Cadmium tolerance and hyperaccumulation by Thlaspi caerulescens populations grown in hydroponics are related to plant uptake characteristics in the field

Keller, Catherine; Diallo, Saliou Bella; Cosio, Claudia; Basic, Nevena; Galland, Nicole

How to cite

KELLER, Catherine et al. Cadmium tolerance and hyperaccumulation by Thlaspi caerulescens populations grown in hydroponics are related to plant uptake characteristics in the field. In: Functional plant biology, 2006, vol. 33, n° 7, p. 673–684. doi: 10.1071/FP05217

This publication URL: https://archive-ouverte.unige.ch//unige:17918
Publication DOI: 10.1071/FP05217

CSIRO PUBLISHING

www.publish.csiro.au/journals/fpb *Functional Plant Biology*, 2006, **33**, 673–684

Cadmium tolerance and hyperaccumulation by *Thlaspi caerulescens* populations grown in hydroponics are related to plant uptake characteristics in the field

Catherine Keller[A,C,D], *Saliou Diallo*[A], *Claudia Cosio*[A], *Nevena Basic*[B] *and Nicole Galland*[B]

[A]Swiss Federal Institute of Technology, ENAC-ISTE-Laboratory of Soil Science, Ecublens, 1015 Lausanne, Switzerland.

[B]University of Lausanne, Department of Ecology and Evolution, Biology Building, 1015 Lausanne, Switzerland.

[C]Present address: CEREGE, Université Paul Cézanne — Aix-Marseille III, Europôle Méditerranéen de l'Arbois, BP 80, 13545 Aix-en-Provence, Cedex 4, France.

[D]Corresponding author. Email: keller@cerege.fr

Abstract. In order to fully understand the hyperaccumulation process and to increase the potential of plants for phytoextraction purposes, there is a need for more investigation of hyperaccumulating species or populations. Five Swiss populations of *Thlaspi caerulescens* J. & C. Presl originating from non-metalliferous but naturally Cd-rich soils (1.1–9.2 mg Cd kg^{-1}) were compared with Ganges and Prayon populations and a non-accumulating species, *Thlaspi perfoliatum* (L.) F.K. Meyer, for their tolerance (shoot and root dry weight and root length) and Cd hyperaccumulation in hydroponics (0, 1, 5, 10, 20 and 50 μM Cd). In the field, the Swiss populations accumulated Zn and clearly hyperaccumulated Cd (up to 505 mg Cd kg^{-1} dry weight). The general response was significantly different between populations but in general an increasing Cd concentration in solution led to a decrease in dry weight production and an increase in Cd concentration in shoots. The shoot dry weight was a more discriminating parameter for tolerance than root dry weight and total root length. The Swiss populations behaved similarly to the Ganges population but differently from the Prayon population. Cadmium concentrations in shoots were above 100 mg kg^{-1} when plants were grown in 1 μM Cd, except for the Prayon population and *T. perfoliatum*. In addition, as 1 μM Cd did not induce any visible toxicity symptoms, it was found to be adequate to test Cd hyperaccumulation. However, the most striking feature was the positive linear relationship observed between the transfer factor (TF) calculated in the field and the response of a population to increasing Cd concentrations in solution, indicating that plant uptake in the field had an influence on the plant response in solution.

Keywords: soil, *Thlaspi caerulescens*, *Thlaspi perfoliatum*.

Introduction

The extraction of heavy metals by plants has been proposed as a technique for remediation of contaminated soils. Hyperaccumulators that are specific to one or several metals are potential candidates, but the agronomic requirements of these species are poorly understood. Additionally they are often composed of different populations restricted to one area and variously adapted to contaminated sites. The stock of seeds available is also limited, and to our knowledge, so far no systematic nursery programs have been launched on a commercial basis. The consequence is that today there exist a very limited number of species and / or populations that could be used in a large-scale remediation scheme. Their number is steadily increasing, but there is still a need for a broader choice of plants (populations and seed reservoirs) (Whiting *et al*. 2004).

Thlaspi caerulescens J. & C. Presl, a hyperaccumulator of Zn, Cd and Ni (depending on the population tested), has been recognised as an interesting model for studying hyperaccumulation (Assunção *et al.* 2003). Approximately 20 populations have been studied, mostly originating from metalliferous or serpentine sites (Baker *et al*. 1994;

Abbreviations used: DW, dry weight; NOEC, no observed effect concentration; TF, transfer factor; TI, tolerance index.

 10.1071/FP05217 1445-4408/06/070673

Meerts and Van Isacker 1997; Schat *et al.* 2000; Roosens *et al.* 2003; Schwartz *et al.* 2003). Few non-metallicolous populations have been investigated for their tolerance and metal uptake ability (Escarré *et al.* 2000). Reeves *et al.* (2001) surveyed populations in France and Luxembourg growing on both metallicolous and non-metallicolous soils. Recently Molitor *et al.* (2005) screened 47 *T. caerulescens* populations grown on non-metallicolous soils in Luxembourg. In all cases except one, *T. caerulescens* populations grown on non-metallicolous soils (low Cd concentration in soil) had Cd concentrations in their shoots below 0.01%.

These populations all exhibited intra- and inter-population variations in the uptake of Cd, Zn and Ni, either together or individually, and in the amounts accumulated (Zhao *et al.* 2003), as well as site characteristics. Thus, it seems probable that populations originating from different areas may present different properties (Schat *et al.* 2000). This is especially true for Cd uptake that varies substantially between populations leading to bioaccumulation factors either below or above one (Zhao *et al.* 2003), which is the ratio set as the limit to define hyperaccumulation (Baker 1981) and tolerance criteria discriminating metallicolous from non-metallicolous populations. The latter populations have been found to be less tolerant but able to accumulate more Zn but less Cd than the former population (Ingrouille and Smirnoff 1986; Meerts and Van Isacker 1997; Escarré *et al.* 2000; Schat *et al.* 2000). However, it is interesting to note that two of the most-studied populations, Prayon and Ganges, are both clearly metallicolous populations growing on soils contaminated with Zn and Cd (Roosens *et al.* 2003). They differ in their Cd uptake ability both in soil and hydroponics, Ganges hyperaccumulating Cd to a higher degree than Prayon (Lombi *et al.* 2000). Thus, the criterion 'metallicolous' or 'non-metallicolous' might not solely explain the differences observed between populations. Furthermore, it is based on soil characteristics but there is no consensus on the metal concentration that sets the limit between the two. Pollard *et al.* (2002) have suggested that a continuum may exist between the two types of populations for both tolerance and hyperaccumulation.

To contribute to the investigation of the extent of Cd tolerance and hyperaccumulation variability and the relationship with their uptake characteristics within the *T. caerulescens* species in the field, five natural populations from the Swiss Jura and the Prealps were collected on non-metalliferous soils with various naturally occurring Cd concentrations and low Cu and Zn concentrations. They were compared to two well-studied populations growing on metalliferous soils: Ganges hyperaccumulating Cd and Zn and Prayon hyperaccumulating only Zn in the field. *Thlaspi perfoliatum* L. was also collected in the Jura for use as a control plant, as it does not show hyperaccumulating traits (Reeves *et al.* 2001; Peer *et al.* 2003). All populations were grown in hydroponics with a range of Cd concentrations and were compared for tolerance by root and shoot dry weight and root length. The level of Cd accumulation was also compared across populations by measuring Cd concentrations in the shoots.

Materials and methods

Origin of the Thlaspi *populations*

Four natural populations of *Thlaspi caerulescens* J. & C. Presl. growing on the Jura chain in Switzerland (le Sentier, les Avattes, Gurnigel 1 and Gurnigel 2) were selected to cover a large range of Cd concentration in the soil of origin and so that they were located far away from each other (Table 1). One population was collected in the Prealps (Leysin) at approximately the same altitude. These five populations were selected from a larger panel of 25 populations originating from both the Jura and the Alps and Prealps (Basic *et al.* 2006*a*, *b*). They were compared with the Cd and Zn hyperaccumulating Ganges population (les Avinières, St-Laurent-le-Minier, France) and the Zn hyperaccumulating Prayon population (Prayon, Belgium). One population of *Thlaspi perfoliatum* (L.) F.K. Meyer, also from the Jura (Bern canton) was used as control, non-hyperaccumulating plant. *Thlaspi arvense* L. is often used as a control plant. However, *T. perfoliatum* is a smaller plant than both *T. arvense* L. and *T. caerulescens* and its growth rate and the time needed to obtain mature plants are more similar to those of *T. caerulescens*. Additionally, the ecology and physiology of *T. perfoliatum* has been studied in detail (Baskin and Baskin 1979; Koch and Hurka 1999; Peer *et al.* 2003) and *T. perfoliatum* was considered a good alternative to *T. arvense* as control plant.

All Swiss test populations were collected on undisturbed soils originating from calcareous bedrock, whereas Ganges and Prayon were collected on metalliferous soils in industrial or mining areas. The five Swiss *T. caerulescens* sites were exploited as meadow. Mature plants from the five local *T. caerulescens*, the *T. perfoliatum* population and the Ganges population were sampled (three or four individuals) and analysed for their heavy metal content (see below for the method). The Prayon population could not be sampled in the field. Seeds from all populations were collected in the field the year the experiment was performed.

Selected soils characteristics are presented in Table 1. For the Jura and the Prealps populations, soil samples were taken immediately adjacent to the plants sampled. Data for the Prayon and Ganges populations are given for comparison and they were either taken from the literature (Prayon; Roosens *et al.* 2003) or from soil samples collected at St-Laurent-le-Minier (les Avinières, 'Ganges') but not necessarily related to the place were the seeds had been collected. The Swiss soils were distinctly different from the metalliferous soils as they had a greater carbon content, a larger cation exchange capacity and lower Zn and Cd total and soluble concentrations. Cadmium concentrations measured in the soils of the Jura populations were high and above the level set for background concentration according to the Swiss legislation (OIS 1998). They were greater than the median value of $0.31\ mg\ kg^{-1}$ Cd calculated for agricultural topsoils in Switzerland (Meyer 1991). This is a common characteristic of the soils from the Swiss Jura (Atteia *et al.* 1994), where the origin of Cd has been shown to be lithogenic (Dubois *et al.* 1998; Baize and Sterckeman 2001) and this is probably also the case for the Prealps site (Doffey 1997). However, the Cd concentrations were low when compared with most contaminated soils. Copper concentrations were low and $\sim 10\ mg\ kg^{-1}$ for all soils except for Leysin ($67 \pm 30\ mg\ kg^{-1}$) and Prayon ($4000 \pm 648\ mg\ kg^{-1}$) (Roosens *et al.* 2003).

The transfer factor (TF) was calculated as the ratio between concentration in plant dry weight (DW) and 2 M-HNO_3-extractable Cd concentration in soil.

Table 1. Selected sites and soils characteristics where *Thlaspi caerulescens* and *Thlaspi perfoliatum* populations were collected and Cd and Zn concentrations in the shoots of *T. caerulescens* and *T. perfoliatum*

CH, Switzerland; F, France; B, Belgium. Values for Cd and Zn concentrations in soil and plants are means ± s.d. of 3–5 soil samples and three or four plant samples expressed in mg kg^{-1} soil and shoot dry weight. For Zn, some populations have no s.d. because $[Zn]_{soluble}$ of some replicates was below detectable limits (bdl). The transfer factor is calculated as the ratio between plant and soil pseudo-total concentrations

		Soil								Plant			
						Pseudo-total[D]		Soluble[E]		Total[F]			
Population	Location	Altitude (m a.s.l.)	pH_{CaCl_2} [C]	C_{org} (%)	CEC[C] ($cmol\,kg^{-1}$)	Cd ($mg\,kg^{-1}$)	Zn ($mg\,kg^{-1}$)	Cd ($\mu g\,kg^{-1}$)	Zn ($mg\,kg^{-1}$)	Cd ($mg\,kg^{-1}$)	Zn ($mg\,kg^{-1}$)	Transfer factor Cd	Transfer factor Zn
T. c. le Sentier	Jura, le Sentier (CH)	1100	6.1	10.2	69	2.1 ± 0.0	72 ± 8	4.1 ± 1.4	0.88 ± 0.30	102 ± 26	2881 ± 1651	38	43
T. c. Leysin	Prealps, Leysin (CH)	1485	7.1	4.7	43	1.1 ± 0.1	158 ± 78	2.0 ± 0.9	0.28 ± 0.39	24 ± 15	3730 ± 2581	19	24
T. c. les Avattes	Jura, les Avattes (CH)	1450	5.8	12.8	55	3.7 ± 0.6	120 ± 33	8.7 ± 3.8	3.00	285 ± 191	4607 ± 2403	117	37
v*T. c.* Gurnigel 1	Jura, Mt d'Amin (CH)	1320	5.9	12.7	60	9.2 ± 4.5	182 ± 59	12.9 ± 6.6	0.37 ± 0.52	505 ± 91	8794 ± 1173	44	41
T. c. Gurnigel 2	Jura, Mt d'Amin (CH)	1393	5.6	9.2	39	2.3 ± 0.3	92 ± 4	10.6 ± 2.0	0.25	327 ± 10	4156 ± 2285	147	45
T. c. Ganges[A]	St-Laurent-le-Minier (F)	1000	6.4	1.6	5.86	20 ± 10	3045 ± 1473	290 ± 140	20 ± 13	1508 ± 364	17 653 ± 8173	75	6
T. c. Prayon[B]	Prayon (B)	–	4.7–6.9	–	–	667 ± 85	75 700 ± 13 500	–	–	–	–	–	–
T. perfoliatum	Witzwilmoos (CH)	433	7.4	4.1	12	bdl	26	–	–	0.6	11	–	0.4

[A]Les Avinières population.
[B]Roosens *et al.* (2003): pH in 1 : 1 (w / v) H_2O and total metal: digestion in 4 : 1 (v / v) HCl/HNO_3.
[C]FAL (1998).
[D]Pseudo-total = 2 M-HNO_3-extractable except *T. c.* Prayon (cf. footnote[B]); FAC (1989).
[E]Soluble = 0.1 M-$NaNO_3$-extractable; FAC (1989).
[F]Pooled sample for *T. perfoliatum*.

Plant cultivation in hydroponics

Seeds were germinated in the dark on filters moistened with deionised water. After 2 weeks, plants were exposed to light and water was replaced by the modified quarter-strength Hoagland's nutrient solution Basal No. 2 (Sigma, St Louis, MO) supplemented with 20 µM Fe–HBED (Strem chemical, Newburyport, MA). Three-week-old seedlings (with two pairs of leaves) were then transferred to 60-mL tubes (one plant per tube) filled with the same nutrient solution. The plants were allowed to grow for 2 weeks (until four pairs of leaves were obtained) in hydroponics before treatment with Cd was started. Six different treatments in four replicates were then performed: control (0 µM), 1, 5, 10, 20 and 50 µM Cd added as [$Cd(NO_3)_2 \cdot 4H_2O$]. Plants were grown for a further 6 weeks before harvest.

Germination and plant culture were performed in a climate chamber (day / night period 16 / 8 h, day / night temperatures 20 / 16°C). The nutrient solution was renewed every 4 d and Fe(III)–HBED was prepared as described by Chaney *et al*. (1998) in such a way that all HBED was saturated with Fe. Because the nutrient solution was changed every 3–4 d, pH (initially at 6) was not controlled further and the solution was not aerated.

Tolerance to Cd

The shoot dry weight is the most widely used parameter for assessing metal tolerance in long-term growth tests (Cieśliński *et al*. 1996; Ebbs and Kochian 1997; Köhl and Lösch 1999). Total root length has been proposed as the best root parameter to quantify tolerance to Cd in long-term growth tests (Köhl and Lösch 1999).

Biomass production

Shoots and roots were harvested separately and quickly rinsed with deionised water. They were dried at 80°C and the individual dry weights were recorded before analysis. The total root length was measured before drying (see below).

A tolerance index (Wilkins 1978; Baker 1981, 1987) was calculated for the shoot dry weight only, as follows:

$$TI_s\ (\%) = \Sigma_1^4[(\text{dry weight of shoots of the Cd-treated plants}) \times 100/(\text{average dry weight of shoots of four control plants})]/4.$$

Total root length

Total root length was measured with a desktop scanning device and is expressed as total root length per plant. Roots were spread in a glass tray in 2–3 mm of water. The software WinRHIZO (version Pro 5.0A, Régent Instruments, Quebec, Canada) was used to analyse images acquired with a desktop scanner STD1600 (Epson) provided by Régent Instruments. Limitations and accuracy of the technique have been tested by Bauhus and Messier (1999). All measurements were carried out at a resolution of 400 dpi. The measurement was performed on all populations except le Sentier and Ganges.

Characterisation of hyperaccumulation

Element concentrations were measured in the shoots of the different populations tested either on the plants collected in the field or on plants grown in hydroponics and harvested after 6 weeks of plant exposure to Cd. Samples were ground in a mill made of titanium (ZM, Retsch, Haan, Germany) and hot-digested in HNO_3 65% (suprapur, Fluka, Buchs, Switzerland) and $HClO_4$ 70% (pro analysis, Fluka, Buchs, Switzerland). Cadmium concentration was measured in the digests by inductively coupled plasma atomic emission spectroscopy (Perkin Elmer Plasma 2000, Wellesley, MA).

Statistical treatment

Analyses of variance (two-way ANOVA) were performed to test the effects of population and Cd treatment on element concentrations in plants as well as on shoot dry weight production and root length of the plants grown in hydroponics (SYSTAT 10.2, SYSTAT Software Inc.; Tables 2, 3). The effect of a given factor was considered significant when the *P*-value was $< 0.001^{***}$, $< 0.01^{**}$, $< 0.05^{*}$. In addition, Student's

Table 2. Analysis of variance (*F* ratio) of Cd concentrations in shoots, shoot dry weight, root dry weight and root length of the *Thlaspi* populations grown in hydroponics with 0 (control), 1, 5, 10, 20 and 50 µM Cd in solution, with the populations, the Cd treatments and the interaction between the two as factors of variability

All calculations performed without Gurnigel 2. Root length analysis was performed without le Sentier and Ganges. M, metallicolous; SP, Swiss population; na, not applicable. $^{***}P<0.001$, $^{**}P<0.01$, $^{*}P<0.05$

	F ratio			
	Cd concentration	Shoot weight	Root weight	Root length
Population	34.6***	31.4***	16.2***	9.00***
Cd treatment	396***	64.8***	14.3***	5.38***
Interaction population × treatment	9.41***	7.49***	1.482	1.77
Error df	93	104	107	69
Origin (M *v.* SP)	33.1***	40.7***	66.2***	na
Cd treatment	126.8***	19.1***	11.5***	na
Interaction origin × treatment	4.76***	3.82**	0.89	na
Error df	116	130	131	
Populations without Prayon	0.076	3.26	9.43***	na
Cd treatment	120.8***	18.5***	15.6***	na
Interaction populations × treatment	4.41***	1.05	1.83*	na
Error df	93	106	88	
Swiss populations	20.6***	5.56**	2.92*	1.59*
Cd treatment	345***	92.8***	15.8***	4.65***
Interaction populations × treatment	7.20***	9.03***	2.95***	3.14**
Error df	60	69	70	52

Table 3. Analysis of variance (*F* ratio) of the shoot dry weight, Cd concentrations in shoots and root length of the *Thlaspi* populations grown in hydroponics with 0 (control), 1, 5, 10, 20 and 50 µM Cd in solution: comparison of the various groups of populations

SP, Swiss population; M, metallicolous (Ganges and Prayon). For a given parameter, the populations or groups of populations are significantly different when the *P*-value is < 0.001***, < 0.01**, < 0.05*

Parameter	Cd concentration in nutrient solution (µM) 0	1	5	10	20	50
Shoot dry weight						
All populations	7.71***	9.87***	10.5***	15.7***	78.1***	4.43**
Swiss populations (SP)	11.3***	12.5***	2.36	2.66	8.58**	5.90*
M *v.* SP	0.08	1.24	13.7***	22.0***	20.3***	9.14**
Ganges *v.* SP	0.39	0.07	1.25	12.4**	0.08	5.41*
Prayon *v.* SP	0.33	3.70	33.7***	161.5***	186***	12.1**
Cd shoot concentrations						
All populations	97.8***	29.4***	77.7***	750***	9.74***	11.2***
Swiss populations (SP)	46.0***	43.7***	25.0***	22.9***	2.37	57.0***
M *v.* SP	7.93*	8.7**	2.32	10.1**	5.41*	15.2***
Ganges *v.* SP	64.0***	0.6	6.54*	1.43	1.10	4.13*
Prayon *v.* SP	0.52	16.8***	24.7***	18.2***	22.2***	18.1***
Root length						
All populations	1.15	2.87	2.47	20.1***	320***	6.54**
Prayon *v.* SP	2.70	0.7	2.18	3.05	2.92	3.39

t-tests were performed to test whether the root length of the plants grown with Cd differed from that of plants grown without Cd (Fig. 1) or whether Cd concentrations in shoots and root dry weight differed between populations for a given treatment (Fig. 3; Table 2). The difference between means was significant when P was < 0.05. Linear regressions were calculated between Log_{10} of the Cd amount in shoots and Log_{10} of Cd concentration in solution for each population. The significance of the correlation coefficient was indicated as: *** for $2\alpha < 0.001$, ** for $2\alpha < 0.01$ and, * for $2\alpha < 0.05$ (Table 5). The slope of the regression was tested against 0 (a slope different from 0 indicating a treatment effect). In addition, a covariance analysis (ANCOVA) was performed to test the significance of the difference between the slopes obtained for the various populations (the difference indicated an interaction between populations and treatments on the amount of Cd accumulated in the shoots of the plants grown in hydroponics at various Cd concentrations). In this later case, the software R 1.7.1 was used (Ihaka and Gentleman 1996).

Results

Metal concentrations in the shoots of the plants collected in the field

The local plants of *T. caerulescens*, except those from Leysin, had Cd concentrations in their shoots higher than

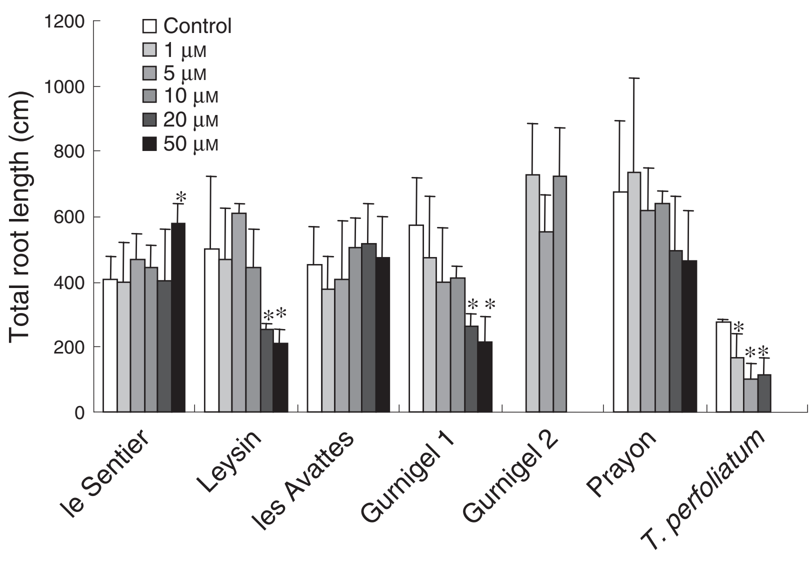

Fig. 1. Total root length (cm) measured for the different populations of *Thlaspi caerulescens* and *Thlaspi perfoliatum* grown in hydroponics with increasing Cd concentrations. Values are means of four replicates with standard deviation. *Difference between treatment and control significant at $2P<0.05$ for a given population.

the hyperaccumulation level set at 0.01% Cd in DW (Reeves and Baker 2000), whereas Zn concentrations, although very high, never exceeded the 1% threshold for hyperaccumulation (Gurnigel 1 plants were relatively close to it) (Table 1). However, the metal TF, calculated as the ratio between metal concentration in *T. caerulescens* shoots and pseudo-total metal concentration in the initial soils, was approximately 40 for Zn and the populations originating from the Jura, whereas for Cd it ranged from 38 to 147. At Leysin, it was lower for both Cd and Zn (19 and 24, respectively). Cadmium and Zn concentrations measured in shoots of *T. perfoliatum* were low and TF was below one for Zn.

Dry weight and root parameters measured on plants grown in hydroponics

For all populations the shoot and root dry weights, and the total root length decreased with increasing Cd concentrations (Figs 1, 2; Table 4). *T. perfoliatum* was the most sensitive with no survival above 10 µM and a sharp decrease of both the shoot and the root dry weights at 1 µM Cd. In general (all populations excluding *T. perfoliatum*), the origin of the population and the Cd treatment factors had an influence on both shoot and root dry weight production, as well as on root length (Table 2). There was also a significant population × treatment interaction, except for

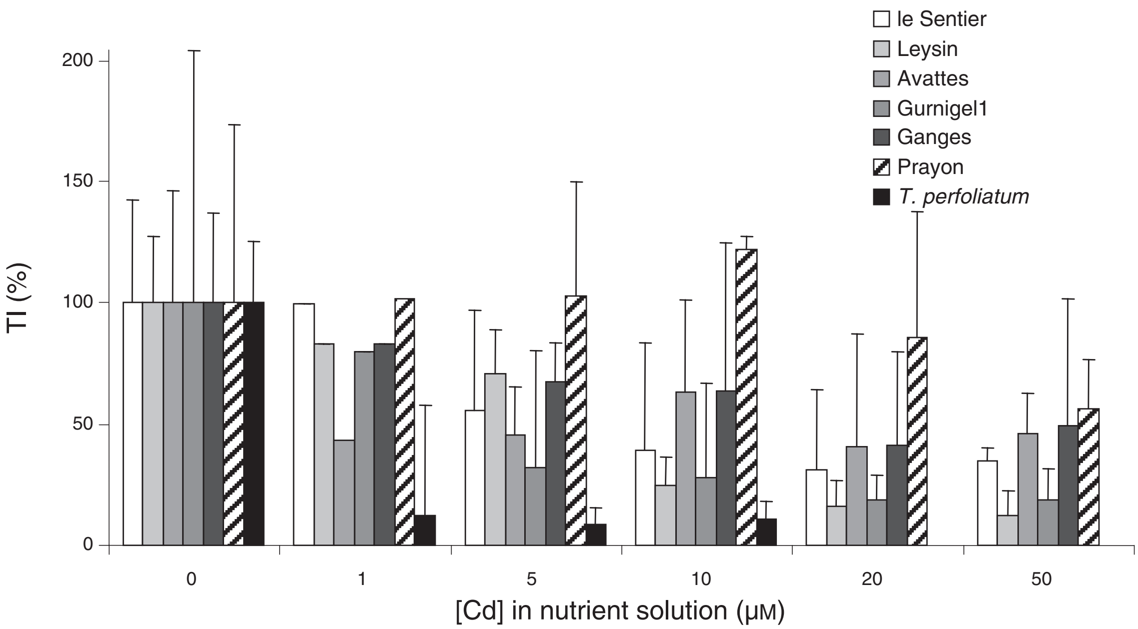

Fig. 2. Tolerance index (% TI) calculated for the shoot dry weight of the different populations grown in hydroponics with increasing Cd concentrations in solution. Values are means of four replicates with standard deviation.

Table 4. Root dry weight production (mg per plant) of the different *Thlaspi caerulescens* and *Thlaspi perfoliatum* populations grown in hydroponics with increasing Cd concentrations

Values are average means of four replicates with standard deviation. Superscript letters refer to the significance of the differences between treatments. For a given population the same superscript letters indicate no difference between the means and different superscript letters indicate significant differences between means at $P<0.05$. 'nd' indicates that no data were collected because plant did not survive, either because of poor initial fitness (Gürnigel 2) or Cd toxicity (*T. perfoliatum*)

Population	Cd concentrations in nutrient solution (µM) 0	1	5	10	20	50
le Sentier	18.4 ± 2.6^{a}	18.8 ± 3.7^{a}	17.0 ± 5.1^{ab}	12.8 ± 4.2^{ab}	11.6 ± 2^{b}	11.6 ± 6.0^{ab}
Leysin	17.2 ± 4.5^{a}	17.1 ± 4.3^{a}	17.8 ± 3.8^{a}	10.5 ± 2.1^{b}	6.4 ± 0.8^{c}	5.7 ± 0.9^{c}
les Avattes	14.4 ± 3.7^{a}	9.7 ± 2.5^{ab}	11.2 ± 4.7^{ab}	14.1 ± 2.6^{b}	11.2 ± 2.6^{ab}	10.7 ± 2.3^{ab}
Gurnigel 1	24.4 ± 6.8^{a}	16.0 ± 9.5^{abc}	11.6 ± 7.1^{bc}	10.2 ± 1.8^{b}	6.9 ± 0.8^{c}	7.6 ± 2.5^{bc}
Gurnigel 2	nd	25.7 ± 3.9^{a}	13.5 ± 3.5^{a}	16.7 ± 6.6^{a}	nd	nd
Ganges	25.3 ± 3.7^{a}	24.3 ± 5.2^{a}	20.8 ± 9.3^{ab}	20.7 ± 6.8^{ab}	12.8 ± 6.4^{b}	14.0 ± 6.5^{b}
Prayon	21.4 ± 7.7^{ab}	23.9 ± 4.6^{a}	22.9 ± 5.0^{ab}	24.2 ± 5.0^{a}	19.1 ± 2.7^{ab}	15.5 ± 4.4^{b}
T. perfoliatum	13.1 ± 3.3^{a}	2.8 ± 1.2^{b}	1.9 ± 0.9^{b}	2.2 ± 1.3^{b}	nd	nd

the root parameters. In order to test whether the Ganges and Prayon populations were responsible for the variation observed between populations, they were removed stepwise from the calculations. When the Prayon population was excluded from the dataset, neither population nor the interaction between population and treatment were significant to explain variation of shoot dry weight. When Ganges was also removed, the two factors and their interaction were significant to discriminate the Swiss populations. When each concentration was tested individually, all populations were significantly different for their shoot dry weight and root length (Table 3). Differences between the Swiss populations were not significant at all concentrations in solution, but when metallicolous populations (Ganges or Prayon, or Prayon only) were compared with the Swiss populations, two groups could be discriminated on the basis of their shoot dry weight above 1 μM Cd in solution.

The shoot dry weight, the root dry weight as well as TI_s (Fig. 2) gave the same order for the populations: Prayon was the most tolerant, followed by Ganges, les Avattes, Gurnigel 2, le Sentier and then Leysin and Gurnigel 1. For the population from les Avattes, the shoot dry weight presented a sharp decrease at 1 μM but then remained constant to 50 μM.

Cadmium concentrations measured in plants grown in hydroponics

The largest differences between the populations were observed at 1 μM Cd; all populations except Prayon and *T. perfoliatum* were able to accumulate 0.01% Cd or more in their above-ground dry matter (Fig. 3). Cadmium concentrations in shoots of *T. perfoliatum* were 0.4, 10, 62 and 129 mg kg^{-1} DW, for 0, 1, 5, and 10 μM treatments, respectively, and were thus, clearly below Cd concentrations in Prayon plants for the same concentrations in solution. In all combinations of populations, the population type (metallicolous or Swiss population) and the treatments were responsible for the differences observed in Cd concentrations in shoots, except when Prayon was removed (Table 2). The interaction between population and treatment had also a significant effect in all cases. These significant differences were observed at all concentrations in solution except when

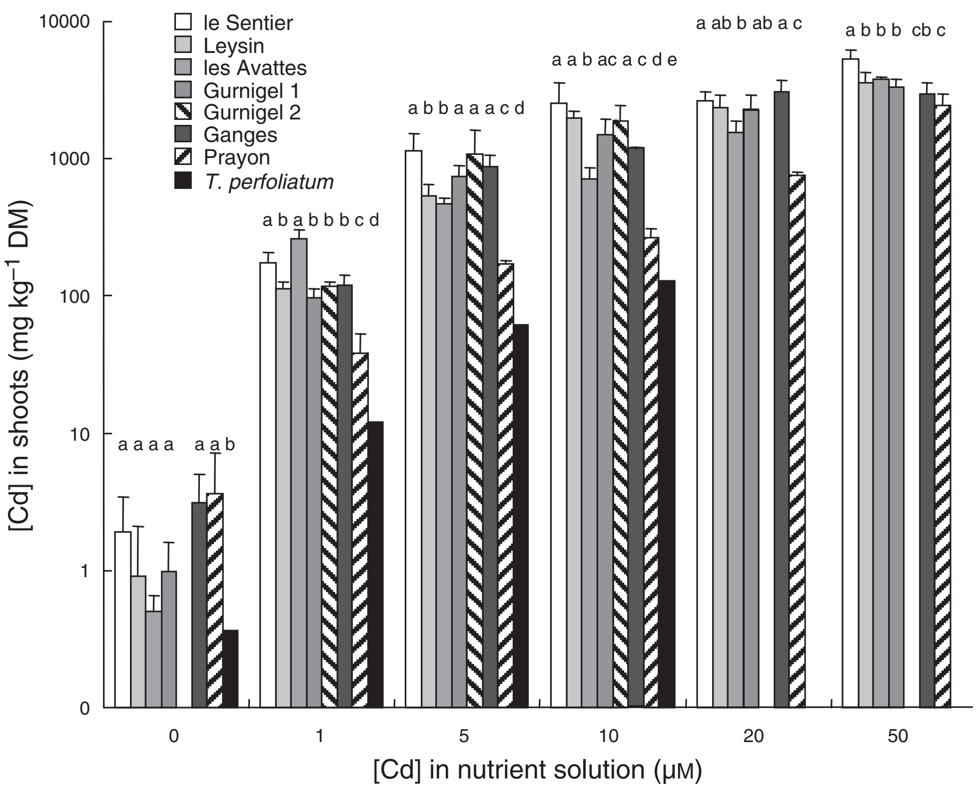

Fig. 3. Cadmium concentrations (mg kg^{-1} DW) in shoots of the different *Thlaspi caerulescens* and *Thlaspi perfoliatum* populations grown in hydroponics with increasing Cd concentrations. Values are means of four replicates with standard deviation. Letters refer to the significance of the differences between populations: for a given treatment, bars topped by the same letters are not significantly different and bars topped by different letters are significantly different at $P < 0.05$. *Thlaspi perfoliatum* replicates died in treatments above 10 mM. For the other treatments samples were pooled.

Ganges was compared to the Swiss populations as soon as Cd was added, and except when Prayon was compared to the Swiss populations in control conditions (Table 3).

In addition, the tolerance index was negatively related to Cd concentrations in shoots when all treatments data were taken (R^2=0.560***). However, when quantities were used, no relation was found with % TI except at 50 μM, for which Cd accumulation (quantities) in shoots was positively correlated with % TI (R^2=0.591*).

Figure 4 presents the amount of Cd accumulated in shoots ($[Cd]_{shoots} \times$ DW plotted *v.* [Cd] in solution). For each population a linear regression was calculated with the Log-transformed data (Table 5). The slope of this line is characteristic of a population's efficiency of accumulation of Cd in solution. All slopes were significantly different from zero except for Leysin, and these slopes were significantly different from one another (Table 5). Consequently, populations could be classified in the following order of efficiency (order of increasing slope): Leysin < Gurnigel 1 < le Sentier < Ganges < les Avattes < Gurnigel 2 < Prayon.

Discussion

Hyperaccumulation in the field

All Jura populations hyperaccumulated in the field, with concentrations above 0.01% Cd in DW and TFs well above one (Reeves and Baker 2000), although Cd soil concentrations were low, with < 10 mg kg^{-1} of 2 M-HNO_3-extractable Cd and < 13 μg kg^{-1} 0.1 M-$NaNO_3$-extractable Cd. Cadmium concentrations measured in shoot DW samples were nevertheless positively correlated with concentrations of 2 M-HNO_3-extractable Cd in soils (R^2=0.908***), but no correlation was found between TF and Cd concentration in soil. The Prealps population (Leysin) did not follow the same pattern and its unique behaviour may be responsible for its distinctive results obtained in hydroponics. These results are notably different from those obtained by Reeves *et al.* (2001), who found only one Cd hyperaccumulating population among five populations growing on non-metallicolous soils, and Molitor *et al.* (2005) who did not find any Cd hyperaccumulating populations among 46 non-metallicolous populations investigated (measurement of the plants collected on their original soil). However, when TFs were compared (calculation was only possible for the populations studied by Reeves *et al.*), they were highly variable but in a similar range in both cases, ranging from 5 to 100 (with an exception of 547) for populations collected in France and Luxembourg (Reeves *et al.* 2001), and from 19 to 147 in the Swiss populations. In contrast, *Thlaspi perfoliatum* originated from a non-metallicolous soil and Cd shoot concentration measured in the field was low and similar to that measured by Reeves *et al.* (2001) in *T. perfoliatum* collected in various areas in France. Transfer factors could not be compared because no soil concentration was given by the authors. Clearly, the Jura populations possess an enhanced hyperaccumulation characteristic for Cd.

Hyperaccumulation and tolerance in nutrient solution and relation with the origin of the populations

In solution, all populations differed in Cd accumulation. However, in general there was an increase in shoot concentration in parallel with the increase in Cd concentration in solution. Hyperaccumulation was achieved for all populations, except Prayon, with 1 μM Cd in solution.

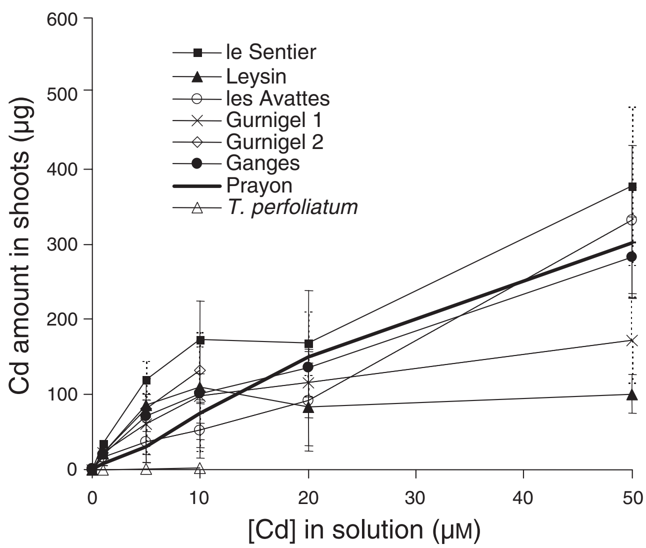

Fig. 4. Amount of Cd accumulated in the shoots ($[Cd]_{shoots} \times$ shoot dry weight) of the *Thlaspi caerulescens* and *Thlaspi perfoliatum* populations tested *v.* Cd concentration in solution.

Table 5. Amount of Cd accumulated in the shoots (dry weight × concentration) *v.* Cd concentration in solution

Calculation of the slope of the linear regression between the Log-transformed data for each population, the regression coefficient (regression performed on mean values) and the significance of the slope was performed. ANCOVA tests the interaction between population and treatment and tests the difference between slopes. Significance of *r*: ***2α < 0.001, **2α < 0.01, *2α < 0.05; Significance of *P*: ***P<0.001, **P<0.01, *P<0.05

Population	Slope	*r*	*P* (significance of each slope against 0)
le Sentier	0.5782	0.9750**	0.004729**
Leysin	0.3798	0.8252	0.0854
les Avattes	0.7286	0.9631**	0.00848**
Gurnigel 1	0.5164	0.9897***	0.00124**
Gurnigel 2	0.7597	0.9996***	0.01671*
Ganges	0.6492	0.9948***	0.000448***
Prayon	0.9342	0.9960***	0.000260***
ANCOVA Interaction population × treatment			P=0.008162** All slopes are different

Concentrations measured in shoots were in the same range as those obtained by Roosens *et al*. (2003) in solution with various *T. caerulescens* populations.

The increase in Cd concentrations led to a decrease in shoot dry weight for all populations although the decrease in % TI was not parallel for all populations. The origin of the populations and the treatments both significantly affected all plant parameters. In addition, the significant interaction between populations and treatments indicated that the populations tested indeed reacted differently to an increase in Cd concentration in solution (Table 2). The Swiss populations presented behaviours similar to that of Ganges for all tested parameters, but differed from Prayon. Also, Cd affected the root system less than it did the shoot dry weight. The limited effect of Cd on the root system is consistent with the idea that *T. caerulescens* roots forage for Cd and Zn (Schwartz *et al*. 1999; Whiting *et al*. 2000), and that consequently roots may develop well even at very high concentrations in the medium. In the field, another explanation would be that concentrations in roots being lower than those in shoots, Cd would reach toxic concentrations in shoots earlier than in roots. This explanation might also hold true for this experiment but could not be verified because no Cd measurement was performed on roots. However, metal concentrations in roots of *T. caerulescens* have been found to be either lower (Zn, Lasat *et al*. 2000; Cd, Roosens *et al.* 2003) or higher (Cd, Lombi *et al*. 2000; C Keller unpubl. data) than concentrations in shoots. The reason could be that roots had or had not been treated with EDTA before metal analysis (not always specified in the literature). Indeed, Cosio *et al*. (2006) have shown that half of Cd found in *Salix* roots was desorbed by EDTA washing.

Growing plants in 1 μM Cd seemed to be an adequate medium to discriminate the various populations tested for hyperaccumulation because (1) this is a concentration close to concentrations likely to be found in soil solutions, (2) it discriminated between Ganges and Prayon populations, which hyperaccumulate differently in field conditions, and (3) except for *T. perfoliatum*, this concentration did not induce any obvious toxicity effect and thus, can be considered as below the no observed effect concentration (NOEC), which allows the distinction to be made between hyperaccumulation as a physiological trait from metal-accumulation as the result of a toxicity effect. In the case of *T. perfoliatum*, the population we tested had a limited tolerance to Cd in solution; dry weight production was severely depressed above 1 μM Cd in solution. At this concentration in solution, concentrations in shoots were lower than in *T. caerulescens* and in the range found for non-accumulating plants (Sauerbeck 1989). The large Cd concentrations above 1 μM Cd in solution can probably be attributed to a general breakdown of the root system. However, these results confirmed that *T. perfoliatum* would be a good alternative to *T. arvense* as a control plant, although it has a smaller size and reaches maturity earlier than *T. caerulescens* (Peer *et al*. 2003).

The most striking feature was the positive relationship observed between TF calculated in the field and the response of a given population to increasing Cd concentrations in solution (Fig. 5). This indicates that a population that had a larger TF in the field was also able to accumulate (in quantity) more Cd in solution. However, there was no relationship between the dry weight produced in hydroponics at a given Cd concentration and TF, except for 5 μM (R^2=0.494, n = 6). This is the first time that a relationship has been clearly demonstrated between the behaviour of a *Thlaspi* population in the field and in hydroponics. It also shows that the comparison of populations has to be based on quantities and not only on concentrations. At the same time, and for a given treatment, an increase in Cd concentration in shoots led to a decrease in % TI for all populations, indicating that the populations that were the most tolerant were also those with the lowest concentrations in shoots, even if they were all able to hyperaccumulate. For treatments above 5 μM, % TI was linearly and positively related to the slope calculated over the whole range of treatment (R^2=0.916***, 0.903*** and 0.838*** for 10, 20 and 50 μM, respectively) indicating that, for large concentrations in the growth medium, the most tolerant populations were also those able to accumulate the most Cd with increasing concentrations in solution. In addition, at 50 μM % TI was positively correlated with Cd quantities accumulated in shoots, indicating that this

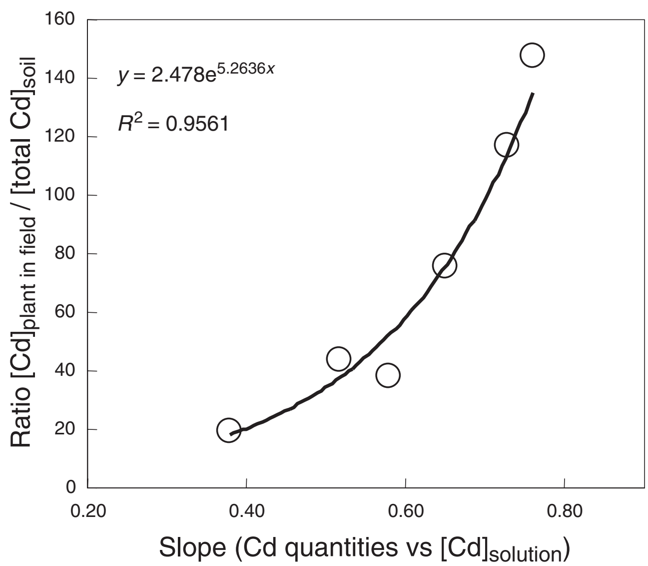

Fig. 5. Relationship between slopes of the linear regression obtained for each population by plotting Log_{10} of the amount of Cd accumulated in the shoots versus Log_{10} Cd concentration in solution [slope (Cd quantities *v*. $[Cd]_{solution}$)], and the Cd transfer factor (TF = $[Cd]_{plant\ in\ the\ field}$ / $[total\ Cd]_{soil}$) calculated from the field data of the Swiss and Ganges populations. The *x*-axis refers to the slope as calculated in Table 5.

concentration may be the starting point for discrimination of the populations according to their tolerance to Cd. However, no relation was found between % TI and TF, either because the number of populations was too low to obtain significant results, or % TI was too simplistic to account for the behaviour of populations in a medium of increasing concentration, whereas the slope calculated from the quantities accumulated with increasing concentrations was most suitable and might be able to characterise tolerance best. Roosens *et al.* (2003) suggested that there was a relationship between Cd content of the parent soil and Cd tolerance. From our results, we can add that TF is the most important factor in discriminating populations for their accumulation ability and that tolerance is a key factor when an efficient population is needed for phytoextraction. As a consequence, it appeared that the origin of the populations influenced their tolerance to Cd in solution, as observed in soils by Escarré *et al.* (2000). However, it is not possible to know whether the TF is a population trait or a result of the soil characteristics. Consequently, the hyperaccumulation capacity might be either a constitutive trait that may evolve differently according to metal concentrations in the soil or was induced by an adaptation to the soil.

Several authors have discriminated between metallicolous and non-metallicolous populations, clearly placing Ganges and Prayon in the former category. The non-metallicolous populations have been found to tolerate less Cd than the metallicolous ones. Their accumulation behaviour is not as clear as for tolerance. Escarré *et al.* (2000) found that the non-metallicolous populations accumulated less Cd than the metallicolous ones, whereas Molitor *et al.* (2005) found the opposite. From our results, it is clear that (1) Prayon is more tolerant than Ganges (les Avinières population) and (2) it accumulates less Cd than Ganges in the presence of close to natural Cd concentrations. However, the Ganges population has evolved in a metalliferous environment as shown by our soil analyses, as well as by other authors (Roosens *et al.* 2003), and according to this sole criterion it should be as tolerant and accumulating as Prayon. Our populations demonstrated a high capability for Cd hyperaccumulation in the field and in solution combined with a tolerance in solution that lies between those of Prayon and Ganges (except les Avattes at 1 μM Cd), while all metal concentrations in initial soils were lower than those found in Prayon and Ganges soils. So, although they did not originate from a metalliferous environment, they behaved similarly to Ganges and Prayon in solution, indicating that tolerance is not only related to initial soil characteristics and confirming that it is a constitutive trait (Schat *et al.* 2000). Our results are also compatible with the findings of Basic *et al.* (2006*a*), who showed that in similar populations (originating from the same area but not the same populations used in this work) Cd concentrations in shoots measured in the field were correlated with total soil concentrations and Zn, Fe and Cu concentrations in shoots. However, when the variance resulting from the soil concentration was removed, the resulting 'hyperaccumulation capacity' calculated on residuals was positively correlated with plant fitness parameters (number and size of leaves) and reproductive traits (number and weight of seeds), indicating better growth and potential reproduction, and suggesting that Cd hyperaccumulation could be a mechanism for increased Cd tolerance.

In general, and from an applied point of view, the first limiting parameter for the use of various populations in phytoextraction is the amount of available seeds and their viability. However, from the results in hydroponics, it seems that limited differences in tolerance and accumulation between populations of *T. caerulescens* (compared with other plants) as well as the difficulty in defining the best conditions for optimal growth in soil and metal removal, may allow and favour the use of a mix of seeds for efficient phytoextraction.

From our results, it is difficult to identify the different behaviour that these various populations may exhibit when growing in contaminated soils. Because of their geological history and their Cd concentrations (Atteia *et al.* 1994), the soils of the Jura chain are unique. Except for *T. caerulescens*, Cd concentrations in shoots of plants growing on these soils are low (Cd remains in the roots) and in the topsoil the available Cd fraction is limited and originates mainly from the turnover of the vegetation (Benitez 1999). Although it has been shown that *T. caerulescens* does not access a different Cd pool from non-accumulating plants (Hamon *et al.* 1997; Gérard *et al.* 2000; Hammer *et al.* 2006), testing Prayon and Ganges on a range of these soils may provide information on Cd availability to *T. caerulescens* and may clarify the specificity of the different *T. caerulescens* populations. Ultimately, our results call for more research on natural populations originating from different geographical regions, since we found that Cd accumulation of the Swiss populations in hydroponics was closely related to their uptake characteristics in the field.

Acknowledgments

This research was part of the project OFES No. C99.0062, financed by the Swiss Federal Office for Education and Science (COST action 837) and by the Geneva University (DESNE). We thank P. Meerts who kindly provided the Prayon seeds.

References

Assunção AGL, Schat H, Aarts MGM (2003) *Thlaspi caerulescens*, an attractive model species to study heavy metal hyperaccumulation in plants. *New Phytologist* **159**, 351–360. doi: 10.1046/j.1469-8137.2003.00820.x

Atteia O, Dubois J-P, Webster R (1994) Geostatistical analysis of soil contamination in the Swiss Jura. *Environmental Pollution* **86**, 315–327. doi: 10.1016/0269-7491(94)90172-4

Baize D, Sterckeman T (2001) Of the necessity of knowledge of the natural pedo-geochemical background content in the evaluation of the contamination of soils by trace elements. *The Science of the Total Environment* **264**, 127–139. doi: 10.1016/S0048-9697(00)00615-X

Baker AJM (1981) Accumulators and excluders — strategies in the response of plants to heavy metals. *Journal of Plant Nutrition* **3**, 643–654.

Baker AJM (1987) Metal tolerance. *New Phytologist* **106**(suppl), 93–111.

Baker AJM, Reeves RD, Hajar ASM (1994) Heavy-metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J. & C. Presl. (Brassicaceae). *New Phytologist* **127**, 61–68. doi: 10.1111/j.1469-8137.1994.tb04259.x

Basic N, Keller C, Fontanillas P, Vittoz P, Besnard G, Galland N (2006*a*) Cadmium hyperaccumulation and reproductive traits in natural *Thlaspi caerulescens* populations. *Plant Biology* **8**, 64–72. doi: 10.1055/s-2005-872892

Basic N, Salamin N, Keller C, Galland N, Besnard G (2006*b*) Cadmium accumulation and genetic differentiation of *Thlaspi caerulescens* populations. *Biochemical Systematics and Ecology* (in press).

Baskin JM, Baskin CC (1979) The ecological life cycle of *Thlaspi perfoliatum* and a comparison with published studies on *Thlaspi arvense. Weed Research* **19**, 285–292.

Bauhus J, Messier C (1999) Evaluation of fine root length and diameter measurements obtained using RHIZO image analysis. *Agronomy Journal* **91**, 142–147.

Benitez N (1999) Cadmium speciation and phyto-availability in soils of the Swiss Jura: hypothesis about its dynamics. PhD thesis, Ecole Polytechnique Fédérale de Lausanne.

Chaney R, Brown S, Angle JS (1998) Soil–root interface: ecosystem health and human food-chain protection. In 'Soil chemistry and ecosystem health'. (Ed. P Huang) pp. 279–311. (Soil Science Society of America: Madison, WI)

Cieśliński G, Neilsen GH, Hogue EJ (1996) Effect of soil cadmium application and pH on growth and cadmium accumulation in roots, leaves and fruit of strawberry plants (*Fragaria* × *ananassa* Duch.). *Plant and Soil* **180**, 267–276. doi: 10.1007/BF00015310

Cosio C, Vollenweider P, Keller C (2006) Localization and effects of phyto-extracted cadmium in leaves of tolerant willows (*Salix viminalis* L.) I. Macrolocalisation and phytotoxic effects of cadmium. *Environmental and Experimental Botany* (in press).

Doffey C (1997) Etude de la variabilité spatiale du cadmium dans les sols de la région de la Dent de Lys. MSc thesis, University of Geneva and Lausanne University.

Dubois J-P, Okopnik F, Benitez N, Védy J-C (1998) Origin and spatial variability of cadmium in some soils of the Swiss Jura. Proceedings of the 16th World Congress on Soil Science, Montpellier, France.

Ebbs SD, Kochian LV (1997) Toxicity of zinc and copper to *Brassica* species: implications for phytoremediation. *Journal of Environmental Quality* **26**, 776–781.

Escarré J, Lefèbvre C, Gruber W, Leblanc M, Lepart J, Rivière Y, Delay B (2000) Zinc and cadmium hyperaccumulation by *Thlaspi caerulescens* from metalliferous and nonmetalliferous sites in the Mediterranean area: implications for phytoremediation. *New Phytologist* **145**, 429–437. doi: 10.1046/j.1469-8137.2000.00599.x

FAC (Eidgenössische Forschungsanstalt für Agrikulturchemie und Umwelthygiene) (1989) 'Methoden für die Bodenuntersuchungen.' (Schriftenreihe der FAC 5: Bern-Liebefeld, Switzerland)

FAL (Eidgenössische Forschungsanstalt für Agrarökologie und Landbau) (1998) 'Manuel pour l'analyse des sols, des plantes et de l'eau de percolation lysimétrique.' (Les Cahiers de la FAL 27: Zürich-Reckenholz, Switzerland)

Gérard E, Echevarria G, Sterckeman T, Morel J-L (2000) Cadmium availability to three plant species varying in cadmium accumulation pattern. *Journal of Environmental Quality* **29**, 1117–1123.

Hammer D, Keller C, McLaughlin MJ, Hamon RE (2006) Fixation of metals in soil constituents and potential remobilization by hyperaccumulating and non-hyperaccumulating plants: results from an isotopic dilution study. *Environmental Pollution* (in press).

Hamon RE, Wundke J, McLaughlin M, Naidu R (1997) Availability of zinc and cadmium to different plant species. *Australian Journal of Soil Research* **35**, 1267–1277. doi: 10.1071/S97052

Ihaka R, Gentleman R (1996) R: a language for data analysis and graphics. *Journal of Computational and Graphical Statistics* **5**, 299–314. doi: 10.2307/1390807

Ingrouille MJ, Smirnoff N (1986) *Thlaspi caerulescens* J. & C. Presl. (*Thlaspi alpestre* L.) in Britain. *New Phytologist* **102**, 219–233. doi: 10.1111/j.1469-8137.1986.tb00812.x

Koch M, Hurka H (1999) Isoenzyme analysis in the polyploid complex *Microthlaspi perfoliatum* (L.) F.K. Meyer: morphology, biogeography and evolutionary history. *Flora* **194**, 33–48.

Köhl KI, Lösch R (1999) Experimental characterization of heavy metal tolerance in plants. In 'Heavy metal stress in plants: from molecules to ecosystems'. (Eds MNV Prasad, J Hagemeyer) pp. 371–389. (Springer-Verlag: Berlin)

Lasat MM, Pence NS, Garvin DF, Ebbs SD, Kochian LV (2000) Molecular physiology of Zn transport in the Zn hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany* **51**, 71–79. doi: 10.1093/jexbot/51.342.71

Lombi E, Zhao FJ, Dunham SJ, McGrath SP (2000) Cadmium accumulation in populations of *Thlaspi caerulescens* and *Thlaspi goesingense*. *New Phytologist* **145**, 11–20. doi: 10.1046/j.1469-8137.2000.00560.x

Meerts P, Van Isacker N (1997) Heavy metal tolerance and accumulation in metallicolous and non-metallicolous populations of *Thlaspi caerulescens* from continental Europe. *Plant Ecology* **133**, 221–231. doi: 10.1023/A:1009717619579

Meyer K (1991) 'La pollution des sols en Suisse.' (PNR 22: Liebefeld-Bern)

Molitor M, Dechamps C, Gruber W, Meerts P (2005) *Thlaspi caerulescens* on nonmetalliferous soil in Luxembourg: ecological niche and genetic variation in mineral element composition. *New Phytologist* **165**, 503–512. doi: 10.1111/j.1469-8137.2004.01240.x

OIS (1998) Swiss Federal Legislation OIS (Ordinance relating to impacts on the soil). SR 814.12.

Peer WA, Mamoudian M, Lahner B, Reeves RD, Murphy AS, Salt DE (2003) Identifying model metal hyperaccumulating plants: germplasm analysis of 20 Brassicaceae accessions from a wide geographical area. *New Phytologist* **159**, 421–430. doi: 10.1046/j.1469-8137.2003.00822.x

Pollard AJ, Powell KD, Harper FA, Smith JAC (2002) The genetic basis of metal hyperaccumulation in plants. *Critical Reviews in Plant Sciences* **21**, 539–566. doi: 10.1080/0735-260291044359

Reeves RD, Baker AJM (2000) Metal-accumulating plants. In 'Phytoremediation of toxic metals — using plants to clean up the environment'. (Eds I Raskin, BD Ensley) pp. 193–230. (John Wiley and Sons Inc.: New York)

Reeves RD, Schwartz C, Morel JL, Edmondson J (2001) Distribution and metal-accumulating behavior of *Thlaspi caerulescens* and associated metallophytes in France. *International Journal of Phytoremediation* **3**, 145–172.

Roosens N, Verbruggen N, Meerts P, Ximenez-Embun P, Smith JAC (2003) Natural variation on cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. *Plant, Cell & Environment* **26**, 1657–1672. doi: 10.1046/j.1365-3040.2003.01084.x

Sauerbeck D (1989) Der Transfer von Schwermetallen in die Pflanze. In 'Beurteilung von Schwermetallkontaminationen im Boden.' pp. 281–316. (DECHEMA Fachgespräche Umweltschutz.: Stuttgart am Mainz)

Schat H, Llugany M, Bernhard R (2000) Metal-specific patterns of tolerance, uptake, and transport of heavy metals in hyperaccumulating and nonhyperaccumulating metallophytes. In 'Phytoremediation of contaminated soil and water'. (Eds N Terry, G Banuelos) pp. 171–188. (Lewis Publisher: Boca Raton)

Schwartz C, Morel J-L, Saumier S, Whiting SN, Baker AJM (1999) Root development of the zinc hyperaccumulator plant *Thlaspi caerulescens* as affected by metal origin, content and localization in soil. *Plant and Soil* **208**, 103–115. doi: 10.1023/A:1004519611152

Schwartz C, Echevarria G, Morel J-L (2003) Phytoextraction of cadmium with *Thlaspi caerulescens. Plant and Soil* **249**, 27–35. doi: 10.1023/A:1022584220411

Whiting SN, Leake JR, McGrath SP, Baker AJM (2000) Positive response to Zn and Cd by roots of the Zn and Cd hyperaccumulator *Thlaspi caerulescens*. *New Phytologist* **145**, 199–210. doi: 10.1046/j.1469-8137.2000.00570.x

Whiting SN, Reeves RD, Richards D, Johnson MS, Cooke JA, *et al.* (2004) Research priorities for conservation of metallophyte biodiversity and their potential for restoration and site remediation. *Restoration Ecology* **12**, 106–116. doi: 10.1111/j.1061-2971.2004.00367.x

Wilkins DA (1978) The measurement of tolerance to edaphic factors by means of root growth. *New Phytologist* **80**, 623–633. doi: 10.1111/j.1469-8137.1978.tb01595.x

Zhao FJ, Lombi E, McGrath SP (2003) Assessing the potential for zinc and cadmium phytoremediation with the hyperaccumulator *Thlaspi caerulescens. Plant and Soil* **249**, 37–43. doi: 10.1023/A:1022530217289

Manuscript received 31 August 2005, received in revised form 6 January 2006, accepted 26 April 2006