Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations?

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Summary

- The ability of metallicolous and nonmetallicolous populations of *Arabidopsis halleri* to accumulate zinc (Zn), cadmium (Cd) and lead (Pb) is compared here in order to explore the extent and variability of this trait in wild *A. halleri* plants.
- Aerial plant parts and the soil around the harvested plants were collected and analysed for metal concentrations or total and extractable metal concentrations, respectively, for 20 metallicolous and 13 nonmetallicolous populations.
- Results show that metallicolous and nonmetallicolous populations have the same ability to accumulate Zn and Cd but that neither population type is able to accumulate Pb. Between populations within type, an homogenous accumulating response is observed for Zn, whereas the ability to accumulate Cd is variable.
- Zn and Cd accumulation to very high concentrations is a constitutive property of the species. The Zn and Cd hyperaccumulator trait of *A. halleri* from contaminated sites was confirmed. Interestingly, nonmetallicolous plants are Zn and Cd hyperaccumulators. The possibility of using *A. halleri* in phytoremediation is discussed.

Key words: *Arabidopsis halleri*, natural populations, cadmium (Cd), zinc (Zn), lead (Pb), hyperaccumulation, phytoextraction.

Introduction

Responses by plants to exposure to metal-contaminated soil vary from total incapacity to grow, to survival accompanied by exclusion or by accumulation. Metal accumulation in a nontoxic form in the upper parts of the plants was described as one of the strategies used by plants to tolerate large amounts of metals in their environment (Baker, 1981). Metal accumulation can be defined as the capacity of a plant to accumulate metal in its shoots to concentrations greater than those found in the soil or the nutritive medium where the plant grows (Baker *et al.*, 2000). Certain plants, termed hyperaccumulators, are able to accumulate exceptional concentrations of metals in their above-ground biomass, when growing in their natural habitat, compared with nonaccumulator plants growing in the same habitat (Brooks, 1998). More precisely, a zinc (Zn) hyperaccumulator is defined as a plant that contains > 10 000 $\mu g\ g^{-1}$ dry wt (1% w : w) whereas a cadmium (Cd) hyperaccumulator is defined as a plant that contains > 100 $\mu g\ g^{-1}$ dry wt (0.01% w : w). Each threshold represents a value of approximately 10 times higher than the one usually found in nonaccumulating plants growing in the same habitat. Metal hyperaccumulation is not a common feature in terrestrial higher plants. So far, approximately 400 taxa have been identified as hyperaccumulators (Baker *et al.*, 2000). Among them, almost all taxa are endemic to metalliferous or metal contaminated soils and thus are metal tolerant.

Recently, plants that hyperaccumulate metals have attracted considerable interest because of their potential use in phytoremediation, as they are able to extract metals from the soil and to concentrate them in their upper parts (McGrath *et al.*, 2001). In addition, hyperaccumulation constitutes an intriguing phenomenon as it raises the question of its ecological significance and function (i.e. why do these plants accumulate one or several metals to concentrations that are toxic to most other plants?). Moreover, hyperaccumulator plants represent good models to study metal tolerance, uptake, translocation, accumulation and detoxification.

In the Brassicaceae, several hyperaccumulators have been identified. Among them, are *Thlaspi caerulescens*, able to hyperaccumulate Zn, Cd and nickel (Ni), and *Arabidopsis halleri*, on which this study is focused. The latter is a well known species for its metal tolerance and Zn hyperaccumulation, occurring on Zn-, lead (Pb)- and Cd-contaminated sites (Ernst, 1976; Brooks, 1998; Bert *et al.*, 2000). In a hydroponic experiment, *A. halleri* shoot concentration increased from 300 $\mu g\ g^{-1}$ dry wt at 1 μM Zn to 32 000 $\mu g\ g^{-1}$ at 1000 μM without suffering from phytototoxicity (Zhao *et al.*, 2000). In addition, *A. halleri* plants originating from contaminated sites are known to accumulate Cd to very high concentrations or to hyperaccumulate this metal in their natural habitat (Dahmani-Muller *et al.*, 1999) as well as in hydroponic culture (Küpper *et al.*, 2000). By contrast to Zn and Cd, plants from metallicolous populations are not able to accumulate Pb, with the Pb concentration in soils always being higher than that of the upper parts of the plants (Dahmani-Muller *et al.*, 1999). Interestingly, this species is not restricted to contaminated soils and can be found on normal soils. The metal accumulation abilities of nonmetallicolous populations are relatively unexplored. Previously, Bert *et al.* (2000) have shown that Zn leaf concentration of nonmetallicolous populations was much higher than that expected for nonaccumulating plants on uncontaminated soil (≥ 0.3% and < 0.01% w : w Zn in dry matter, respectively). They also found a greater accumulation ability of the nonmetallicolous population in hydroponics when it was compared with the metallicolous one. To our knowledge, no data are available on Cd and Pb accumulation in nonmetallicoulous populations of *A. halleri*.

As was suggested by Escarré *et al.* (2000) and McGrath *et al.* (2001), it is essential to study different genotypes within a species that is already classed as a metal hyperaccumulator. Indeed, the differences in metal accumulation ability between populations of metallicolous and nonmetallicolous origins and within populations of the same origin can be potentially useful in terms of phytoremediation and may help to select the more efficient plants as well as those able to hyperaccumulate multiple heavy metals.

The aim of this study was to investigate the Zn, Cd and Pb aerial part contents of field-collected plants from numerous metallicolous and nonmetallicolous populations of *A. halleri* in order to explore the extent and variability of metal accumulation in natural populations of the species. To compare metal accumulation abilities of plants originated from contaminated and normal soils, ratios between metal content in aerial parts and in soils were calculated. More precisely, the following questions were addressed:

- Is Zn accumulation to very high concentration a constitutive property of the species?
- What is the pattern of Cd and Pb accumulation in nonmetallicolous plants when they grow in their natural uncontaminated soil? (Cd and Pb are present in trace quantities in such soils)
- Do the metallicolous and nonmetallicolous populations differ in metal accumulation?
- Are Zn and Cd concentrations in aerial parts correlated?

Materials and Methods

Collection of plant and soil samples from field sites

In July 1999, 33 populations of *A. halleri* (L.) (O'Kane & Al-Shehbaz) (= *Cardaminopsis halleri* (L.) Hayek), were found in Eastern Europe of which 13 were in Germany, 11 in the Czech Republic, four in Slovakia and five in Poland (Table 1). In Germany, seven populations were found in the Bohemian forest (200–350 m above sea level) and six populations in the Harz, an ancient mining region (600–700 m above sea level). These two regions are separated by about 300 km. In the Bohemian forest, populations were separated from each other by between 5 km and 40 km. In the Harz, the minimum and the maximum distances between populations were 4 km and 13 km, respectively. In the Czech Republic, all populations were located in the Bohemian forest (500–1200 m above sea level) (Table 1). The distance that separated Bohemian populations from Germany and the Czech Republic ranged from 20 to 100 km. Minimum and maximum distances between populations were 5 km and 98 km, respectively. In Slovakia, populations were located in the Tatras mountains (300–1000 m above sea level) (Table 1). They were separated by between 14 km and 88 km. In Poland, populations were found in Silesia, a region highly polluted by long-term mining and industrial activities (200–300 m above sea level) (Table 1); populations were separated by between 4 km and 32 km. Precise locations of all sites are given in Table 1.

At each study site, one to three individual plants were randomly collected to assess concentrations of Zn, Cd and Pb in the aerial parts (Table 1). In addition, soil samples were collected, at a depth of 0–20 cm, from around the roots of *A. halleri* plants previously harvested (Table 1).

Analysis of total and extractable metal concentrations and pH in soil samples

Soil samples were air-dried at 70°C to constant weight. Dried soils were sieved through a 1-mm mesh and ground in a porcelain pestle and mortar.

Total metals From each prepared soil, 0.5 g (three replicates per sample) were cold-digested with HCl and HNO_3 (3 : 1), for 12 h, then digested at 120°C for 2 h. Acid digests were analysed for Zn, Pb and Cd by flame atomic absorption

Table 1 Details of the sites where *Arabidopsis alleri* populations were found. For each of them, pH and number of collected plant (n_p) and soil (n_s) samples are indicated; GPS (Global Positioning System) co-ordinates are given to locate precisely each site

Site[1]	n_p	n_s	Region	Substratum	pH	GPS
G1	2	2	Bohemian forest	Nitrogenous Regen river's bank	4.8	12°09′88″ E, 49°10′64″ N
G2	2	2	Bohemian forest	Regen river's bank, in front of G1	5.5	12°09′52″ E, 49°11′31″ N
G3	2	2	Bohemian forest	Shady lawn next to Vltava river bank	4.7	12°39′66″ E, 49°13′1″ N
G4	2	2	Bohemian forest	Regen river bank	4.9	12°47′75″ E, 49°09′85″ N
G5	2	2	Bohemian forest	Regen river bank	5.0	12°44′75″ E, 49°12′53″ N
G6	2	2	Bohemian forest	Cham river bank	5.3	2°49′78″ E, 49°17′42″ N
G7	3	1	Bohemian forest	Regen river bank	5.4	12°22′43″ E, 49°19′27″ N
G8	2	2	Harz	Old mine	5.8	10°29′04″ E, 51°53′79″ N
G9	2	2	Harz	Roadside	5.1	10°25′11″ E, 51°53′46″ N
G10	1	2	Harz	Old mine	5.6	10°25′13″ E, 51°53′72″ N
G11	1	1	Harz	Undergrowth	4.5	10°21′95″ E, 51°51′27″ N
G12	2	2	Harz	Mine rubbles	5.4	10°17′9″ E, 51°51′91″ N
G13	2	2	Harz	Roadside and lawn	5.5	10°18′5″ E, 51°55′22″ N
Cz1	1	1	Bohemian forest, Sumava	Forest above a railway	5.3	13°46′ E, 48°59′ N
Cz2	1	1	Bohemian forest, Sumava	Meadow alongside a railway	4.9	13°46′ E, 48°59′ N
Cz3	1	1	Bohemian forest, Sumava	Meadow alongside a railway	4.9	13°46′3″ E, 49°03′55″ N
Cz4	1	1	Bohemian forest, Sumava	Shady roadside	5.7	13°46′39″ E, 48°59′258″ N
Cz5	1	1	Bohemian forest, Sumava	Beside road alongside a hay meadow	5.1	13°46′ E, 48°59′ N
Cz6	1	1	Bohemian forest, Sumava	Meadow	4.7	13°48′ E, 48°57′ N
Cz7	1	1	Bohemian forest, Sumava	River bank alongside a meadow	4.0	13°48′ E, 48°57′ N
Cz8	2	2	Bohemian forest, Sumava	Slope in a cool, shady wood	4.3	12°42′92″ E, 49°28′37″ N
Cz9	2	2	Bohemian forest, Sumava	Sloppy meadow	4.3	13°33′31″ E, 49°03′35″ N
Cz10	1	1	Bohemian forest, Sumava	Meadow	6.1	13°31′65″ E, 49°06′23″ N
Cz11	2	2	Bohemian forest, Sumava	Woody footpath	4.6	13°32′18″ E, 49°05′7″ N
Sl1	2	2	Tatras	Bank liable to flooding	4.6	20°53′95″ E, 48°45′19″ N
Sl2	2	2	Tatras	Shady meadow	5.1	21°07′81″ E, 48°46′17″ N
Sl3	1	1	Tatras	Copper mine	6.1	20°55′64″ E, 48°51′41″ N
Sl4	2	2	High Tatras	Tatransla javorina (Nature Reserve)	4.8	20°09′24″ E, 49°16′96″ N
P1	2	2	Silesia	Wood in Katowice suburb	4.6	18°57′04″ E, 50°14′8″ N
P2	1	1	Silesia	Metallurgical factory	4.6	18°56′67″ E, 50°29′68″ N
P3	1	1	Silesia	Metallurgical factory	4.4	18°57′58″ E, 50°29′5″ N
P4	1	1	Silesia	Metallurgical factory	5.6	18°55′79″ E, 50°29′98″ N
P5	1	1	Silesia	Old mine	5.8	19°01′52″ E, 50°16′95″ N

[1]G, Germany; Cz, Czech Republic; Sl, Slovakia; P, Poland.

spectrometry (FAAS). The limit of detection for Cd was 1 µg g^{-1}, and any value less than this was recorded as 0.1 µg g^{-1} for statistical analysis.

Because there is no international standard by which a metal-contaminated soil is defined, we have classified our soil samples following the French agricultural approved NFU 44 041 norm. This norm characterizes Zn-, Pb- and Cd-contaminated mud and it is commonly used to describe metal-contaminated soils. Thus, a soil will be considered as a contaminated one if it contains more than 300 µg g^{-1} Zn or 100 µg g^{-1} Pb or 2 µg g^{-1} Cd. These values are expressed in total concentrations of metal.

Extractable metals From each prepared soil, 0.2 g (four replicates per sample) were extracted with 20 ml of ammonium acetate (1 M) in the presence of ethylenediaminetetraacetic acid (EDTA) (0.01 M) and shaken at 20 ± 1°C for 12 h (pH 7). Concentrations of Zn, Pb and Cd in extractable solutions were analysed by FAAS. Values below 1 µg g^{-1} Cd were replaced by 0.1 µg g^{-1} for statistical analysis.

PH Soil pH was measured on 1 : 1 soil–distilled water mixtures (Table 1).

Plant sample analysis

Aerial parts of each plant collected were rinsed thoroughly in distilled water and dried at 50°C. For analysis, three subsamples (0.2 g each) of each rinsed plant were taken and cold-digested with HNO_3 and $HClO_4$ (4 : 1) for 12 h, then digested at 190°C until a final volume of 1 ml was reached. The samples were then diluted to 10 ml using 2 M HCl. Concentrations of Zn, Cd and Pb were analysed by FAAS.

Data analysis

Nine quantitative variables were obtained from soil and plant measurements as described in Table 2. In order to compare metallicolous and nonmetallicolous populations for the amount of heavy metal accumulation, the ratio of the metal

Table 2 List of traits measured in this study with their abbreviations

Trait	Abbreviation
Soil traits	
Total concentration of zinc in soil sample	ZnT
Total concentration of cadmium in soil sample	CdT
Total concentration of lead in soil sample	PbT
Extractable concentration of zinc soil sample	ZnE
Extractable concentration of cadmium in soil sample	CdE
Extractable concentration of lead in soil sample	PbE
Plant traits	ZnAp
Zinc aerial part concentration	CdAp
Cadmium aerial part concentration	PbAp
Lead aerial part concentration	
Calculated ratios	
Zinc aerial part concentration : total concentration of zinc in soil sample ratio	ZnAp : ZnT
Zinc aerial part concentration : extractable concentration of zinc in soil sample ratio	ZnAp : ZnE
Cadmium aerial part concentration : total concentration of cadmium in soil sample ratio	CdAp : CdT
Cadmium aerial part concentration : extractable concentration of cadmium in soil sample ratio	CdAp : CdE

All concentrations are expressed as $\mu g\ g^{-1}$ on a dry weight basis. Additional traits calculated as ratios from measured traits are also listed.

concentration in aerial parts over the metal concentration in soils was calculated for Zn and Cd. Given that both total and extractable metal concentrations were considered for soil samples, four new variables were created, leading to a total of 13 characters (Table 2). All variables were log-transformed to improve the fit to a normal distribution (Sokal & Rohlf, 1995; Underwood, 1997). Zero values for soil Cd concentration were given a value of 0.1 before log transformation.

To test whether metal contents in soils and plants differed among soils and populations of plants, according to their metallicolous or nonmetallicolous type, a nested analysis of variance type III SS of SAS Proc GLM for unequal sample sizes (SAS Institute, 1992) was performed on each variable. The model was type, soil within type or population within type. Type (uncontaminated or contaminated for soils, or metallicolous or nonmetallicolous for plants) and soil or population were, respectively, fixed and random effects. For CdT and CdE (Table 2), only the variation among contaminated soils could be analysed, because of the very low Cd concentrations found in uncontaminated soils.

Canonical discriminant analyses (procedure CANDISC of SAS; SAS Institute, 1992) were also performed on means of total soils and plant populations to summarize the results of the analysis of variance using linear combinations of the quantitative variables instead of each variable separately. This analysis enables us to test whether soils and plant populations are grouped according to their contaminated or metallicolous origin, without any a priori hypothesis about their type.

In order to analyse the relationships among variables, phenotypic correlations (procedure CORR of SAS; SAS Institute, 1992) were calculated using the whole data set and each type separately. As they are combinations of measured variables, and thus correlated to those characters, ratios were not included into these analyses. Given the large number of correlations, sequential Bonferroni tests were applied to adjust the probability of type-1 error to the number of comparisons (Sokal & Rohlf, 1995; Underwood, 1997).

Results

Soil samples

Validity of the soil classification In order to test whether the NFU classification was valid, a canonical discriminant analysis was performed on total metals in soils (ZnT, CdT and PbT) (Table 2). This analysis was made without any a priori hypothesis about the contaminated or uncontaminated type of soils. Figure 1 shows that soils were significantly differentiated according to the axes 1 and 2, which represented 88% and 8% of the between-soil variation, respectively. According to axis 1, all soils can be grouped into the soil types defined by the NFU norm, indicating that this classification is robust.

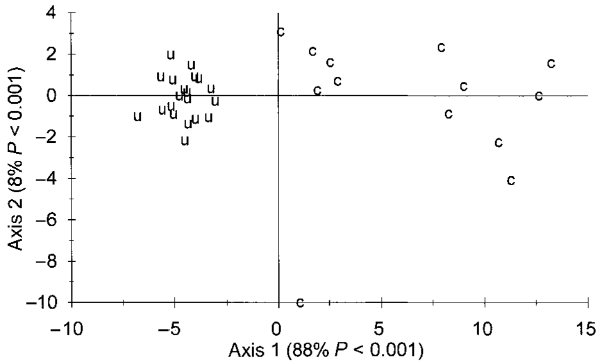

Fig. 1 Canonical discriminant analysis on total metals (zinc, lead and cadmium) in soils. Axes 1 and 2 represent 88% and 8% of the between soil variation, respectively; u, uncontaminated soils; c, contaminated soils.

Table 3 Metal concentrations in the soil/plant column. Zinc (Zn), lead (Pb) and cadmium (Cd) concentrations in the aerial parts of wild *Arabidopsis halleri* plants and total and extractable Zn, Pb and Cd concentrations in the corresponding rhizospheric soils. Mean ± SE were calculated when the number of samples was > 1 (µg g^{-1} dry wt; $n = 3$)[1]

	Total metal in soil			Extractable metal in soil			Aerial plant part concentration		
Site[2]	Zn	Pb	Cd	Zn	Pb	Cd	Zn	Pb	Cd
G1	117 ± 17	29 ± 9	< 1	3 ± 2	1 ± 0	< 1	6 364 ± 1380	10 ± 1	56 ± 11
G2	176 ± 2	35 ± 2	< 1	5 ± 2	1 ± 0	< 1	6 276 ± 230	10 ± 2	69 ± 16
G3	175 ± 15	40 ± 6	< 1	3 ± 0	1 ± 0	< 1	9 640 ± 987	13 ± 2	89 ± 47
G4	113 ± 64	28 ± 11	< 1	3 ± 1	1 ± 0	< 1	9 116 ± 1476	9 ± 1	49 ± 15
G5	201 ± 30	50 ± 4	< 1	7 ± 4	1 ± 0	< 1	10 876 ± 578	12 ± 1	75 ± 41
G6	83 ± 5	33 ± 1	< 1	1 ± 0	2 ± 0	< 1	6 667 ± 484	9 ± 0	55 ± 9
G7	74	31	< 1	1	0	< 1	2 394 ± 845	12 ± 0	23 ± 4
G8	2 062 ± 1331	2121 ± 725	15 ± 0	123 ± 9	117 ± 49	7 ± 2	11 561 ± 2171	38 ± 21	267 ± 86
G9	549 ± 38	1487 ± 30	4 ± 1	52 ± 0	56 ± 4	3 ± 0	6 893 ± 462	33 ± 11	11 ± 2
G10	8 762 ± 192	3894 ± 1245	29 ± 6	1894 ± 407	60 ± 35	14 ± 3	11 456	20	27
G11	69	144	< 1	7	5	< 1	5 467	16	4
G12	7 045 ± 5070	6592 ± 352	34 ± 2	1272 ± 1047	679 ± 37	17 ± 1	10 003 ± 3662	164 ± 7	78 ± 6
G13	3 427 ± 1462	9377 ± 3242	10 ± 4	405 ± 207	803 ± 520	5 ± 1	5 978 ± 5714	70 ± 5	20 ± 18
Cz1	104	49	< 1	1	2	< 1	6 254	11	70
Cz2	90	22	< 1	1	1	< 1	1 937	8	20
Cz3	63	37	< 1	2	2	< 1	4 740	9	20
Cz4	75	15	< 1	1	2	< 1	1 995	5	16
Cz5	148	54	< 1	2	1	< 1	4 216	7	6
Cz6	97	34	< 1	2	0	< 1	2 523	13	4
Cz7	28	24	< 1	1	1	< 1	6 438	10	12
Cz8	157 ± 80	228 ± 89	2 ± 2	11 ± 8	12 ± 2	1 ± 0	5 014 ± 3029	14 ± 4	20 ± 7
Cz9	81 ± 3	29 ± 1	< 1	1 ± 0	1 ± 0	< 1	2 703 ± 315	9 ± 1	3 ± 0
Cz10	249	34	< 1	5	1	< 1	4 499	9	31
Cz11	70 ± 0	40 ± 7	< 1	1 ± 0	1 ± 0	< 1	4 854 ± 2211	13 ± 1	20 ± 2
Sl1	40 ± 0	21 ± 0	< 1	2 ± 0	1 ± 0	< 1	4 464 ± 987	14 ± 2	22 ± 13
Sl2	51 ± 6	26 ± 1	< 1	1 ± 0	1 ± 0	< 1	8 859 ± 4734	11 ± 3	79 ± 24
Sl3	31 500	196	5	5000	3	2	13 168	12	8
Sl4	46 ± 6	34 ± 2	< 1	1 ± 0	2 ± 0	< 1	1 806 ± 769	8 ± 0	36 ± 1
P1	273 ± 25	189 ± 30	7 ± 0	22 ± 1	2 ± 0	2 ± 0	6 783 ± 150	11 ± 2	37 ± 10
P2	307	275	4	49	45	2	10 570	107	177
P3	180	235	4	33	47	3	4 825	145	44
P4	2 325	1533	24	481	266	13	15 744	376	36
P5	22 685	4395	64	2490	380	27	9 701	52	87

[1]Following the French NFU 44 041 norm, a soil is considered as contaminated if it contains more than 300 µg g^{-1} Zn or 100 µg g^{-1} Pb or 2 µg g^{-1} Cd (expressed in total concentrations of metal). The mean Zn, Pb and Cd concentrations expected in nonaccumulationg plants growing on uncontaminated soil are < 100, < 10, and < 1, respectively, and on contaminated soil are < 1000, < 100, and < 10, respectively (µg g^{-1} dry wt; Brooks,1998). Thresholds of hyperaccumulation are > 10 000, > 1000 and > 100 for Zn, Pb and Cd, respectively (Brooks, 1998).
[2]G, Germany; Cz, Czech Republic; Sl, Slovakia; P, Poland.

Concentrations of total and extractable Zn, Pb and Cd in soil samples

Among all the soils analysed, 13 had at least one total metal concentration above the NFU thresholds (Table 3). As a result, such soils were considered contaminated. The other 20 soils, with concentrations of total Zn, Pb and Cd below the NFU thresholds (Table 3) were considered uncontaminated. A nested analysis of variance (type, soil within type) was carried out on characters describing metal content in soil samples. The results are presented in Table 4. For all soil traits (ZnT, ZnE, CdT, CdE, PbT and PbE) (Table 2), both type and soil were significant and *F*-ratio values showed that variation between types was higher than variation among soils within type (e.g. ZnT: *F*-ratio for type = 41.87***; > *F*-ratio for soil (type) = 7.62***). In addition, for all these characters, the mean of the contaminated type was significantly higher than the mean of the uncontaminated type. It thus appeared that the soils are differentiated according to their metal content.

Relationships between total and extractable metals in soils Using a correlation analysis, both on all data and on those from only contaminated soils, a very high and positive linear relationship between variables measuring total and extractable metals in soils was observed (Table 5a,b; significant correlations with Bonferonni's corrections). When only

Table 4 Results of the nested variance analysis performed on metal content in soils and plants[1]

Character	Mean of U/M type	Mean of C/NM type	F-ratio type	F-ratio pop (type)
ZnT	5090.00 (n = 20)	99.00 (n = 31)	41.87***	7.62***
ZnE	780.90 (n = 20)	2.50 (n = 31)	66.66***	5.93***
ZnAp	8549.00 (n = 19)	5576.00 (n = 33)	4.12 ns	1.01 ns
CdT	15.19 (n = 20)	0.00 (n = 31)	123.16***	3.6 **
CdE	7.22 (n = 20)	0.00 (n = 31)	106.48***	5.88***
CdAp	67.17 (n = 19)	40.32 (n = 33)	1.30 ns	3.84**
PbT	2722.50 (n = 20)	36.80 (n = 31)	60.05***	18.23***
PbE	209.99 (n = 20)	1.40 (n = 31)	46.15***	10.16***
PbAp	72.75 (n = 19)	10.66 (n = 33)	27.39***	8.89***
ZnAp/ZnE	203.7 (n = 19)	3212.1 (n = 30)	62.61***	3.65**
CdAp/CdE	15.75 (n = 17)	_	_	3.38 ns
ZnAp/ZnT	13.17 (n = 19)	69.25 (n = 30)	28.36***	3.26**
CdAp/CdT	7.94 (n = 17)	_	_	3.67 ns

,,***$P < 0.05$, $P < 0.01$, $P < 0.001$, respectively; ns, not significant ($P > 0.05$).
[1]For soil traits, type refers to uncontaminated (U) or contaminated (C) soils. For plant traits, type refers to metallicolous (M) or nonmetallicolous (NM) populations. The model is type, population within type.

uncontaminated soils are considered, ZnT and ZnE were positively correlated (Table 5c; significant correlations with Bonferonni's corrections).

Relationships between metals in soil When all data were considered and after Bonferonni's corrections, ZnT, CdT and PbT were all positively correlated (Table 5a). The same result was obtained with ZnE, CdE and PbE (Table 5a; significant correlations with Bonferonni's corrections). When data from contaminated soils were considered, CdT and ZnT and CdT and PbT were positively correlated after Bonferonni's corrections (Table 5b). In uncontaminated soils, all correlations were nonsignificant (Table 5c). This result means that a soil contaminated with one of these metals also tends to be contaminated with the two others. By contrast, no correlation was observed between Zn, Cd and Pb on uncontaminated soils, although these metals are present in trace quantities.

Plant samples: zinc, cadmium and lead contents in aerial parts

Plant differentiation Results of the canonical discriminant analysis on plant populations for metal concentrations in aerial parts (ZnAp, CdAp and PbAp) are presented in Fig. 2. As was found for total metal in soils, the populations of plants are significantly differentiated for aerial part metal concentration (76% and 20% of the between population variation are significantly explained by axis 1 and axis 2, respectively). Unlike Fig. 1, populations were less well grouped according to their metallicolous or nonmetallicolous types, as defined by the NFU norm for metals in soils. This means that the level of metals in aerial parts is probably only slightly correlated with soil metal content.

Table 5 Results of the correlation analysis performed on all variables from Table 2 except calculated ratios. Significant correlations ($P < 0.05$) calculated on (a) all data, (b) contaminated/metallicolous type data and (c) uncontaminated/nonmetallicolous type data

	Traits							
Traits	ZnAp	ZnT	ZnE	CdAp	CdT	CdE	PbT	PbE
(a)								
ZnT	0.36							
ZnE	0.39	0.96*						
CdAp	0.53*							
CdT	0.33	0.86*	0.91*					
CdE	0.32	0.86*	0.92*		0.99*			
PbT		0.85*	0.89*		0.92*	0.93*		
PbE		0.75*	0.81*		0.86*	0.88*	0.94*	
(b)								
ZnT								
ZnE		0.96*						
CdL	0.57							
CdT		0.68*	0.78*					
CdE		0.70*	0.80*		0.98*			
PbT		0.63	0.66		0.74*	0.78*		
PbE			0.50		0.59	0.67	0.88*	
(c)								
ZnT	0.40							
ZnE	0.55	0.67*						
CdAp	0.48							
CdT								
CdE								
PbT		0.41	0.54					
PbE							0.44	

The probability for experiment-wise significances using the Bonferroni test: (a) *$P < 0.0006$; (b) *$P < 0.001$; (c) *$P < 0.00011$.

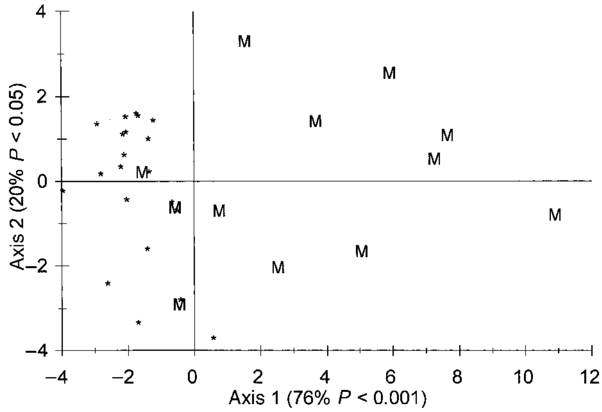

Fig. 2 Canonical discriminant analysis on metal concentrations in aerial plant parts (zinc, lead and cadmium) of the *Arabidopsis halleri* populations. Axes 1 and 2 represent 76% and 20% of the between population variation, respectively; asterisk, nonmetallicolous populations; M, metallicolous populations.

Zinc aerial part content The mean ZnAp concentration of metallicolous populations ranged from 4825 to 15 744 µg g^{-1} dry wt, whereas that of the nonmetallicolous populations ranged from 1806 to 10 876 µg g^{-1} dry wt. (Table 3). These results explained the nonsignificant effect of soil type for ZnAp in the nested analysis of variance (type, populations within type) (Table 4). There were also nonsignificant differences between populations within type. Whatever the type of population, all plants (see Table 3) showed very high amounts of Zn relative to the expected concentrations for nonaccumulating plants growing on unpolluted or polluted soils (< 100 or < 000 µg g^{-1} dry wt, respectively (Brooks, 1998)). Considering individual plant data, Zn concentrations ranged from 264 to 15 744 µg g^{-1} and a total of 12 plants out of 49 (24%) (seven out of 18 plants from contaminated soils and five plants out of 31 from normal habitats) showed Zn concentrations higher than 10 000 µg g^{-1}, which is the common threshold used to define Zn hyperaccumulation (Brooks, 1998) (Fig. 3).

Cadmium aerial part content The mean CdAp concentration of metallicolous populations ranged from 4 to 267 µg g^{-1} dry wt. whereas those of nonmetallicolous populations ranged from three to 89 µg g^{-1} dry wt. (Table 3). Similar to ZnAp, there was no effect of soil type (Table 4), although the population effect was significant. Unlike ZnAp, CdAp showed variation among populations within type. Regardless of the type of population, all plants (see Table 3) showed very high amounts of Cd relative to expected concentrations for nonaccumulating plants growing on unpolluted or polluted soils (< 1 or < 10 µg g^{-1} dry wt, respectively) (Brooks, 1998). Considering the metallicolous populations, two populations out of 13 showed Cd concentrations higher than 100 µg g^{-1} dry wt, which is the common threshold used to define Cd hyperaccumulation (Brooks, 1998). Interestingly, five out of 20 populations from the normal soils had Cd values higher

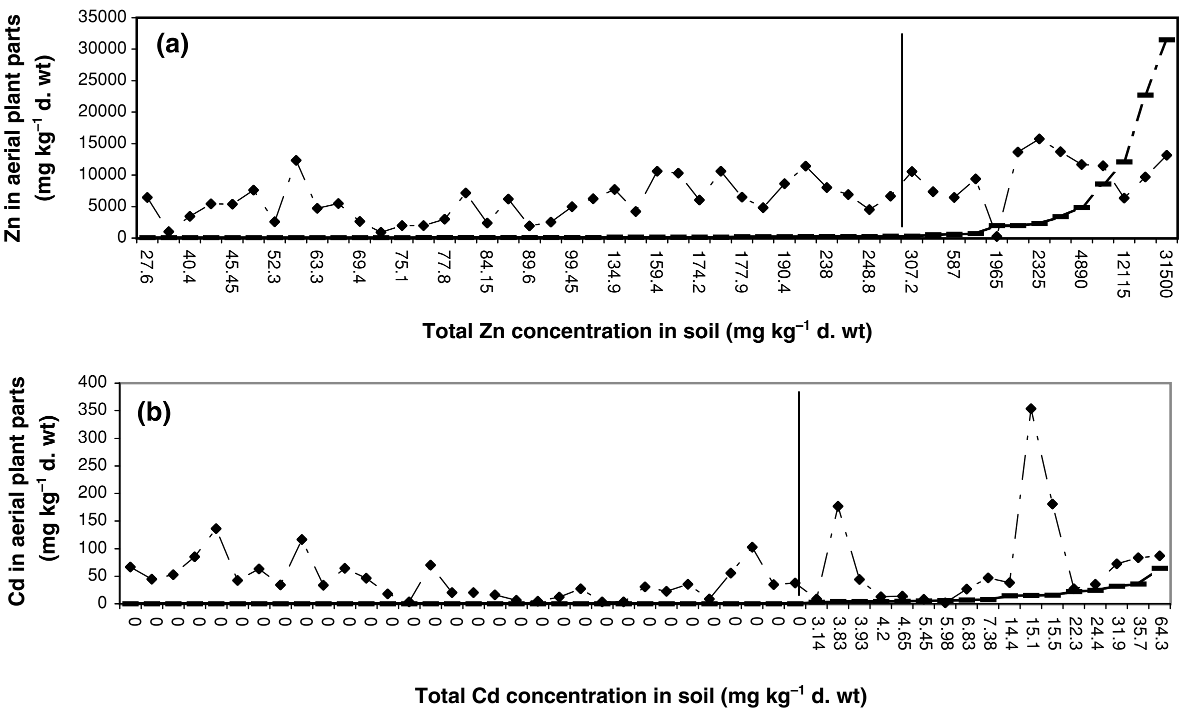

Fig. 3 Metal content in aerial plant parts of each plant collected and associated total soil metal content. Soil values are classified following an increasing order. (a) Values for zinc (Zn); (b) values for cadmium (Cd). The vertical line represents the limit between a contaminated and a normal soil following the French NFU norm (300 µg g^{-1} and 2 µg g^{-1} Zn and Cd, respectively); rectangles, metal in soil; diamonds, metal in plants.

than 69 μg g^{-1} (i.e. very close to 100 μg g^{-1} dry wt), even though the total Cd values for the soils were extremely low (Table 3, e.g. for G3, CdAp = 89 ± 47 μg g^{-1} dry wt and CdT < 1 μg g^{-1} dry wt). Considering individual plant data, Cd concentration ranged from 2 to 354 μg g^{-1} in metallicolous populations and from 3 to 136 μg g^{-1} in nonmetallicolous populations (Fig. 3). A total of six plants out of 49 (12%) (three out of 18 plants from contaminated soils and three plants out of 31 from normal habitats) showed Cd concentrations higher than 100 μg g^{-1}.

Lead aerial part content The mean PbAp concentration of metallicolous populations ranged from 11 to 376 μg g^{-1} dry wt, whereas that of the nonmetallicolous populations ranged from 5 to 14 μg g^{-1} dry wt (Table 3). For PbAp, both soil type and population were significant (Table 4). For this character, variation between type was higher than variation among population within type (*F*-ratio for type = 27.39*** > *F*-ratio for pop (type) = 8.89***). The mean of the metallicolous type was significantly higher than the mean of the nonmetallicolous type. Several metallicolous populations (P1, P2, P3, P4, G9 and G11) are located close to a road or an active metallurgical factory. Thus, part of the PbAp concentration measured is certainly due to atmospheric deposition or pollution from leaded fuel. For the other contaminated sites, Pb contamination can occur through soil particles adhering to the leaf. This observation can explain why type and population effects are significant. A significant result is that most of the populations (see Table 3) showed low or similar amounts of Pb relative to expected concentrations for nonaccumulating plants growing on unpolluted or polluted soils (< 10 or < 100 μg g^{-1} dry wt, respectively; Brooks, 1998) and their PbAp concentrations are always lower than their associated PbT.

Relationship between ZnAp and CdAp

After Bonferroni's corrections, when all data were considered, ZnAp and CdAp were positively correlated (Table 5a).

Zinc and cadmium contents in aerial parts of wild *Arabidopsis halleri* plants related to Zn and Cd concentrations in soil

Relationship between Zn and Cd content in plants and Zn and Cd content in soils After Bonferroni's corrections, no significant correlation between soil metal contaminants and plant metal concentration were found (Table 5).

Metal accumulation capabilities of plants Ratios between the metal content in aerial plant parts and metal content in soil were calculated in order to estimate the metal accumulation capacities of plants on the soil where they grow. Results of the nested analysis of variance (soil type, population within type) performed on these ratios are summarized in Table 4. For ZnAp : ZnE and ZnAp : ZnT ratios, both soil type and population were significant. Variation between soil type was higher than variation between population within type (e.g. ZnAp : ZnE, *F*-ratio for type = 62.61*** > F-ratio for pop (type) = 3.65***). For all ratios, the mean of the nonmetallicolous type was significantly higher than the mean of the metallicolous type.

Considering the contaminated soils, the values of ZnAp : ZnT and CdAp : CdT ratios were > 1 (Table 4). Thus, aerial parts of the metallicolous plants had a higher Zn and Cd concentration than in the soil. Interestingly, the value of the ZnAp : ZnT ratio for normal soils was >> 1 (Table 4). Thus, nonmetallicolous plants also showed more Zn in their aerial parts than in the soil. In addition, the average value of the ratio was about five times larger than that of the contaminated soil. Considering Cd, if the average CdT of the nonmetallicolous soils was equal to 1 μg g^{-1} then the CdAp : CdT ratio would be equal to 40. Thus, the ratio of nonmetallicolous plants is five times higher than that of metallicolous plants. When extractable values of metals in soils are considered instead of total metals, ratios became higher than those described above, given that extractable metals are always in lower quantities than total metals (Table 4). In this case, the ratio in plants from normal soils is 15 times higher for Zn and 25 times higher for Cd than the ratios in plants from contaminated soils.

Figure 3 presents metal content in aerial parts of each plant related to the total metal content in their respective soil. For Zn and Cd, plants (except four for Zn) showed more Zn and Cd in their leaves than in their respective soil. For Zn, three plants had an aerial part content less than that of soil content when the soil value was above 12 115 μg g^{-1}. It appears that as soil values increase, aerial plant part values stay comparable with those obtained for lower soil values. This result would suggest that plant Zn levels cannot increase above some plateau. This was not observed for Cd in the range of soil values we have collected.

Discussion

In this study, we have investigated a large number of natural populations of *Arabidopsis halleri* from contaminated and normal habitats. On contaminated habitats, our results have confirmed that the soils where *A. halleri* grows are characterized by elevated concentrations of Zn, Cd and Pb.

Metal accumulation and hyperaccumulation in natural populations of *A. halleri*

In a previous study, Bert *et al.* (2000) investigated three nonmetallicolous and two metallicolous populations of *A. halleri* for accumulation ability. Studying field data, they showed that Zn leaf concentration of nonmetallicolous populations (> 3000 μg g^{-1}) was much higher than that expected for nonaccumulating plants on normal soil (< 100 μg g^{-1}). They found no plants with Zn leaf concentration > 10 000 μg g^{-1} (i.e. above the hyperaccumulator threshold). They confirmed the Zn hyperaccumulator status (> 10 000 μg g^{-1}) of *A. halleri* from metallicolous populations. They performed hydroponic experiments on seedlings raised from seeds from the same field material and found a greater accumulation ability of the nonmetallicolous population compared with a metallicolous one. These results, combined with those obtained from the field, predicted that the nonmetallicolous populations would hyperaccumulate Zn if they were transplanted to a contaminated site. This work posed the question: Is the accumulation to very high concentration (> 100 and < 10 000 μg g^{-1}) a constitutive trait present in all members of populations of *A. halleri* growing either on contaminated or normal soils?

The present study, performed on 33 populations, finds that populations from each type accumulate Zn at broadly similar and very high level in their shoots, although they grow on soils with extremely different Zn content. Thus, by studying a large number of metallicolous and nonmetallicolous populations, we conclude that Zn accumulation to very high concentration is a constitutive property in *A. halleri*. In addition, a very homogeneous accumulating response is observed between populations within type. A similar result was obtained by Lombi *et al.* (2000) who compared four metallicolous populations of *Thlaspi caerulescens* and found that they had the same ability to hyperaccumulate Zn.

Once more, the Zn hyperaccumulator trait of the metallicolous populations, according to the definition of Zn hyperaccumulation by Baker & Brooks (1989) (> 10 000 μg g^{-1} Zn dry wt) has been confirmed. Indeed, 24% of the *A. halleri* plants (12 out of 49; 39% of the metallicolous individuals and 16% of the nonmetallicolous individuals) exceeded the Zn hyperaccumulator threshold, whereas most of the plants had aerial part Zn concentrations approaching the threshold of hyperaccumulation (10 000 μg g^{-1}). To our knowledge, this is the first time that Zn hyperaccumulation is described for nonmetallicolous individuals of *A. halleri*, growing on soils with extremely low metal contents.

Although *A. halleri* has long been known as a Zn hyperaccumulator (Brooks, 1998), Zn hyperaccumulation in natural populations is still relatively unexplored. The Zn shoot concentrations for four plants collected from a single Zn–Pb–Cd contaminated site in Austria ranged from 5320 to 8570 μg g^{-1} Zn (Wenzel & Jockwer, 1999). That study found Zn concentrations approaching the limit of hyperaccumulation, as we found for most of the plants studied in this paper, but no individual with Zn concentration exceeding 10 000 μg g^{-1}. By contrast, Dahmani-Muller *et al.* (1999) analysed three plants from a single Zn–Pb–Cd contaminated site in France and found an average of 21 500 μg g^{-1} Zn in the leaves of *A. halleri*. This value greatly exceeds 10 000 μg g^{-1} and the highest value for hyperaccumulation found by us on metallicolous plants (15 744 μg g^{-1}).

As for Zn accumulation, we can conclude that Cd accumulation to very high concentration is a constitutive property of *A. halleri*. The present work is the first to give information about the variability of Cd accumulation in natural populations of *A. halleri*. By contrast to Zn, the Cd accumulation abilities of the populations within type were very variable. Such differences have also been observed in *Thlaspi caerulescens* among metallicolous populations (Lombi *et al.*, 2000). It is the first time that populations from uncontaminated soil have been measured for their aerial part Cd content. We have shown that some of them (five out of 20) had CdAp concentration approaching the threshold of hyperaccumulation (> 100 μg g^{-1} Cd dry wt) whereas 10% of the plant individuals exceeded 100 μg g^{-1} Cd. To our knowledge, it is the first time that Cd hyperaccumulation is described for nonmetallicolous populations of *A. halleri*. We have also confirmed the hyperaccumulator status of metallicolous populations even if only two populations out of 20 and 17% of the plant individuals had CdAp concentration > 100 μg g^{-1}. This trait seems not to be very widespread in *A. halleri* whatever the type and the population considered. This result could explain why *A. halleri* is only sometimes cited as a Cd hyperaccumulating species. Wenzel & Jockwer (1999) studied at least four plants from a single metallicolous population and found that the best performer contained 80.3 μg g^{-1} Cd in its shoots and the worst contained 70.3 μg g^{-1} Cd, whereas Dahmani-Muller *et al.* (1999) found more than 250 μg g^{-1} Cd in the leaves of *A. halleri* (mean of three samples) from a highly contaminated site. Differences in Cd content could result from the variability between metallicolous populations, as found by us, and/or be explained by the fact that the authors did not analyse the same plant organs (leaves and shoots).

Lead is usually extremely immobile and not easily accumulated by plants. These considerations could explain why hyperaccumulation of Pb is particularly rare. The Pb hyperaccumulation threshold is fixed at 1000 μg g^{-1} dry wt. (Baker & Brooks, 1989). Although some *A. halleri* plants were shown to be Zn- and Cd-hyperaccumulators, the species was not able to hyperaccumulate Pb (average Pb aerial part content was 72.75 μg g^{-1} and 10.66 μg g^{-1} for metallicolous and nonmetallicolous types, respectively). Moreover, *A. halleri* did not behave as an accumulator of Pb *sensu* Baker (1981) since the aerial part Pb concentration was always lower than PbT. Thus, the Pb pattern in *A. halleri* aerial parts completely differed from those of Zn and Cd. Dahmani-Muller *et al.* (1999) came to a similar conclusion after they measured 138 μg Pb g^{-1} in leaves from metallicolous plants whereas the total Pb content of the soil was more than 900 μg Pb g^{-1}. To our knowledge, no data have previously been published on Pb aerial part concentrations from nonmetallicolous populations. It is also the first time that Pb aerial part pattern and its variability have been investigated in natural nonmetallicolous and metallicolous populations of *A. halleri*. As with Cd, Pb leaf concentration was very variable between populations within soil type.

The definition of accumulators *sensu* Baker is based on the ratio between aerial plant part content over total metal content in soil (Baker, 1981). Using these ratios, we have shown that metallicolous plants contained more Zn and Cd in their aerial parts than in the soil where they grow, and thus these plants could be considered as Zn and Cd accumulators (Baker, 1981). Interestingly, we have found that plants from nonmetallicolous populations are also accumulators of Zn and Cd and that their capabilities of accumulation appear to be greater than those of metallicolous ones, under the conditions of the study (i.e. on uncontaminated soil).

Extractable metals represent the fraction of metal available for plant uptake. Thus, ratios calculated with extractable metals instead of total metals should be more realistically related to plant accumulation abilities. When relating metal concentrations accumulated in the leaves to extractable metal concentrations, nonmetallicolous plants concentrated much more Zn and Cd than the metallicolous ones.

The results presented in Fig. 3 suggest that with increasing total Zn soil content, the Zn content of the aerial parts of the plants reaches a plateau. This was not observed for Cd. Hamon *et al.* (1999) suggest that a plateau response could be due to plant physiology and be explained by blocking of the translocation of metals from the roots to the shoots and/or saturation of the metal uptake mechanism at the root surface. Thus, plateau response could be a safety mechanism that limits plant metal uptake and prevents phytotoxicity at high metal soil concentration. From our results, we could suggest that nonmetallicolous populations will not possess this safety mechanism for Zn. For Cd, one hypothesis could be that this plateau will appear later in the range of concentrations tested. However, to verify what we assume about underlying mechanisms of accumulation in the two population types, hydroponic experiments will have to be performed on seedlings raised from seeds collected from field material as previously performed by Bert *et al.* (2000).

Finally, we have shown that plants from nonmetallicolous populations appear to concentrate Zn and Cd more effectively than those from metallicolous populations. This result posed the question of whether nonmetallicolous plants transplanted to a contaminated site will be able to survive and maintain this accumulating potential. Will they accumulate more Zn and Cd in their shoots than the metallicolous ones? Reciprocally, what will be the accumulation phenotype of metallicolous plants on nonpolluted or low pollution soils?

The Zn and Cd hyperaccumulator plant *A. halleri* as a potential agent for phytoremediation?

Our study contributes to discussions on the possibility of using *A. halleri* to phytoremediate Zn- and Cd-contaminated soils. We have shown that some genotypes from metallicolous and nonmetallicolous populations were able to hyperaccumulate Zn and Cd, and so are potentially good candidates for phytoextraction. In addition, *A. halleri* plants have the ability to co-accumulate Zn and Cd, which is of great practical importance because soil contamination is rarely restricted to a single metal. Furthermore, using ratio calculations, we have shown that plants from uncontaminated sites seemed to be more efficient at Zn and Cd accumulation than metallicolous plants and so could represent a valuable resource of genotypes for phytoextraction.

However, these genotypes have first to be tested on contaminated soils in order to see if they will be able to survive and at least maintain their metal accumulation ability. The fact that most of the plants from both origins are able to accumulate very large amount of Zn and that a very homogenous accumulating response between populations within type was found suggests that to increase the performance of the species by selecting more efficient genotypes may be difficult. By contrast, the large variability in Cd accumulation of the populations within type suggests that it will be possible to select more efficient genotypes while maintaining the efficiency of Zn accumulation.

In conclusion, to explore more fully the extent of genetic variability (between plants within population, between population within type) of metal accumulation in *A. halleri*, it is absolutely necessary to compare this trait for metallicolous and nonmetallicolous populations growing in the same concentrations of heavy metal, in order to reduce the environmental variance. Controlled experiments using maternal progenies are in progress; they should confirm the metal accumulation ability of nonmetallicolous genotypes and their potential use for phytoextraction.

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