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Thallium Accumulation in Floral Structures of *Hirschfeldia incana* (L.) Lagrèze-Fossat (Brassicaceae)

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The upper part of the Guadiamar Basin, in the south of Spain, is located in the pyritic mining belt, which has been mined for copper and other ores since Roman times (ca. 2000 years ago). In April 1998, a mine spill (of ca. 5 x 10^6 m^3 of slurry) affected about 55 km^2 downstream of Aznalcollar (Seville) along the course of the Guadiamar River (for a review of the accident, see the special issue edited by Grimalt et al., 1999). This had a major ecological impact because the river discharges into the Guadalquivir marshes of Doñana National Park, which is a wildlife site of international importance and a wintering area for many European water birds.

Soils were polluted by the trace elements As, Bi, Cd, Cu, Pb, Sb, Tl and Zn (Cabrera et al., 1999). Soon after the mine spill, an emergency soil clean-up operation started (CMA, 2001). Toxic sludge was mechanically removed and disposed off in an open-pit mine. This operation, which lasted six months, removed the sludge and a major portion of the contaminated surface soil; however, the spill-affected zone was found to be still polluted by trace elements with a fairly irregular distribution (Madejón et al., 2002).

Despite elevated Tl concentration in the sludge (up to 60 mg kg^{-1} Cabrera et al., 1999), its concentration in most of the plants examined in the affected area was very low. Different plant organs of a variety of species were analysed: the leaves and stems of poplar (*Populus alba*) (Madejón 2003); the shoots and roots of bermudagrass (*Cynodon dactylon*) (Madejón et al., 2002) and the leaves, stems and reproductive structures of sunflower (*Helianthus annuus*) (Murillo et al., 1999; Madejón et al., 2003). In all these species Tl concentration was within the range 0.02 – 0.7 mg kg^{-1}. This could be partly explained by the low mobility of Tl in the spill-affected soils (Vidal et al., 1999), although other authors have claimed greater mobility of Tl in the environment (Kabata-Pendias and Pendias, 2001).

A recent study by Soriano and Fereres (2003) reported relatively high Tl accumulation in the aerial biomass of the crop plants *Brassica napus* and *B. carinata*, cultivated in experimental plots within the spill-affected soils of the Guadiamar Valley. Here we explore the accumulation of Tl and other trace

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elements in wild members of the *Brassicaceae* family. We selected *Hirschfeldia incana* and *Raphanus raphanistrum* L., subsp. *raphanistrum* (a taxon which belongs to the same tribe as *Hirschfeldia*), both abundant in the spill-affected area. We also analysed *Plantago lanceolata* L. (*Plantaginaceae*), to confirm evidence of Tl accumulation found in this species elsewhere (Wierzbicka et al., 2004).

MATERIALS AND METHODS

Following clean up, remediation of polluted soils and afforestation, the spill-affected area within the Guadiamar and Agrio floodplains was protected and named "Paisaje Natural del Corredor Verde del Guadiamar" (The Guadiamar Green Corridor Nature Landscape) (CMA, 2001). Five study sites were selected within this general area: Site 1 (37° 29' 55" N; 6° 13' 17" W), although close to the Aználcollar mine, was upstream from the spill source and so not affected; it has been taken as a reference control; Site 2 (37° 28' 48'' N; 6° 12' 43'' W) was about 2 km downstream from the mine and affected by the spill. Site 3 (37° 28' 9'' N; 6° 12' 42'' W) was located where there had been a temporary storage pool for sludge, prior transport and disposal, and was therefore extremely polluted. Sites 4 and 5 (37° 23' 13'' N; 6° 13' 36'' W) were 15 km downstream from the mine and were polluted to different levels. Cabrera et al. (1999) and Galán et al. (2002) give general information on composition of sludge and affected soils in the Guadiamar Basin.

Plant sampling was carried out during May 2004. The selected study sites had patched, sparse vegetation. Plants were sampled from areas within each site that showed adequate plant growth as well as species diversity. A minimum of 3 such areas were sampled within each of the 5 chosen sites. *Hirschfeldia* was collected at all five sites (15 samples), while *Brassica* and *Plantago* were only found at sites 1, 2 and 3 (9 samples). The aerial parts of the plants were collected and transported to the laboratory. Plant material received 3 consecutive 10 s washes with a solution of phosphate-free detergent, with a 0.1 N HCl solution and with distilled water. The plant material was sorted into leaves, stems and flowers, dried at 70° C, then ground and passed through a 500 μm stainless-steel sieve.

Nitrogen concentration was measured by Kjeldahl digestion. Macronutrients (P, K, S and Ca), micronutrients (Cu, Fe, Mn and Zn) and other elements (As, Cd, Pb and Tl) were extracted by wet oxidation with concentrated HNO_3 using microwave assisted pressure digestion. Analysis of nutrients (including Fe and Mn) in the extracts was obtained by ICP-OES (inductively coupled plasma spectrophotometry), while the analysis of the other elements (As, Cd, Cu, Pb, Tl and Zn) was performed by ICP-MS (inductively coupled plasma-mass spectroscopy). 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), (Table 1). Student *t*-tests were used to assess differences between paired mean values from the affected and unaffected soils. A significance level of $p<0.05$ was used throughout the study.

Table 1. Analysis of BCR reference samples (mean values ± 95% confidence interval, in mg kg^{-1} of dry matter) for trace elements.

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	0.118 ± 0.014
Cd	0.274 ± 0.022	0.202 ± 0.007	0.120 ± 0.003	0.117 ± 0.005
Cu	13.14 ± 0.37	11.63 ± 0.73	9.65 ± 0.38	9.76 ± 0.09
Fe	(2300 ± 100)	2113 ± 72.3	(164 ± 13)	150 ± 28
Mn	(2030 ± 31.5)	1758 ± 64.8	81.6 ± 2.6	79.2 ± 5.7
Pb	13.48 ± 0.36	12.47 ± 1.09	2.38 ± 0.11	2.29 ± 0.07
Tl	(0.038 ± 0.005)	0.027 ± 0.005	-	-
Zn	51.3 ± 1.2	52.18 ± 3.29	31.5 ± 1.4	32.7 ± 0.2

(Values in brackets are indicative. Experimental values are calculated from N=6 (sea lettuce) and N=5 (ryegrass)).

RESULTS AND DISCUSSION

The floral structures of *Hirschfeldia* were very rich in nitrogen, and consequently in proteins (estimated as ca. 30 %) in both types of soil (Table 2). The two brassicaceae species had relatively high values of N, P, S and K in flowers, with much lower values found in *Plantago* (Table 2). High nutrient levels in there organs will probably produce seeds with nutrient-rich reserves and foster seedling establishment. On the other hand, the nutrient-rich organs of these plants will be consumed preferentially by primary consumers.

Table 2. Nutrient concentration in the floral structures of *Hirschfeldia incana* (Hi), *Raphanus raphanistrum* (Rr) and *Plantago lanceolata* (Pl), collected in unaffected (UN) and spill-affected (A) soils.

Species	Soil	N	P	S	K	Ca
Hi	UN	4.74±0.02	0.68±0.02*	1.17±0.04	2.78±0.09	0.78±0.02*
	A	4.47±0.15	0.58±0.02	1.09±0.03	2.70±0.06	1.03±0.05
Rr	UN	3.49±0.32	0.51±0.02	1.15±0.02	2.77±0.12	0.92±0.06
	A	3.86±0.29	0.50±0.03	1.31±0.10	2.60±0.02	1.11±0.09
Pl	UN	1.66±0.17*	0.37±0.01	0.30±0.01*	1.38±0.07	0.99±0.05
	A	0.89±0.06	0.37±0.02	0.62±0.08	1.20±0.08	1.53±0.16

(Mean values ± standard error, expressed as a percentage of dry matter. For each species, significant differences between soils are marked by an asterisk. For the UN soils, N=3 for all species; for the A soils, N=12 for Hi, and N=6 for Rr and Pl).

Calcium was slightly higher in the *Plantago* spikes than in the brassicaceae flowers, in particular in the spill-affected soils. It is remarkable that the Ca concentrations were systematically greater in the affected soils; this pattern was also observed for the leaves and stems in the three species (data not shown). The higher Ca levels in the plant tissues could have a favourable, protective effect against trace metal toxicity.

The concentration of micronutrients in the floral structures of plants growing in spill-affected soils was very high, even reaching toxic levels in some cases (Table 3). Copper levels were significantly higher in the spill-affected soils than in the control, for all the species, and the highest value (23.3 mg kg^{-1} for *Hirschfeldia*) was close to the maximum level tolerated by sheep (25 mg kg^{-1}, according to Chaney, 1989). The highest accumulation of Fe was found in the *Plantago* spikes, up to 2670 mg kg^{-1}, above the maximum level tolerated by cattle, sheep and chicken (500-1000 mg kg^{-1}; Chaney, 1989). The Brassicaceae species had some differential accumulation of Zn in the spill-affected soils (although not statistically significant), but the levels were below the toxicity threshold for animals (300-1000 mg kg^{-1}; Chaney, 1989).

Table 3. Micronutrient concentration in floral structures of *Hirschfeldia incana* (Hi), *Raphanus raphanistrum* (Rr) and *Plantago lanceolata* (Pl), collected in unaffected (UN) and spill-affected (A) soils.

Species	Soil	Cu	Fe	Mn	Zn
Hi	UN	8.15±0.48*	155±29	55.8±2.0	75.8±2.5
	A	18.3±0.92 (23.3)	502±135 (1470)	94.0±19.6 (194)	102±7.4 (143)
Rr	UN	7.84±0.43*	77.0±3.1	73.3±13.9	54.3±3.5
	A	12.3±1.0 (16.3)	192±46 (340)	63.7±29.6 (175)	82.1±11.4 (124)
Pl	UN	9.78±0.86*	406±116	115±13	97.8±11.8
	A	13.1±1.73 (20.2)	1306±318 (2670)	78.4±15.1 (147)	84.2±9.25 (124)

(Mean values ± standard error, expressed as mg kg^{-1} of dry matter. For each species, significant differences between soils are marked by an asterisk. For the UN soils, N=3 for all species; for the A soils, N=12 for Hi, and N=6 for Rr and Pl. The maximum value reached in the affected soils is shown in brackets).

In general, all the species had a significantly higher accumulation of trace elements in their organs, when growing in the contaminated soils (Table 4). The highest value of As was found in the spikes of *Plantago*, up to 22.8 mg kg^{-1}, exceeding phytotoxic levels (3-10 mg kg^{-1}), but below the tolerated levels for animals (50 mg kg^{-1}; Chaney, 1989). The leaves of *Raphanus* had the highest value for Cd, up to 5.13 mg kg^{-1}, being above the tolerated level for animals (0.5 mg kg^{-1}). The spikes of *Plantago* also had the highest concentration of Pb, up to 47.5 mg kg^{-1}, with potential toxicity for animals (above 30 mg kg^{-1}; Chaney, 1989). The most striking result was the very high accumulation of Tl in the reproductive structures of *Hirschfeldia*, up to 46.5 mg kg^{-1}, well above the phytotoxic level (20 mg kg^{-1}; according to Kabata-Pendias and Pendias, 1992).

Thallium tended to accumulate in leaves and stems of plants growing in polluted soil, between of 8 to 34 times higher than in the control soil, *Plantago* having the lowest values. The flowers of *Raphanus* (4 times higher than the control) and *Plantago* (30 times) accumulated Tl within the same range; the striking exception was the unprecedented accumulation of Tl in *Hirschfeldia* flowers: at least 1340 times higher than the control. Exceptional accumulation of Tl in the flowers has also been documented for *Galium* sp. (*Rubiaceae* family), (Kabata Pendias and Pendias 2001).

Table 4. Trace element concentrations in the floral structures (F), leaves (L) and stems (S) of *Hirschfeldia incana* (Hi), *Raphanus raphanistrum* (Rr) and *Plantago lanceolata* (Pl), collected in unaffected (UN) and spill-affected (A) soils.

Organ	Species	Soil	As	Cd	Pb	Tl
F	Hi	UN	0.29±0.02*	0.05±0.02*	0.49±0.09*	0.01±0.001*
		A	3.34±1.11	0.19±0.02	4.31±1.47	13.4±5.3
			(11.8)	(0.36)	(14.8)	(46.5)
F	Rr	UN	0.76±0.05	0.07±0.006*	0.54±0.06*	0.02±0.02*
		A	2.41±1.12	0.59±0.08	3.08±1.95	0.08±0.02
			(6.83)	(0.88)	(10.8)	(0.15)
F	Pl	UN	0.62±0.01	0.13±0.02	1.88±0.58*	0.01±0.001
		A	9.00±3.54	0.21±0.05	17.7±7.4	0.30±0.19
			(22.8)	(0.42)	(47.5)	(1.21)
L	Hi	UN	0.49±0.14*	0.18±0.04*	1.01±0.32*	0.02±0.007*
		A	3.45±0.58	1.04±0.13	5.20±1.45	0.53±0.17
			(7.48)	(1.68)	(15.0)	(1.94)
L	Rr	UN	1.40±0.18*	0.57±0.06*	1.62±0.20	0.04±0.009
		A	4.36±1.11	3.75±0.71	4.65±1.88	0.58±0.35
			(8.01)	(5.13)	(11.5)	(2.04)
L	Pl	UN	0.69±0.03*	0.71±0.13	1.37±0.32*	0.04±0.004
		A	5.07±1.43	1.04±0.13	10.7±3.36	0.34±0.13
			(10.2)	(1.48)	(23.3)	(0.85)
S	Hi	UN	0.16±0.02*	0.04±0.008*	0.11±0.02*	0.01±0.001*
		A	0.36±0.08	0.41±0.08	0.52±0.17	0.34±0.11
			(1.10)	(1.14)	(2.11)	(1.10)
S	Rr	UN	0.48±0.02	0.26±0.03*	0.31±0.03*	0.02±0.006*
		A	0.72±0.16	1.59±0.32	0.99±0.41	0.29±0.14
			(1.24)	(2.13)	(2.81)	(0.97)
S	Pl	UN	0.21±0.01*	0.35±0.09	0.48±0.07	0.01±0.002
		A	0.66±0.13	0.36±0.08	1.18±0.26	0.08±0.03
			(1.18)	(0.60)	(2.27)	(0.15)

(Mean values ± standard error, expressed as mg kg^{-1} of dry matter. For each species, significant differences between soils are marked by an asterisk. For the UN soils, N=3 for all species; for the A soils, N=12 for Hi, and N=6 for Rr and Pl. The maximum value reached in the affected soils is shown in brackets).

Given the high Tl concentration found in the reproductive structures of *Hirschfeldia*, a more exhaustive monitoring of plants growing in the soils most affected by the mine spill is recommended; in particular, the monitoring of other species within the family *Brassicaceae*.

The toxicity of Tl^+ on humans and animals results from Tl^+ mimicking K^+ in metabolic processes. It may also bind with sulfhydryl groups of proteins to inactivate many enzymatic reactions. Nonetheless, for many years Tl has not been considered an environmental pollutant. For example, in Poland, only recent biomonitoring studies using magpie (*Pica pica*) feathers as a bioindicator has indicated the need for such studies (Wierzbicka et al., 2004).

Thallium was recognised as constituent of the sludge polluting the Guadiamar Valley (S. Spain) after the mine spill. However, early surveys of plants growing in the polluted soils only detected low levels in leaves, stems and fruits of several woody and herbaceous species: white poplar; bermudagrass; holm oak; wild olive and sunflower (Madejón, 2003; Madejón et al., 2002, 2003). Our finding shows that *Hirschfeldia incana*, a member of the *Brassicaceae* family, accumulates high concentrations of Tl in its flowers, when growing in Tl-polluted soils. This may have significant consequences for the environment. Extreme values of Tl have been reported for plants growing in mineralized or industrialized areas (Kabata Pendias and Pendias, 2001; Wierzbicka et al., 2004; Xiao et al., 2004). The concentration of Tl in the nutritive N-rich organs, such as the reproductive structures, which are consumed by herbivor, deserves special monitoring of the Tl dynamics in the food chain. Xiao et al. (2004) consider that a full understanding of the behavior of Tl in a local ecosystem is important for the identification, remediation and management of Tl-related health and environmental problems. The utility of separating plant organs for analysis to detect differential Tl allocation, which has ecophysiological and ecosystemic consequences, needs to be emphasised.

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