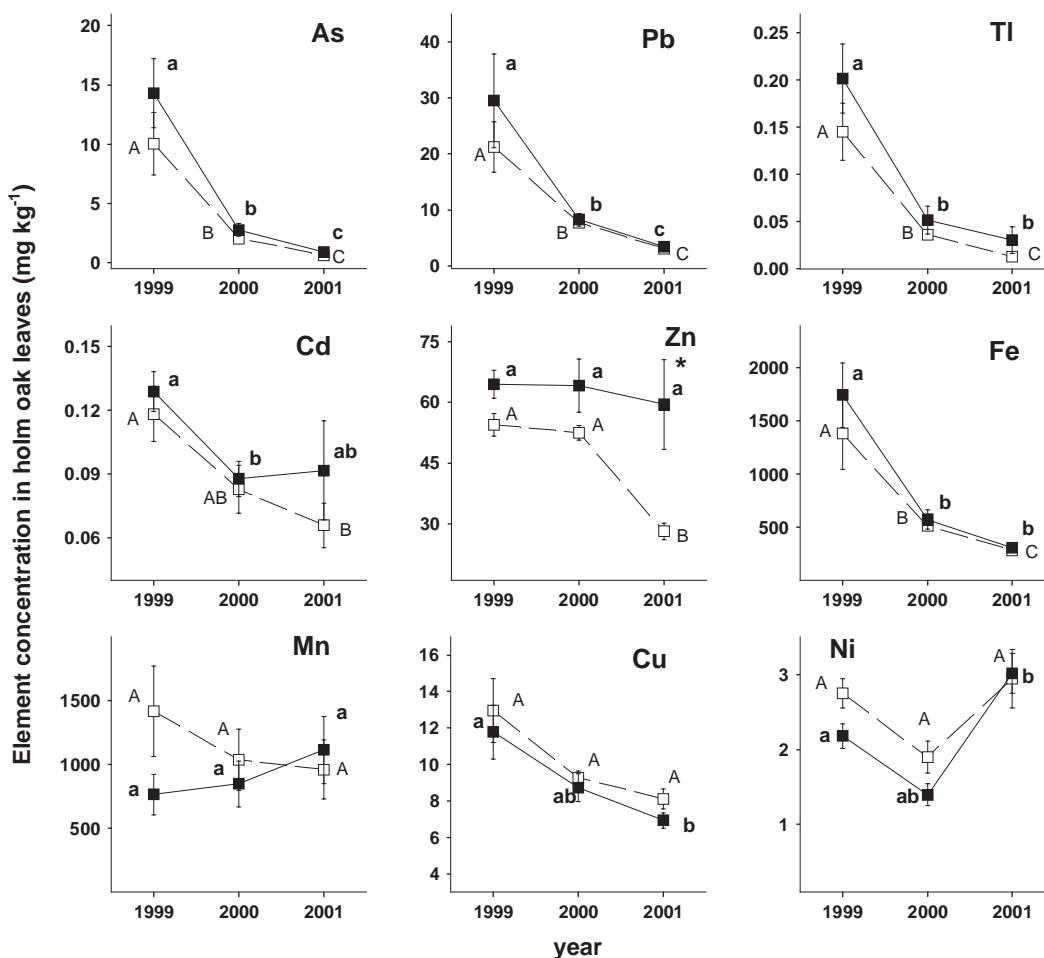


Fig. 2. Temporal variation in the concentration of nine trace elements, in the (washed) leaves of *Quercus ilex* (mean and S.E., $N=5$). Significant differences among years are marked with different letters. Trees from spill-affected soils (in black) are compared with trees from control soils (in white), and significant differences are marked with asterisks.

by a pericarp in oak) also influence the dust deposition and the element accumulation in plant tissues. There was a high concentration of Fe, up to 4200 mg kg^{-1} , in the olive fruits of the polluted site, and up to 2500 mg kg^{-1} in control trees. This cumulative effect could be reinforced by the general dryness during 1999 (annual rainfall of 485 mm, 18% lower than in 2000 and 32% lower than in 2001). In dry conditions, the olive fruits produced were of a smaller size (20% lower than the average for 2000 and 30% lower than the average for 2001), had a lower water content (13% versus 30% in normal fruits) and a rougher surface.

The dust source of trace elements could mask the effect of direct uptake from the soil and translocation to the leaves and fruits. In the case of As, significant differences were detected between the fruits from olive trees growing in spill-affected soils (0.24 mg kg^{-1} in 2000), compared to the control (0.09 mg kg^{-1} in the same year; Fig. 3).

The seeds of oak were protected by the pericarp and did not suffer direct dust deposition, having lower concentration of elements than olive fruits (compare Figs. 3 and 4). The decrease with time on Fe concentration in oak seeds (Fig. 4) could be explained because iron is a micronutrient and was probably

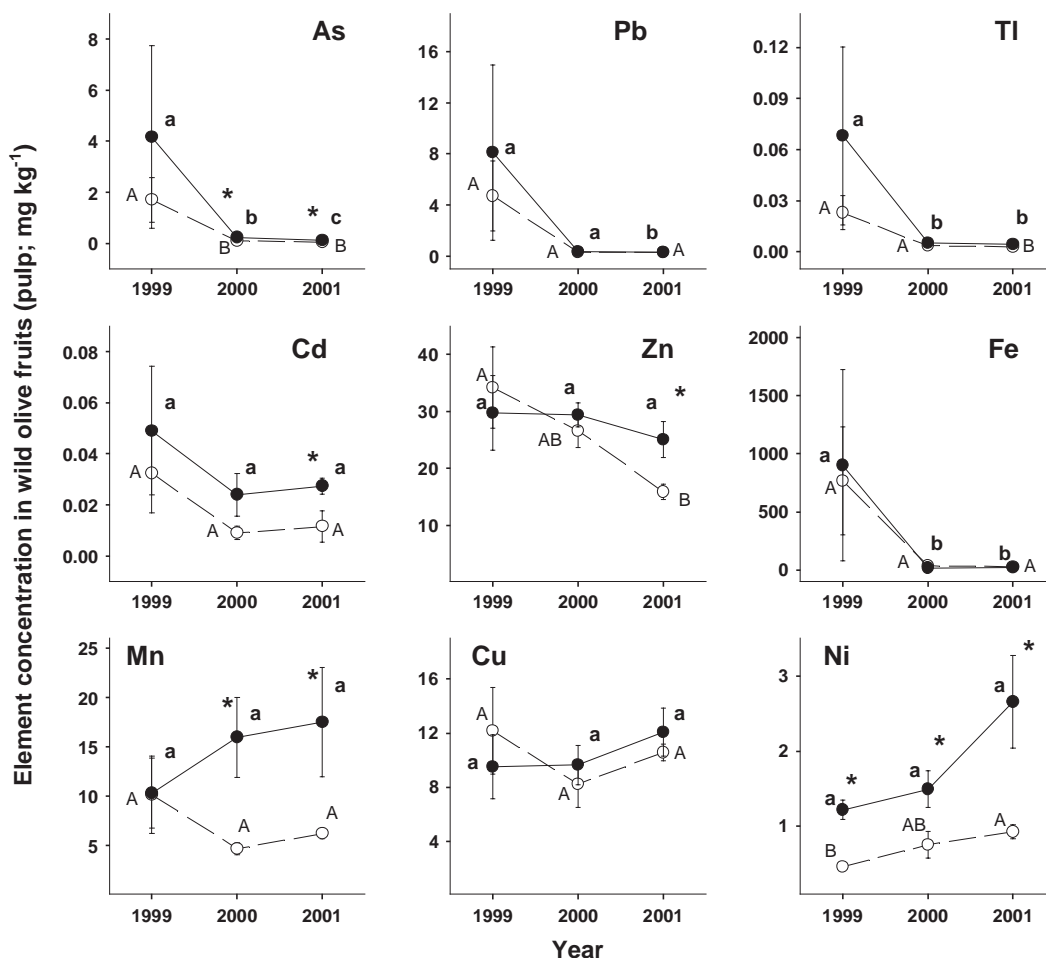


Fig. 3. Temporal variation in the concentration of nine trace elements, in the (washed) fruits of *Olea europaea* (mean and S.E., $N=5$). Significant differences among years are marked with different letters. Trees from spill-affected soils (in black) are compared with trees from control soils (in white), and significant differences are marked with asterisks.

taken in from dust deposition on the leaves and translocated to the fruits; thus, the decreasing accumulation in the protected seeds indirectly reflected the air pollution trend. On the contrary, spill-flooded oak trees did not accumulate higher amounts of As or Pb in their seeds than those of the control; in the case of Pb (during 2001), they even had a significantly lower concentration than the control. This pattern seems to indicate a mechanism to exclude the translocation of these potentially toxic elements into the seeds.

Thallium is a trace element of special interest because the scant information about its bioaccumulation in Mediterranean trees. Despite the very high

concentration of Tl in the sludge (up to 60 mg kg^{-1} , Cabrera et al., 1999), this element seems to have scarce mobility in the spill-affected soils and in consequence should have little accumulation in plants (Alastuey et al., 1999; Vidal et al., 1999). The highest peak of Tl pollution in leaves was reached during 1999, as a probable result of air dust. However, the absolute concentration was relatively low (up to 0.3 mg kg^{-1} in oak) and far lower than the reported limit of phytotoxicity: 20 mg kg^{-1} (Kabata-Pendias and Pendias, 1992). Oak seeds did accumulate a significantly higher amount of Tl at the spill-flooded sites than the control, indicating a certain capacity to take

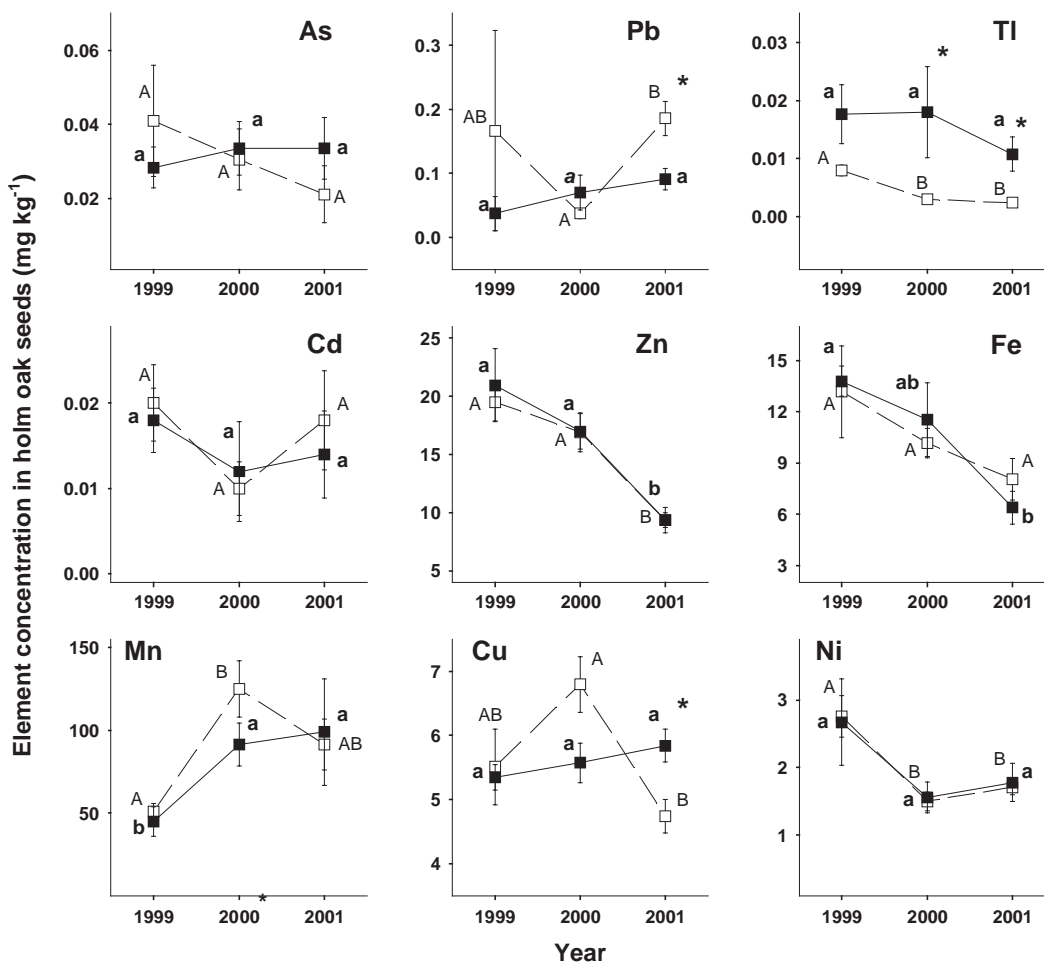


Fig. 4. Temporal variation in the concentration of nine trace elements, in the seeds of *Quercus ilex* (mean and S.E., $N=5$). Significant differences among years are marked with different letters. Trees from spill-affected soils (in black) are compared with trees from control soils (in white), and significant differences are marked with asterisks.

up and transport Tl into the seeds. Although the maximum level reached (0.05 mg kg^{-1} in 2000) was low and stayed below the threshold value considered safe for the trophic web (2.5 mg kg^{-1} of dry matter; Makridis and Amberger, 1996). Nevertheless, possible negative effects on seedling growth and development should be studied. Relatively low values of Tl have also been found in different tree and herb species monitored in the spill-affected soils of the Guadiamar Valley: poplar (Madejón et al., 2004), wild grasses (Madejón et al., 2002) and sunflower (*Helianthus annuus*; Madejón et al., 2003). However, some accumulator plants (such as cultivated *Brassica* spp.)

have been documented as having a comparatively high Tl uptake in the Guadiamar basin (Soriano and Fereres, 2003). A high Tl concentration (ca. 50 mg kg^{-1}) has also been recorded in the flowers of *Hirschfeldia incana* (L.) Lagr. Foss., a wild crucifer growing abundantly in heavily polluted soils in the Guadiamar basin (Madejón et al., in press). These flowers are often consumed by animals and Tl accumulation should be examined because of the possible impact on the trophic web.

The bioaccumulation of trace elements in the leaves and fruits of the trees is the combined result of the uptake process via the roots from the soil which

is then transported to the leaves and translocated to the fruits, and the direct capture from the air or precipitation (Reimann et al., 2001b). This bioaccumulation increases with the increasing metal concentration in the external medium (Greger, 1999). The separation between the proportion of elements taken in after airborne deposition, from those translocated via the roots from the soil is difficult to ascertain (Bargagli, 1998). Further monitoring of the oak and olive leaves and fruits, once air pollution caused by soil remediation practices has finished, will help to understand the relative importance of both pollution sources.

3.2.2. Elements Cd and Zn

There were no differences in the Cd and Zn concentrations in the olive leaves among years in either the spill-affected or the control soils (Fig. 1). Neither were there inter-year differences for the Zn concentration in oak leaves in the spill-flooded soils (Fig. 2). However, at the non-flooded sites, the Zn in oak leaves significantly decreased in 2001 respect to previous years. Also, Cd levels in oak leaves decreased with time at both types of sites.

The absolute concentration of Cd in the leaves (up to 0.16 mg kg^{-1} in oak, during 1999) was relatively low and far lower than the phytotoxic level: 5 mg kg^{-1} (as reported by Kabata-Pendias and Pendias, 1992). Despite the important proportion of Cd in the mine sludge (Table 3), and the fact that acid soils tend to enhance Cd uptake by plants (Das et al., 1997), these particular species of trees, i.e., olive and oak, do not accumulate a significant amount of Cd in their leaves. In contrast, poplar leaves sampled at a spill-affected site nearby, accumulated more than 10 mg kg^{-1} of Cd (30 times more than oak and 80 times more than olive) (Madejón et al., 2004).

Compared with other studies, Cd in the leaves of the spill-affected olive trees (mean value of 0.04 mg kg^{-1} in 1999) lies within the range reported for background areas under Mediterranean conditions: $0.02\text{--}0.10 \text{ mg kg}^{-1}$ (Bargagli, 1995). Oak leaves had a mean value of 0.13 mg kg^{-1} Cd in 1999, in the spill-affected site, which is similar to the values reported for *Q. ilex* in non-polluted areas of Italy (0.10 mg kg^{-1} ; Monaci and Bargagli, 1997).

The relatively lower uptake of Cd could be related to interactions with other trace elements. Cadmium is

absorbed passively via the roots and freely translocated to the leaves (Das et al., 1997); however, high concentrations of Zn, Cu and other trace elements can reduce the Cd uptake from both the root and foliar systems (Kabata-Pendias and Pendias, 1992).

In contrast, the absolute values of Zn concentration in the spill-affected trees were relatively high: mean values (in 1999) of 57.5 mg kg^{-1} for olive and 64.5 mg kg^{-1} for oak. These surpassed the range values reported for olive in background areas of Italy ($14.7\text{--}38.2 \text{ mg kg}^{-1}$; Bargagli, 1995) as well as the average values for *Q. ilex*, also in Italy ($26 \pm 2.9 \text{ mg kg}^{-1}$; Monaci et al., 2000).

In 2001, when air pollution caused by the soil cleaning operations had finished, the olive leaves in the sites not flooded by the spill had significantly lower concentrations of Cd and Zn than in the flooded sites nearest to the river (Fig. 1). Oak leaves also had a much lower Zn concentration in the non-flooded soils than at the directly polluted site (Fig. 2), although significant differences in Cd levels were not found. These patterns support the important contribution of root uptake to the resulting Cd and Zn accumulation in the leaves of olive and oak, although further monitoring of Cd in oak leaves should be continued.

The concentrations of Cd were rather low in the fruits of olive and the seeds of oak, far lower than the critical level for animal consumption (0.5 mg kg^{-1} ; Chaney, 1989), although similar to the acceptable level in most fruits and some edible leafy plants (0.05 mg kg^{-1} ; Pillay et al., 2002). The olive fruits in 2001 accumulated more Cd at the spill-flooded site than in the control, indicating a certain capacity to take this up from the polluted soil and transport it into the fruits. On the contrary, there was no difference between sites for Cd in oak seeds, probably reflecting a mechanism excluding Cd from the seeds.

As in the case of Fe, the pattern of Zn (another micronutrient) in oak seeds diminished with time at both sites, probably reflecting the indirect intake from dust Fe deposited on the leaves and translocated into the seeds. The temporal pattern in the non-flooded olive trees was similar; but not in the flooded trees, which kept a relatively high amount of Zn in their fruits, probably compensated for by direct Zn uptake from the polluted soil.

3.2.3. Elements Cu, Mn and Ni

The concentrations of Mn in the leaves of olive and oak did not vary significantly with time (Figs. 1 and 2). However, the spill-induced effects were contrasted between the tree species: in olive, there was a significant increase (almost four times greater in 2001) in the leaves from the spill-flooded site, while in oak there was no significant difference.

The absolute Mn levels in the leaves were also very different between species. Oak leaves had a very high concentration of Mn (up to 2530 mg kg⁻¹ in one tree at the control site in 1999), while olive leaves were below 500 mg kg⁻¹ (total mean of 74.5 for *N*=30). Thus, in comparison to the range considered as “normal” for concentration of Mn in plant tissues: 17–600 mg kg⁻¹ for woody angiosperms (Bowen, 1979), olive falls within the lower limit, while oak is well above the upper limit.

Trees in the genus *Quercus* are well-known as accumulators of Mn in leaves (Bargagli, 1998). For example, a mean concentration of 1830 mg kg⁻¹ Mn was found in the leaves of *Q. suber* (cork oak) from a pyritic mining area in the south of Spain (Soldevilla et al., 1992), or a maximum of 1200 mg kg⁻¹ Mn in leaves of *Q. canariensis* growing in sandstone-derived soils in the Aljibe Mountains, southern Spain (Marañón, unpublished). The uptake capacity of Mn could already be at saturation (maximum values for control trees in 1999) and the effect of the spill, despite increasing slightly the available Mn (Table 3), was negligible in the leaves.

On the contrary, olive trees must have a quite different physiology for Mn uptake and accumulation. The Mn levels in control leaves were relatively low. However, the small increase in available Mn in the spill-flooded soils, reinforced by the lower pH, induced a much higher accumulation of Mn in the leaves (Fig. 1).

Oak seeds (unlike the leaves) showed an increasing accumulation of Mn with time, at both sites (Fig. 4). This trend is the reverse of the Zn and Fe patterns, probably reflecting some antagonistic mechanisms in the uptake and translocation. In the olive fruits, there was no significant difference among years, but there was a divergent trend in 2000 and 2001 making the spill-flooded trees accumulate more Mn in their fruits than the control. This pattern indicates a certain uptake of Mn from the polluted

soil and translocation into the fruits. Unlike the other trace elements, Mn levels in the protected oak seeds were much higher than in the exposed olive fruits, supporting the nature of oak trees as Mn-accumulators in their seeds too.

The decreasing trend of Cu with time, in the oak leaves, resembled the pattern discussed above for As, Fe, Pb and Tl (Fig. 2). Because Cu was an important element in the sludge (Table 3) and the pattern was consistent at both sites (despite the soil differences in Cu), we can infer a major effect of the atmospheric contamination on the leaves.

The pattern of Cu was quite different for olive leaves (Fig. 1). The accumulation of Cu in leaves from the control site was relatively low, compared with the range considered as “normal”: 6–14 mg kg⁻¹ for woody angiosperms (according to Bowen, 1979). Interestingly, the Cu levels were even lower in the spill-flooded sites, although there was some convergence with time (Fig. 1). This contradictory pattern of leaves having a lower Cu concentration in those soils with higher Cu could respond to antagonistic interactions between trace elements. Thus, the higher concentration of Fe and Zn in 1999 could inhibit the uptake of Cu (Kabata-Pendias and Pendias, 1992), and this antagonistic effect would be weaker with time as Fe and Zn pollution decreased.

Olive fruits showed no differences among years or between sites in their Cu concentrations (Fig. 3). Oak seeds showed no differences in Cu concentration between 1999 and 2000, but they did in 2001 when Cu levels decreased in control trees (compared to the spill-flooded trees), indicating some uptake from the polluted soil.

The relatively low accumulation of Cu in the aerial parts of plants has also been reported for other species in the Guadiamar basin, despite the significant enrichment of Cu in the spill-flooded soils: for example in poplar leaves (mean of 7.6 mg kg⁻¹; Madejón et al., 2004) and in bermudagrass shoots (mean of 13.5 mg kg⁻¹ in remediated soils; Madejón et al., 2002). However, in some cases, the upper limit of the normal range (20 mg kg⁻¹ for herbaceous; Bowen, 1979) has been exceeded, like in the shoots of bermudagrass growing in sludge-covered soils (mean value of 28.4 mg kg⁻¹; Madejón et al., 2002) and in the leaves of sunflower cultivated in the spill-affected soil (29 mg kg⁻¹; Madejón et al., 2003).

Although Ni was not an abundant element in the mine sludge, the spill-flooded soils had a slightly higher Ni concentration than control soils (Table 3), probably due to their lower pH. Olive leaves had higher Ni at the spill-flooded sites than at the control sites, probably corresponding to a higher Ni uptake in the Ni richer soils. The increasing Ni accumulation with time could be explained by antagonistic interactions (as discussed above for Cu), which weaken as the main pollutants Zn and Fe diminish.

Oak leaves, however, did not show a significant difference in the Ni concentration of their leaves between sites (Fig. 2). There was an increase with time (from 1999 to 2001) in the Ni content in their leaves, for the spill-flooded sites, that could reflect a release of the antagonistic interactions by the diminishing pollution of Fe and Zn. In all the cases, olive and oak leaves had a relatively low content of Ni, within the normal range of 0.1–5 mg kg⁻¹ (Kabata-Pendias and Pendias, 1992).

Olive fruits (like leaves) also showed an increasing concentration of Ni with time and was higher in the spill-flooded trees. That pattern was reverse to that of Zn, indicating a probable antagonistic mechanism among those elements. On the contrary, in oak seeds, there was a reduction with time and no differences between sites. For both species, the content of Ni in both the fruits and seeds was relatively low.

Interestingly, despite being minor components of the mine sludge, Mn and Ni were accumulated in the spill-flooded olive leaves, and also translocated to the pulp of the fruits. In these spill-affected olives, the relative concentration of Mn in the fruits was less by one order of magnitude than in the leaves, while the levels of Ni in both plant organs was rather similar.

3.3. Implications for the food web

One of the main environmental problems with pollution by trace elements is that the accumulation in plant organs can produce toxic effects in animals feeding on them. To evaluate the potential impact of (presumably) spill-induced accumulation of trace elements in olive and oak trees on the food web, we analysed plant samples without being washed in the laboratory. Thus, we included the soil or atmospheric

dust deposited on the surface, which would also be ingested by the consumers. Some details of trace element concentrations in the unwashed leaves of olive and oak, and in the unwashed fruits of olive are given below. We have not considered the oak seeds here, because they are protected inside the fruit by a hard pericarp, and their values have been already discussed above.

3.3.1. Unwashed leaves

The range of trace elements accumulated in the leaves of the trees growing in the spill-affected area of Guadiamar river are shown in Table 4. In general, oak leaves accumulated a higher amount of trace elements than olive leaves, with the exception of Cd and Zn.

Iron accumulation was very high in oak leaves, during 1999 at both sites (up to 3160 mg kg⁻¹), exceeding the threshold considered toxic for livestock (Table 4). Although this decreased in 2001 (up to 764 mg kg⁻¹ in affected and 715 mg kg⁻¹ in control), it was still potentially toxic for more sensitive animals like sheep. These results justify the ban on grazing and browsing by domestic animals in the spill-affected zone. However, the precise definition of the critical toxicity concentration for Fe is difficult, as the relationship between the proportion of Fe (III), which precipitates preferentially in the apoplast, and the highly toxic Fe²⁺, which freely circulates in the cytoplasm and cell organelles, is not known (Römheld and Marschner, 1991).

Arsenic was one of the trace elements that caused most social alarm during the mine spill, due to its known toxicity. However, the maximum values reached during 1999 in the oak leaves (31.2 mg kg⁻¹) were below the toxic threshold for livestock (Table 4). The As pollution, and the risk of toxicity for animals, decreased progressively with time, after the soil clean-up had finished and soil stabilisation and vegetation cover had improved: e.g., maximum of 3.6 mg kg⁻¹ in 2001 (Table 4).

The maximum accumulation of Cd in the olive leaves was higher than in the oak leaves. In 1999, the olive (but not oak) leaves had a Cd content above the toxic threshold, but with time this decreased below toxic levels. The toxicity levels for Cd in wildlife must be reconsidered, according to Beyer (2000), who argues that they have been exaggerated.

Table 4

Range (minimum and maximum) values of trace elements obtained in wild olive and holm oak unwashed leaves in affected (A) and control (C) soils ($N=5$)

Species	Soil	Year	Fe	As	Cd	Cu	Mn	Pb	Zn
Wild olive	A	1999	186–292*	0.92–2.89	0.01–0.54	3.93–6.08	44.5–125*	2.80–17.5	21.7–110
		2000	151–224*	1.07–2.86*	0.06–0.16	6.24–9.05	57.9–287	2.90–16.5	21.4–128
		2001	98–143	0.38–1.33	0.02–0.11	4.19–6.72	46.2–418	1.63–2.42	22.5–82.2
	C	1999	119–222	0.51–1.15	0.01–0.53	4.49–7.46	20.8–39.3	3.14–15.2	16.0–28.3
		2000	115–174	0.72–1.36	0.05–0.10	7.38–11.6	25.9–48.3	1.21–4.76	17.8–36.7
		2001	107–147	0.26–0.67	0.05–0.07	5.68–8.42	30.0–40.7	1.35–2.10	14.2–29.3
Holm oak	A	1999	1100–3160	9.42–31.2	0.14–0.19	11.3–25.4	451–1450	21.0–93.0	56.8–76.6*
		2000	627–1140	4.40–7.10*	0.21–0.37	11.9–17.5	495–1460	9.60–15.0	26.2–87.2
		2001	319–764	1.60–3.58	0.13–0.25*	8.60–11.9	318–2000	4.70–20.5	44.0–113*
	C	1999	798–2760	5.41–23.6	0.09–0.23	11.6–25.3	754–2400	18.7–48.5	32.8–59.9
		2000	623–745	3.30–4.20	0.16–0.25	12.4–18.4	583–1990	9.50–31.1	39.9–55.6
		2001	330–715	1.33–1.96	0.05–0.017	9.20–16.4	602–1990	3.80–7.60	26.7–45.3
Tolerance level			500 (sheep), 1000 (cattle)	50	0.50	25 (sheep), 100 (cattle)	1000	30	300 (sheep), 500 (cattle)

Values marked with asterisks are significantly different from the control ($P < 0.05$ of Student's t -test). Maximum levels tolerated by livestock (tolerance level) according to Chaney (1989) are indicated at the bottom of the table.

The leaves of oak (but not of olive) at both sites had Pb contents above toxic levels in 1999. However, Pb in the leaves decreased to below toxic level in 2001. Soil cleaning and stabilisation had ameliorated Pb pollution in the plants. In addition, most Pb taken up by the plants remained in the root system, and this soil–plant barrier could act protecting the food chain against toxicity (Adriano, 1986; Chaney, 1989).

The leaves of oak (but not of olive) had a Cu content just above the toxic threshold for sheep (Table 4), decreasing with time.

Manganese, despite not being one of the polluting heavy metals in the mine spill, was highly accumulated in oak leaves, exceeding the maximum level tolerated by livestock (Table 4). This pattern is associated with the particular physiology of the *Quercus* genus, with high uptake and accumulation of Mn, which is not observed in other species of tree, such as olive, with low values of Mn.

In contrast, Zn was one of the most significant elements in the mine sludge (Cabrera et al., 1999), although its accumulation in the leaves of both species of tree was far lower than the critical threshold for livestock (Table 4). There were other species of tree, like white poplar, which did accumulate a very high Zn content in the spill-affected zone (up to 1312 mg kg⁻¹; Madejón et al., 2004). This physiological feature, of high uptake and accumulation of Zn, is characteristic of the Salicaceae family.

Nickel and Tl values in the leaves of the trees were far lower than the critical threshold for toxicity and so are not shown in Table 4.

To sum up, during the first year after the mine spill (1999), there were high accumulations of trace elements (Cd, Pb, Cu, Mn and Fe) in the leaves, which were above potentially toxic levels. Thus, the legal ban on grazing and browsing by domestic animals in the spill-affected area was justified. Moreover, this study has shown that during the soil remediation operations (first 2 years), air pollution affected the nearby trees not directly flooded by the spill. A buffer zone, including the potentially air-polluted trees, should be delimited and monitored in similar cases in the future.

3.3.2. Unwashed olive fruits

Some fruits harvested in 1999 showed very high values of Cd (up to 0.58 mg kg⁻¹) and Pb (up to 40.7 mg kg⁻¹), exceeding the tolerance level for chicken (Table 5). The toxic effects of trace elements on birds depend on the dose; these can cause mortality (Ramey and Sterner, 1995; Mateo et al., 1998), sublethal effects (Eisler, 1994; Bokori et al., 1995) or affect reproduction negatively (Burger, 1995).

After the Aznalcóllar mine spill, the levels of Pb and Cd in blood from birds feeding in the area were higher than those normally found in uncontaminated areas (Benito et al., 1999). When studying the effects

Table 5

Range (minimum and maximum) values of trace elements obtained in the pulp of wild olive unwashed fruits in affected (A) and control (C) soils ($N=5$)

Species	Soil	Year	Fe	As	Cd	Cu	Mn	Pb	Zn
Wild olive	A	1999	53.8–3120	1.03–24.9	0.006–0.58	7.60–22.1	5.20–20.9	0.30–40.7	12.6–52.5
		2000	25.9–89.7	0.76–6.61	0.045–0.15*	7.70–42.5	5.30–66.3	0.62–1.15	19.9–74.6
		2001	21.5–53.7	0.12–0.21*	0.007–0.04*	6.70–16.4	5.20–27.5	0.20–0.43	9.40–30.3
	C	1999	45.9–2210	1.40–7.38	0.043–0.34	9.30–29.3	4.30–22.8	0.42–15.0	11.7–37.2
		2000	21.2–49.1	0.21–1.26	0.003–0.03	3.30–12.6	0.90–6.20	0.43–0.85	10.5–21.9
		2001	25.5–52.3	0.01–0.10	0.004–0.01	4.50–11.0	3.40–5.80	0.18–0.51	6.10–18.6
Tolerance level			–	50	0.5	300	2000	30	1000

Values marked with asterisks are significantly different from the control ($P<0.05$ of Student's t -test). Maximum levels tolerated by chicken (tolerance level) according to Chaney (1989) are indicated at the bottom of the table.

of an episode of local contamination on various wildlife species, it is necessary to bear in mind that there are numerous sources of individual variation (rates of absorption and assimilation, trophic position and weight, sex and age, distance from the spill, time of exposition, etc.).

Dietary Pb accumulates primarily in the bones, as well as in soft tissues, notably the kidneys. The harmful effects of chronic Pb exposure on birds includes growth impairment and subtle reproductive effects, such as reduced clutch size and inadequate Ca deposition in eggshells (Wright and Welbourn, 2002). Zn is also known to be toxic for birds (Ramey and Sterner, 1995).

Fortunately, the toxicity risk has decreased with time, as shown by the lower concentration of trace elements in the olive fruits for the year 2001 (Table 5). Nevertheless, the biomonitoring of trace elements in the fleshy fruits of wild olives and other shrubs and trees in the spill-affected area, should be continued, because these are the main source of food for a wide range of wintering birds.

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