

Factors affecting accumulation of thallium and other trace elements in two wild Brassicaceae spontaneously growing on soils contaminated by tailings dam waste

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Abstract

Thallium is a scarce, highly toxic element. There are several investigations that report Tl accumulation in plants of the family *Brassicaceae*. These plants could pose a risk in areas where Tl is present at higher concentrations than normal soils. The present study reports analyses of two wild Brassicaceae, *Hirschfeldia incana* and *Diplotaxis catholica*, growing spontaneously at five sampling sites moderately polluted with Tl and other trace elements in the Green Corridor of the Guadiamar river, Seville, S. Spain. In general, trace element content was unremarkable in all part plants, despite the concentrations present in soil. Thallium was the only element whose concentration in both plant species was above normal for plants (maximum values of 5.00 mg kg⁻¹ in *H. incana* flowers). There were significant positive correlations between total Tl in soil and Tl in both plant species. Transfer Coefficients (TC) for all elements were, in general, <1 for both species, except for Tl in flowers and fruits at some sites. The highest Enrichment Factor (EF) was found for Tl in *H. incana* fruits (EF = 607) and *D. catholica* flowers (EF = 321). *H. incana* was studied in a previous growing season (2004) in the same area, although the rainfall was 3 times more than in the year of the present study (2005), giving a maximum Tl content of 46.5 mg kg⁻¹ in *H. incana* flowers. The data presented here show that Tl content of plants growing in semi-arid conditions can be significantly influenced by precipitation. In dry years, plant Tl accumulation may be significantly reduced.

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1. Introduction

Thallium is a scarce element in nature, considered an environmental pollutant due to its significant potential for toxicity (Smith and Carson, 1977; Mulkey and Oehme, 1993). This element is toxic to humans, plants, and animals, and plays no role in their metabolism. Thallium is more toxic to mammals than cadmium, lead, copper, zinc or even mercury (Nriagu, 1998; Kazantzis, 2000).

Recently, more attention has been given to the occurrence of elevated concentrations of Tl in the environment. Environmental accumulation of Tl is the result of both geogenic and anthropogenic inputs (farming, mining, manufacturing processes, combustion of coal, and cement production) (Nriagu, 1998). The major pathway of exposure for animals and humans is the ingestion of plants grown in Tl-contaminated soils (Scheckel et al., 2004). When thallium is present in soils it may be easily taken up by plants because it is generally present as thermodynamically stable Tl (I), an analogue of potassium.

It is well known that the uptake rate of heavy metals can vary between plant species (Marschner, 1986). Tremel and Mench (1997a) and Tremel and Mench (1997b) showed

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that Tl is accumulated by sulphur rich plants from the family *Brassicaceae*. Several studies have investigated Tl accumulation in different members of this family. Crop plants such as green cabbage (Xiao et al., 2004), white cabbage (*Brassica oleracea capitata*) and kale (*Brassica oleracea acephala*) (Kurz et al., 1999) have been shown to accumulate Tl in edible parts (shoots and foliage). Soriano and Fereres (2003) reported relatively high Tl accumulation in the aerial biomass of *Brassica napus* and *B. carinata*. Other brassicas such as *Iberis intermedia* and *Biscutella laevigata* have been found to hyperaccumulate Tl in their shoot tissues (Anderson et al., 1999; Scheckel et al., 2004).

We studied two members of the Brassica family (*Hirschfeldia incana* and *Diplotaxis catholica*) growing spontaneously in soils moderately polluted with Tl and other trace elements ('Green Corridor' of the Guadiamar river, SW Spain, CMA, 2003). In a previous study, carried out in the spill affected soils at the same sites, we found that *H. incana* accumulated Tl in flowers up to a concentration of 50 mg kg⁻¹ (Madejón et al., 2005) despite the fact that the soil was only moderately polluted with Tl (Vidal et al., 1999; Martín et al., 2004). That study was carried out during a growing season with greater rainfall than the average for the area.

The Mediterranean climate shows a complex pattern of spatial and seasonal variability, with wide and unpredictable rainfall fluctuation from year to year (Martínez-Casnovas et al., 2002). The soils in the Mediterranean area are generally characterized by low water availability that can alter the availability of trace elements. Moreover, under drought conditions the capacity of plants to absorb nutrients and trace elements is severely diminished (Thomas, 1997). The year of the present study (2005) was significantly drier than that of the previous study (2004).

The aims of this study were the following: (1) to determine Tl and other trace elements content in plants of *H. incana* and *D. catholica* that had spontaneously re-colonised this polluted area during a year with lower than average rainfall and (2) to evaluate the potential risk from food chain transfer of Tl, based on field measurements.

2. Material and Methods

2.1. Study area

The study area is located in the Guadiamar river Valley (Seville, SW Spain). The area has a semi-arid Mediterranean climate with, in general, mild rainy winters and warm dry summers. The average annual rainfall was 484 mm for the period 1976–2001 (hydrological year). The hydrological year of the present study (1 September 2004–31 August 2005) was relatively dry; the total rainfall was only 246 mm (Fig. 1).

The upper part of the Guadiamar Basin is located in the South-Eastern edge of the Iberian Pyrite Belt, that constitutes the largest and most important volcanogenic massive sulphide province in Western Europe (López-Pamo et al.,

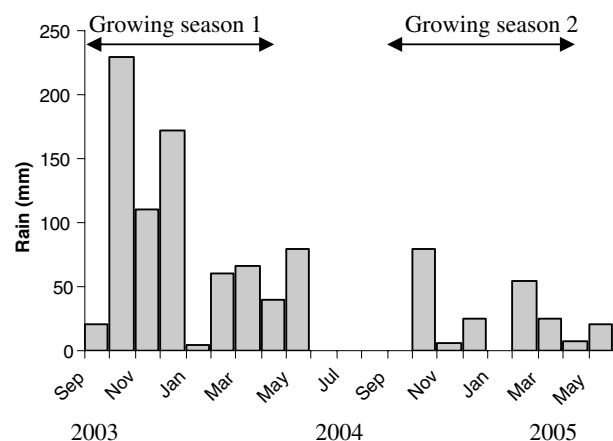


Fig. 1. Monthly precipitation September 2003–May 2005 (data from Met. Station at Coria del Rio, Seville). Sampling time and duration of growing season indicated.

1999), which has been exploited for copper and other ores since Roman times (ca. 2000 year ago). After the Aznalcóllar mine disaster (Grimalt and Macpherson, 1999) a large-scale plan of action was launched to mitigate damage from the mine accident, including the compulsory purchase of affected land (formerly devoted to crops and pasture) and the designation of a public wildlife reserve. This so-called 'Guadiamar Green Corridor' connects the lowlands (Doñana National Park) with the mountains of Sierra Morena (CMA, 2003).

2.2. Plants

Plants belonging to two wild members of the family *Brassicaceae* were sampled: *Hirschfeldia incana* (L.) Lagrèze-Fossat (Mediterranean mustard) and *Diplotaxis catholica* (L.) DC. Both are invasive weeds, frequent colonists of disturbed and cultivated areas, regenerating mainly from persistent seed banks in the soil.

A previous study identified *H. incana* as a Tl accumulator (Madejón et al., 2005). A further, more intensive sampling program, including comparison with the apparent non-accumulator *D. catholica* was undertaken to confirm the degree of Tl-accumulation in *H. incana*. Both species are annuals that produce abundant flowers and seeds.

2.3. Sampling sites and analysis

Five sampling sites were selected along a 20 km stretch in the Guadiamar Green Corridor. Site 1 (6°13' 17" N, 37° 31' 12" W), close to the Aznalcóllar mine (0.7 km), was upstream from the 1998 spill source and not affected by the spill. Sites 2 (6° 13' 18" N, 37° 29' 23" W), 3 (6° 13' 12" N, 37° 28' 14" W), 4 (6° 13' 05" N, 37° 26' 10" W) and 5 (6° 13' 57" N, 37° 23' 22" W) were all affected by the spill and were located 2.1, 3.3, 8.3 and 12.9 km respectively downstream from the mine. In general, CaCO₃ and soil organic carbon contents of the soils were <1% and

around 0.5%, respectively. Soil texture, sandy loam, was the same at all sampling sites.

Plant sampling was carried out in spring 2005 (April for *D. catholica* and May for *H. incana*). Whole plants at ripening stage (about stage 5.3 as defined by Harper and Berkenkamp, 1975) for *Brassica campestris* and *B. napus* were sampled from each site. At each sampling location, three patches of each species, separated by a distance of at least 50 m, were sampled. From every patch, three whole plants and the corresponding soil (0–25 cm depth) were taken. *H. incana* was collected at all five sites (15 samples), while *D. incana* was taken at four sites (12 samples, absent from site 5).

Prior to analysis, whole plants were partitioned into roots, stems, leaves, flowers and fruits. Plant material was washed in deionised water (twice) to remove any extraneous soil particles; particular attention was paid to roots, where effective soil removal was very important. The prepared plant samples were oven-dried at 70 °C, then ground to pass through a 500 µm stainless-steel sieve.

Composite soil samples (0–25 cm depth) were taken from the immediate area where plants were sampled. These were oven-dried to constant weight at 40 °C, ground to pass a 2 mm sieve, and then ground to <60 µm prior to trace element determination.

Plant samples were prepared for trace element (As, Cd, Cu, Tl and Zn) analysis by wet oxidation with concentrated HNO₃ under pressure in a microwave digester. Analysis of the digests was performed by ICP-MS (inductively coupled plasma-mass spectroscopy; Perkin Elmer, Sciex-Elan 5000), using an internal standard (Rh) and multielement standard solutions for calibration. Three reference standard materials were routinely analysed for quality assurance, BCR (Community Bureau of Reference) reference samples: BCR 62 (Olive leaves), CRM 279 (Sea lettuce) and CRM 281 (Ryegrass) (reference and experimental values are given in Madejón et al., 2006).

pH was analysed potentiometrically in a 1:2.5 soil-water solution. Total trace element content of soils (<60 µm) was determined by ICP-MS after digesting the samples to dryness with a mixture of concentrated HNO₃ and HF and subsequently redissolution in 4% concentrated HNO₃. EDTA-extractable trace element concentrations were determined by ICP-MS after extracting the samples (<2 mm) with a 0.05 M EDTA solution at pH 7.0. The soil sample (5 g) – EDTA solution (50 ml) mixture was shaken end-over-end at 30 rev/min for 1 h at room temperature (Ure et al., 1993). Total and EDTA-extracted trace element concentrations were calculated on a dry weight basis.

2.4. Metal transfer and enrichment factor coefficients

In order to find out what proportion of the total soil metal concentration was available and transferred to plant aboveground parts, the transfer coefficient (TC) was calculated. This is defined as the ratio of metal concentration in the plant $[M]_{\text{plant}}$ to the total metal concentration in the soil

$[M]_{\text{soil}}$ (Adriano, 2001). In this study we calculated TC for different plant organs, for example, $TC_{\text{flower}} = [M]_{\text{flower}} / [M]_{\text{soil}}$.

To evaluate differences in metal accumulation between plants growing in non polluted and polluted soils, the enrichment factor (EF) was calculated. This is defined as the ratio of metal concentration in organs of plants growing in contaminated soil to those in organs of plants growing in non-polluted soils.

2.5. Statistical analysis

Differences between trace element concentrations in soils and plants at different sampling sites were assessed by ANOVA. When soil and plant concentrations between sites were found to be significant, post hoc multiple comparison of mean values by Tukey's test was used. Data normality was tested prior to analysis and, when necessary, variables were logarithmically transformed. If after transformation, the data did not have a normal distribution, we used the non-parametric Kruskal–Wallis ANOVA by ranks test for analysis of variance.

Correlation analysis (Pearson *r*) was performed between the concentration of trace elements in soils (total and EDTA extraction) and in each plant organ. All statistical analyses were carried out using the SPSS 12.0 for Windows program.

3. Results and discussion

3.1. Thallium and other trace element in soils

Results of pH and trace element contents in soils are shown in Table 1. Although site 1 soils were up river from the dam burst, their trace element content, except for Tl, was some greater than the accepted background values for soils in this area, probably as a result of their proximity to both the mine and due to particulate re-entrainment from adjacent soils that had been affected by the spill. Background values: As (18.9 mg kg⁻¹), Cd (0.33 mg kg⁻¹), Cu (30.9 mg kg⁻¹), Tl (0.60 mg kg⁻¹) and Zn (109 mg kg⁻¹) are given by Cabrera et al. (1999); Simón et al. (1999).

In the spill affected soils (sites 2–5) arsenic concentration is always above potentially phytotoxic levels (Table 1) and exceeds both the proposed statutory maximum allowable value (<20 mg kg⁻¹) and the proposed intervention value (>50 mg kg⁻¹) suggested for Andalusian soils (Galán et al., 2002). Concentrations of the other elements were below intervention values for Andalusian soils: > 10 mg kg⁻¹ (Cd), >500 mg kg⁻¹ (Cu), >5 mg kg⁻¹ (Tl) and >1000 mg kg⁻¹ (Zn). Only Cu in soils 3, 4 and 5, and Zn in soils 3 and 5, are higher than the maximum allowable values (<100, 200 and 300 mg kg⁻¹, respectively) (Galán et al., 2002).

Soil concentrations at these sites could be potentially phytotoxic for Cu or Zn (Table 1). There is no current regulatory value for Tl in Andalusian soils (Table 1).

Table 1
pH, total and EDTA-extractable concentrations (mg kg⁻¹) of trace elements in soils (depth 0–25 cm) (mean ± SD, n = 6)

Soil	Fraction	pH	As	Cd	Cu	Tl	Zn
1	Total	7.7 ± 0.11	26.5 ± 13.1 a	0.49 ± 0.21 a	54.0 ± 15.7 a	0.61 ± 0.20 a	158 ± 48.2 a
2		5.9 ± 1.31	79.8 ± 60.0 a	0.69 ± 0.27 ab	71.3 ± 31.8 a	1.23 ± 0.85 ab	218 ± 81.6 ab
3		7.4 ± 0.07	194 ± 83.8 b	2.22 ± 0.81 c	162 ± 49.7 bc	2.29 ± 0.90 b	739 ± 234 c
4		6.1 ± 0.95	96.1 ± 37.1 ab	0.79 ± 0.19 ab	115 ± 29.3 ab	1.14 ± 0.46 ab	243 ± 51.5 ab
5		6.1 ± 0.95	134 ± 40.4 ab	1.35 ± 0.08 bc	204 ± 23.0 c	1.40 ± 0.34 ab	333 ± 38.2 b
1	EDTA		0.69 ± 0.80 a	0.21 ± 0.09 a	21.0 ± 13.1 a	0.015 ± 0.005 a	31.1 ± 13.4 a
2			1.10 ± 0.73 ab	0.32 ± 0.16 ab	22.4 ± 9.97 a	0.022 ± 0.02 a	37.9 ± 21.7 a
3			0.44 ± 0.29 a	0.86 ± 0.37 c	27.9 ± 8.90 a	0.023 ± 0.01 a	120 ± 53.9 b
4			3.77 ± 2.04 b	0.35 ± 0.10 abc	39.3 ± 8.45 ab	0.010 ± 0.003 a	34.5 ± 13.5 a
5				0.53 ± 0.10 a	0.66 ± 0.60 bc	68.3 ± 1.94 b	0.024 ± 0.01 a
Toxic for plants (range)			15–50	3–8	60–125	–	70–400

Total concentrations potentially toxic for plants (Kabata-Pendias and Pendia, 1992) are shown. For each element, values followed by the same letter do not differ significantly ($P < 0.05$).

The EDTA-extractable trace element concentrations of the soils are shown in Table 1. Availability of trace elements depends on numerous interrelated factors, and does not always relate to total soil concentrations. In this study, there are positive correlations for total and EDTA extraction in case of Cd ($r = 0.96$, $P < 0.05$), Zn ($r = 0.84$, $P < 0.05$), Cu ($r = 0.73$, $P < 0.05$) and Tl ($r = 0.68$, $P < 0.05$).

It is very probable that EDTA concentrations of extractable trace elements in neutral-alkaline soils are higher than the actual bio-available fraction (Vidal et al., 1999; Martín et al., 2004), so the concentrations in Table 1 are indicative, rather than absolute in relation to potential availability. In general, it seems that Tl and As have a low extractability compare to other elements. This accords with Vidal et al. (1999); Martín et al. (2004) who concluded in their studies that both elements showed low mobility in the spill-affected area.

The recent history of the site also needs to be considered here. The soils downstream from the dam burst had the layer of toxic sludge and approximately 10–25 cm of underlying topsoil mechanically removed shortly after the accident (Madejón et al., 2004). Whilst every effort was made to remove the sludge, there were inevitable instances where soils were inadvertently left in a contaminated state (difficulties in mechanical removal) or where sludge was physically incorporated into the exposed subsoil. This left a very heterogeneous distribution of trace elements in the soils from sites 2 to 5, which inevitably resulted in a considerable degree of variability between and within the different sites in terms of soil and plant metal concentrations.

3.2. Thallium and other trace element content in plants

Thallium was the only trace element where concentrations, in general, were above normal levels found in plants (0.05 mg kg⁻¹, Adriano, 2001). The highest concentrations were found in *H. incana* flowers and fruits (maximum values of 5.00 mg kg⁻¹ in flowers and 3.77 mg kg⁻¹ in fruits). This accords with a previous study (Madejón et al., 2005). These concentrations are above the 'security threshold' for

the food chain (2.5 mg kg⁻¹; Makridis and Amberger, 1996). Flowers of different *Brassicaceae* are not only can be eaten by animals, humans also eat them; in some parts of Spain they are called 'quesitos' (Tardío et al., 2005). These types of plants can be a potential risk for the food chain in soils where Tl exceeds background concentrations.

In general trace element content was unremarkable in all plant parts of both studied species (Tables 2 and 3), despite elevated concentrations of trace elements in soils at some of the sampled sites. Arsenic concentrations in both species were not elevated; in most cases these were very similar to normal concentrations found in plants (Tables 2 and 3), although soil As concentration was relatively high (Table 1). In the case of *D. catholica*, As concentrations in roots were higher than normal levels (this could be due, in part, to the presence of adhering particles). For this species the highest As concentrations in all plant parts were found in plants growing at site 3 (Table 3), where total As concentration was highest, although this was not the same for EDTA-extractable As (Table 1). Correlation between total soil As and plant As in this species was significant ($p < 0.01$), although there were no significant correlations with the EDTA extractable concentrations.

Cadmium, Cu and Zn concentrations in all plant organs of both species did not exceed normal plant values. In general, we found highest concentrations in plants growing at affected sites; in some cases there were significant differences between sites (Tables 2 and 3). The pattern of low accumulation of Cu in plants in this affected area has been reported for other plant species such as sunflower (Madejón et al., 2003) and wild grasses (Madejón et al., 2006).

3.3. Correlation of soil and plant Tl concentrations

There were significant positive correlations between total soil Tl concentrations and Tl content of some plant parts of *H. incana*: root ($r = 0.69$, $P < 0.01$), foliar ($r = 0.72$, $P < 0.01$) and stem ($r = 0.75$, $P < 0.01$). *D. catholica* also showed significant correlation between total soil Tl and root, foliar, stem ($r > 0.95$, $P < 0.01$), and fruit Tl

Table 2
Trace element concentrations (mg kg⁻¹ dry matter) in each plant part of *H. incana* (mean ± SD, n = 3)

Organ	Soil	As	Cd	Cu	Tl	Zn
Roots	1	0.05 ± 0.02 a	0.05 ± 0.01 a	3.85 ± 0.10 a	0.02 ± 0.003 a	29.7 ± 8.24 a
	2	0.21 ± 0.14 ab	0.18 ± 0.03 ab	4.54 ± 0.46 a	0.08 ± 0.04 b	105 ± 53.5 a
	3	0.67 ± 0.13 ab	0.32 ± 0.14 b	6.16 ± 1.58 ab	0.16 ± 0.11 b	93.6 ± 22.5 a
	4	0.65 ± 0.25 ab	0.18 ± 0.10 ab	5.30 ± 0.76 ab	0.02 ± 0.01 a	42.3 ± 20.3 a
	5	0.79 ± 0.46 b	0.36 ± 0.12 b	7.34 ± 1.30 b	0.07 ± 0.05 ab	101 ± 17.0 a
Leaves	1	0.77 ± 0.12 a	0.34 ± 0.23 a	10.0 ± 1.00 a	0.05 ± 0.01 a	130 ± 84.4 a
	2	0.89 ± 0.46 a	0.59 ± 0.03 ab	13.4 ± 7.38 a	0.17 ± 0.03 ab	387 ± 60.5 c
	3	1.37 ± 0.18 a	0.84 ± 0.24 b	12.3 ± 4.33 a	0.65 ± 0.33 b	302 ± 50.9 bc
	4	1.29 ± 0.47 a	0.41 ± 0.13 ab	11.9 ± 3.45 a	0.13 ± 0.05 a	62.4 ± 30.6 a
	5	1.24 ± 0.31 a	0.62 ± 0.10 ab	17.3 ± 4.85 a	0.11 ± 0.08 a	137 ± 78.5 ab
Stems	1	0.001 ± 0.0002 a	0.05 ± 0.02 a	4.40 ± 1.70 a	0.03 ± 0.01 a	27.6 ± 26.4 a
	2	0.012 ± 0.02 a	0.12 ± 0.03 ab	4.30 ± 0.79 a	0.03 ± 0.01 a	58.3 ± 15.5 a
	3	0.08 ± 0.07 a	0.36 ± 0.27 b	4.04 ± 0.36 a	0.37 ± 0.13 b	88.4 ± 41.3 a
	4	0.10 ± 0.08 a	0.15 ± 0.05 ab	4.10 ± 0.60 a	0.05 ± 0.02 a	21.1 ± 7.63 a
	5	0.011 ± 0.02 a	0.20 ± 0.09 b	4.11 ± 0.87 a	0.06 ± 0.03 a	40.3 ± 26.1 a
Flowers	1	0.10 ± 0.03 a	0.04 ± 0.03 a	8.78 ± 0.45 a	0.01 ± 0.01 a	75.6 ± 12.4 a
	2	0.18 ± 0.12 a	0.05 ± 0.01 a	11.7 ± 2.87 a	0.012 ± 0.01 ab	107 ± 37.1 a
	3	0.40 ± 0.10 a	0.08 ± 0.06 a	11.4 ± 1.26 a	0.72 ± 0.71 b	99.9 ± 22.1 a
	4	0.13 ± 0.04 a	0.06 ± 0.02 a	11.3 ± 1.88 a	2.86 ± 2.54 c	70.2 ± 9.80 a
	5	0.68 ± 0.94 a	0.32 ± 0.45 a	12.1 ± 2.66 a	0.015 ± 0.01 ab	70.1 ± 11.1 a
Fruits	1	0.08 ± 0.07 a	0.06 ± 0.04 a	5.85 ± 0.94 a	0.003 ± 0.002 a	51.9 ± 15.2 a
	2	0.06 ± 0.03 a	0.05 ± 0.03 a	8.34 ± 0.79 a	0.004 ± 0.002 a	74.8 ± 14.2 a
	3	0.18 ± 0.06 a	0.18 ± 0.16 a	7.56 ± 1.50 a	0.60 ± 0.53 a	74.3 ± 24.8 a
	4	0.17 ± 0.09 a	0.07 ± 0.02 a	7.63 ± 0.90 a	1.81 ± 1.88 a	41.0 ± 11.6 a
	5	0.06 ± 0.02 a	0.05 ± 0.01 a	7.78 ± 2.09 a	0.005 ± 0.002 a	54.9 ± 22.8 a
^A Normal levels in plants		0.01–1	0.1–1	3–20	0.05	15–150
^B Phytotoxic levels		3–10	5–700	25–40	20	500–1500

Values followed by the same letter, for each element, do not differ significantly ($P < 0.05$). Normal levels in plants and phytotoxic levels (from Chaney, 1989 and other authors) are shown.

^A Typical concentration for a wide range of plants (Adriano, 2001).

^B Excessive or toxic levels for mature plants (Kabata-Pendias and Pendia, 1992).

($r = 0.68$, $P < 0.05$). In contrast, correlation between EDTA-extractable and plant Tl was only significant in *H. incana* roots ($r = 0.71$, $P < 0.01$). This data suggests that soil concentration values after extraction with EDTA do not reflect bioavailable Tl in plants of both *Brassicaceae* species; total Tl concentration in soil may have a positive influence in the content of Tl in the aerial part of the plant. Tremel et al. (1997) found similar results with rape. *Thlaspi caerulescens* (another member of the *Brassicaceae*) has been shown to be more effective in mobilising Zn from less soluble, rather than mobile, fractions in the soil (McGrath et al., 1997). In a study of As accumulated by ferns, most of the As taken up was from the amorphous hydrous-oxide bound fraction rather than the more potentially bioavailable non-specifically bound fraction (Silva et al., 2006).

3.4. Transfer coefficients for Thallium and other trace elements

Thallium showed a different pattern of accumulation to all other metals that were studied. It exhibited high TC values in flowers (both species) and fruits (*H. incana*) (Table 4). These high ratios were found exclusively at site 4, although soil Tl concentrations (Total and EDTA-extractable) were

not the highest. The lowest TC values were found at site 1. These data show that when soil Tl is comparatively high, *H. incana* can take it up efficiently, but the striking result is the accumulation in the reproductive tissues (Madejón et al., 2005).

For the remainder of the trace elements, transfer coefficients for each plant fraction were < 0.01 , indicating low mobility from soil to aerial plant parts. In case of As, the range of TC values was between 0.00003 and 0.02 for *H. incana* and 0.002–0.02 for *D. catholica*. For Cu, Cd and Zn, TC values were somewhat higher, but always < 1 , except in leaves for Cd in *D. catholica* at site 2 (TC = 2.12) and for Zn in *H. incana* at site 2 (TC = 1.7). These results suggest that both plants showed limited accumulation of these elements in their aboveground parts and thus present a low food chain hazard, at least during periods of low rainfall.

3.5. Enrichment factors for thallium and other trace elements

The EF has been calculated using trace element contents found in plants growing at site 1 (non-affected by the spill). As we mentioned above these soils have higher trace ele-

Table 3
Trace element concentrations (mg kg⁻¹ dry matter) in each plant part of *D. catholica* (mean ± SD)

Organ	Soil	As	Cd	Cu	Tl	Zn
Roots	1	0.53 ± 0.07 a	0.18 ± 0.03 a	6.24 ± 0.27 a	0.02 ± 0.003 a	91.7 ± 7.03 a
	2	1.81 ± 0.74 ab	0.52 ± 0.17 a	7.29 ± 0.51 a	0.29 ± 0.12 b	164 ± 101 a
	3	6.65 ± 4.78 b	0.60 ± 0.06 a	12.4 ± 4.91 a	0.93 ± 0.78 b	159 ± 49.2 a
	4	2.64 ± 1.59 ab	0.72 ± 0.46 a	9.88 ± 3.64 a	0.26 ± 0.21 b	77.2 ± 24.3 a
Leaves	1	0.24 ± 0.09 a	0.19 ± 0.06 a	9.13 ± 2.26 a	0.01 ± 0.001 a	69.8 ± 9.5 a
	2	0.79 ± 0.36 ab	0.83 ± 0.26 b	16.4 ± 1.01 c	0.31 ± 0.08 bc	162 ± 70.3 a
	3	1.79 ± 0.84 b	0.85 ± 0.22 b	14.9 ± 0.86 bc	1.00 ± 0.79 c	192 ± 94.0 a
	4	0.56 ± 0.22 ab	0.44 ± 0.16 ab	11.5 ± 2.23 ab	0.08 ± 0.03 bc	43.6 ± 9.37 a
Stems	1	0.03 ± 0.01 a	0.07 ± 0.01 a	5.13 ± 0.38 a	0.003 ± 0.001 a	29.8 ± 2.66 a
	2	0.14 ± 0.02 ab	0.24 ± 0.01 b	8.00 ± 0.89 a	0.11 ± 0.05 b	53.3 ± 15.6 ab
	3	0.52 ± 0.39 b	0.24 ± 0.04 b	8.33 ± 0.35 a	0.32 ± 0.26 b	68.3 ± 20.3 b
	4	0.13 ± 0.05 ab	0.20 ± 0.05 b	8.28 ± 0.65 a	0.03 ± 0.03 ab	34.5 ± 3.00 ab
Flowers	1	0.15 ± 0.04 a	0.04 ± 0.02 a	10.2 ± 0.5 a	0.004 ± 0.001 a	80.3 ± 1.65 ab
	2	0.36 ± 0.23 ab	0.40 ± 0.09 b	14.5 ± 2.03 b	0.02 ± 0.005 a	95.2 ± 14.0 b
	3	1.25 ± 0.56 b	0.27 ± 0.11 ab	14.3 ± 0.42 b	0.07 ± 0.05 a	88.8 ± 9.5 ab
	4	0.48 ± 0.13 ab	0.35 ± 0.16 b	13.2 ± 1.82 ab	1.18 ± 2.00 a	70.3 ± 0.59 a
Fruits	1	0.06 ± 0.05 a	0.01 ± 0.005 a	8.09 ± 1.42 a	0.006 ± 0.001 a	52.0 ± 1.50 a
	2	0.48 ± 0.34 a	0.22 ± 0.03 b	12.8 ± 1.31 b	0.02 ± 0.006 ab	67.7 ± 15.0 a
	3	1.06 ± 0.67 a	0.16 ± 0.09 b	10.5 ± 0.57 ab	0.04 ± 0.03 b	60.2 ± 7.98 a
	4	0.45 ± 0.32 a	0.18 ± 0.04 b	11.0 ± 0.65 b	0.05 ± 0.05 b	59.3 ± 6.58 a
^A Normal levels in plants		0.01–1	0.1–1	3–20	0.05 a	15–150
^B Phytotoxic levels		3–10	5–700	25–40	20 b	500–1500

Values followed by the same letter, for each element, do not differ significantly ($P < 0.05$). Normal levels in plants and phytotoxic levels (from Chaney, 1989 and other authors) are shown.

^A Typical concentration for a wide range of plants (Adriano, 2001).

^B Excessive or toxic levels for mature plants (Kabata-Pendias and Pendias, 1992).

Table 4
Transfer coefficients (TC) for Tl for *H. incana* and *D. catholica*

Species	Site	Leaves	Stems	Flowers	Fruits
<i>H. incana</i>	1	0.06	0.03	0.01	0.003
	2	0.13	0.06	0.01	0.003
	3	0.27	0.15	0.32	0.27
	4	0.09	0.03	2.27 (4.9)	1.20 (1.9)
	5	0.08	0.04	0.01	0.004
<i>D. catholica</i>	1	0.02	0.006	0.01	0.01
	2	0.43	0.16	0.03	0.03
	3	0.43	0.13	0.03	0.02
	4	0.09	0.03	1.08 (3.18)	0.006

The maximum values are shown in brackets.

ment levels than other non-affected sites in the Guadiamar valley. The EF values found in this study could be lower than other EFs calculated with plants growing in soils with lower trace element contents.

The EF's for Cu and Zn in both species were low (Table 5), despite the high concentration of both elements in the affected soils (Table 1). The EF for Cu was below 2 (in all plant parts). In case of Zn, an element that is readily taken up by plants, the maximum EF values were approximately 3.0 in roots, leaves and stems, whilst values for flowers and fruits were around 1. These results could be related to the fact that Cu and Zn are micronutrients, whose absorption by plants is closely regulated, although

some plants are able to accumulate these micronutrients, as in the case of Zn in poplar trees (Madejón et al., 2004).

Thallium, As and Cd are not essential for plant nutrition, and they can present a food chain hazard, even at low concentrations. Absorption of these elements is not regulated, as they play no role in plant metabolism (Table 6). In general, greater EF values were found, especially for Tl.

The highest EF values for Tl were found in flowers and fruits (Table 5). The maximum EF values were found in *H. incana* fruits (EF = 607) and *D. catholica* flowers (EF = 321), both at site 4. These results suggest that, in field conditions, metabolic control of Tl absorption does not appear to be regulated in the manner of monovalent macronutrients, such as K (total and available soil K at site 4 were into the ranges found for all the soils, 4–8 g kg⁻¹ and 90–150 mg kg⁻¹, respectively).

Logan (1985) suggested that Tl uptake appears to be under metabolic control for some plants, and it is absorbed as the monovalent cation Tl⁺. He also observed that the uptake pattern is similar to those for other monovalent cations such as K⁺. Uptake into plants may be due to the close geochemical affinity between thallium and potassium. Because of their very similar ionic radii, Tl can readily substitute for K in plants during biogeochemical processes (Leblanc et al., 1999). In this study we could not find any significant correlation between total and available K in soil

Table 5
Enrichment factors for tissues of *H. incana* and *D. catholica*

Plant species	Organ	Soil	As	Cd	Cu	Tl	Zn
<i>H. incana</i>	Root	2	4.4	3.8	1.2	4.6	3.6
		3	13.9	6.7	1.6	9.6	3.2
		4	13.4	3.9	1.4	1.3	1.4
		5	16.3	7.5	1.9	4.0	3.4
		Leaves	2	1.1	1.7	1.3	3.4
	3	1.8	2.5	1.2	13	2.3	
	4	1.6	1.2	1.2	2.6	0.5	
	5	1.6	1.8	1.7	2.2	1.0	
	Stems	2	11.5	2.45	1	2.8	2.1
		3	84	7.4	1	13.8	3.2
		4	100	3.1	1	1.8	0.8
		5	11	4.1	1	2.3	1.4
		Flowers	2	1.8	1.05	1.3	1.2
	3		4.1	1.8	1.3	72.5	1.3
	4		1.2	1.2	1.3	286	0.9
	5		7.0	7.1	1.4	1.5	0.9
	Fruits		2	0.8	0.8	1.4	1.3
		3	2.2	2.8	1.3	200	1.4
		4	2.2	1.1	1.3	607	0.8
		5	0.7	0.8	1.3	1.6	1.0
<i>D. catholica</i>		Root	2	3.41	2.9	1.2	14.5
	3		12.5	3.3	2.0	46.5	1.7
	4		5.0	4.1	1.6	13.0	0.8
	Leaves		2	3.3	4.4	1.8	30.7
	3	7.5	4.5	1.6	100	2.7	
	4	2.3	2.3	1.3	7.9	0.6	
	Stems	2	4.7	3.4	1.6	36.6	2.0
		3	17.9	3.4	1.6	107	2.3
		4	4.3	2.8	1.6	10	1.2
	Flowers	2	2.4	10.5	1.4	5.4	1.2
		3	8.3	6.9	1.4	18.1	1.2
		4	3.2	9.0	1.3	321	1.1
	Fruits	2	8.0	15.7	1.6	3.8	1.3
		3	17.7	11.4	1.3	7.8	1.2
		4	7.5	12.8	1.4	9.0	1.1

(EF = $[M]$ plant grown in contaminated soil/ $[M]$ plant grown in control soil). Higher values are indicated in bold.

Table 6
Trace element concentration (mg kg⁻¹ dry matter) in different plant parts of *H. incana* (mean ± SD) sampled in two consecutive years ($n = 3$) at site 4

Organ	Sampling	As	Cd	Tl
Flower	2004	1.42 ± 0.40*	0.17 ± 0.04*	34.5 ± 16.6
	2005	0.13 ± 0.04	0.06 ± 0.02	2.86 ± 2.54
Stem	2004	0.24 ± 0.07	0.69 ± 0.40	0.27 ± 0.05*
	2005	0.10 ± 0.08	0.15 ± 0.05	0.05 ± 0.02
Leaf	2004	2.53 ± 0.51*	0.71 ± 0.30	0.29 ± 0.14
	2005	1.29 ± 0.47	0.41 ± 0.13	0.13 ± 0.05

Significant differences between sampling times are marked with asterisk.

and Tl in soil (total and EDTA-extractable Tl). Neither could we find any significant correlation between K and

Tl in different plant parts (data not shown). Tremel et al. (1997) found no correlation between Tl and K in seeds, and suggested that transport of K and Tl appeared to occur in different ways. This could explain the present results where no correlations between K and Tl were found in either species. It seems that for both species growing in semi-arid field conditions, there is no direct relation between K and Tl absorption.

3.6. Does annual rainfall affects Tl uptake in annual plants?

We have studied thallium concentration of *H. incana*, growing in the same area of polluted soils for two consecutive years. In general, trace element contents in plants from the first year (Madejón et al., 2005) were always higher than in the second year (data from site 4, maximum plant Tl content in 2004) (Table 6). Arsenic content of flowers from the 2004 sampling was 11 times higher than found in 2005, although the greatest difference was found for Tl. In 2004 mean Tl content in flowers was 34.5 mg kg⁻¹ (maximum 46.5 mg kg⁻¹) while in 2005 this only was 2.86 mg kg⁻¹ (maximum 5.0 mg kg⁻¹). This could be related to differences in rainfall between the years (Fig. 1). Total rainfall before the first sampling in 2004 was 703 mm (eight months), while it was only 219 mm in 2005 (nine months). Similar results were found by Pascual et al. (2004) who concluded that transfer of some metals from soil to plant was reduced under dry conditions.

Monovalent cations, especially those present in low concentrations are not exchangeably adsorbed on to electro-negative sites in the soil matrix, but exist as free ions in solution. This process is facilitated by water fluxes in the soil–plant continuum, which is ultimately caused by loss of water to the atmosphere via transpiration (Epstein, 1972). The movement of metals from the external solution into the cell walls is basically a non-metabolic, passive process, driven by diffusion or mass flow. Soil moisture can also influence the rate of root growth, as showed by Kuchenbuch and Barber (1988) for corn in a nine-yearly root measurements field experiment. Root length density in the upper 15 cm of soil was significantly correlated with precipitation during the 3-week period prior to silking.

Despite the fact that the plants could take up Tl from the less available soil fractions, it seems apparent from data presented here that Tl content of plants growing in semi-arid conditions can be significantly influenced by soil hydrology and hence soil water content. In dry years, plant Tl accumulation may be significantly reduced although studies are still necessary to investigate this further. A programme of plant sampling of these Brassicaceae is recommended to determine their risk for food chain transfer. Additional biomass measurements would also be required to quantify differences in Tl transfer in absolute terms. Despite the variable nature of contamination of both soils and plants in the sampled area, and the differences found between the two growing seasons, the results give a clear indication of the selectivity of Tl uptake by *H. incana*

and its ability to accumulate this element in reproductive structures.

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