Zhao, F-J., Jiang, R. F., Dunham, S. J. and McGrath, S. P. 2006. Cadmium uptake, translocation and tolerance in the hyperaccumulator Arabidopsis halleri. *New Phytologist.* 172 (4), pp. 646-654.

New Phytologist

Cadmium uptake, translocation and tolerance in the hyperaccumulator *Arabidopsis halleri*

F. J. Zhao[1], R. F. Jiang[2], S. J. Dunham[1] and S. P. McGrath[1]

[1]Agriculture and Environment Division, Rothamsted Research, Harpenden, Hertfordshire AL5 2JQ, UK; [2]Key Laboratory of Plant–Soil Interactions of Ministry of Education, College of Resources and Environmental Sciences, China Agricultural University, Beijing 100094, China

Author for correspondence:
Fangjie Zhao
Tel: +44 1582763133
Fax: +44 1582760981
Email: Fangjie.zhao@bbsrc.ac.uk

Received: *26 May 2006*
Accepted: *17 July 2006*

Summary

• *Arabidopsis halleri* is a well-known zinc (Zn) hyperaccumulator, but its status as a cadmium (Cd) hyperaccumulator is less certain. Here, we investigated whether *A. halleri* can hyperaccumulate Cd and whether Cd is transported via the Zn pathway.
• Growth and Cd and Zn uptake were determined in hydroponic experiments with different Cd and Zn concentrations. Short-term uptake and root-to-shoot transport were measured with radioactive ^{109}Cd and ^{65}Zn labelling.
• *A. halleri* accumulated > 1000 mg Cd kg^{-1} in shoot dry weight at external Cd concentrations ≥ 5 μM, but the short-term uptake rate of ^{109}Cd was much lower than that of ^{65}Zn. Zinc inhibited short-term ^{109}Cd uptake kinetics and root-to-shoot translocation, as well as long-term Cd accumulation in shoots. Uptake of ^{109}Cd and ^{65}Zn were up-regulated, respectively, by low iron (Fe) or Zn status. *A. halleri* was much less tolerant to Cd than to Zn.
• We conclude that *A. halleri* is able to hyperaccumulate Cd partly, at least, through the Zn pathway, but the mechanisms responsible for cellular Zn tolerance cannot detoxify Cd effectively.

Key words: *Arabidopsis halleri*, cadmium (Cd), hyperaccumulation, tolerance, transport, zinc (Zn).

New Phytologist (2006) **172**: 646–654

doi: 10.1111/j.1469-8137.2006.01867.x

Introduction

Plant species that hyperaccumulate heavy metals or metalloids have attracted much attention over the last decade, because the metal accumulation trait has the potential to be exploited in phytoremediation of contaminated soils (Salt *et al.*, 1998; McGrath & Zhao, 2003). Furthermore, hyperaccumulator species serve as an interesting model for research into the mechanisms of metal uptake and homeostasis (Clemens *et al.*, 2002; Assunção *et al.*, 2003b).

Arabidopsis halleri is a Zn hyperaccumulator, occurring mainly in the Galmei (zinc) floras of central and western Europe (Ernst, 1974). In hydroponic experiments, *A. halleri* was able to accumulate 32 000 mg Zn kg^{-1} in shoot dry weight (DW) without suffering from phytotoxicity (Zhao *et al.*, 2000). Studies using both field surveys (Bert *et al.*, 2002) and experiments under standardized conditions (Bert *et al.*, 2000; Macnair, 2002) have shown that the ability to hyperaccumulate Zn is a species-wide constitutive trait in *A. halleri*. Macnair *et al.* (1999) performed a genetic analysis on crosses between *A. halleri* and the nonhyperaccumulating, nontolerant species *Arabidopsis lyrata* spp. *petraea*. Their results suggest that Zn tolerance and accumulation are genetically independent, with tolerance possibly being controlled by a single major gene.

Because of the close relatedness of *A. halleri* to *Arabidopsis thaliana*, *A. thaliana* GeneChips have recently been used to identify genes that are more active in the root and shoot tissues of *A. halleri* (Becher *et al.*, 2004; Weber *et al.*, 2004). These studies have shown constitutively high expression of a number of genes that are possibly involved in metal uptake and detoxification in *A. halleri*. Cellular detoxification of Zn appears to involve enhanced nicotianamine synthesis (Becher *et al.*, 2004; Weber *et al.*, 2004), as well as a high expression

of *AhMTP1* (a member of the CDF (cation diffusion facilitators) family) that encodes a vacuolar metal transporter (Dräger *et al.*, 2004). A number of putative metal transporter genes, including *AhZIP9* and *AhNramp3* in roots, and *AhZIP6* and *AhHMA3* in shoots, are also highly expressed (Becher *et al.*, 2004; Weber *et al.*, 2004). The encoded transporters may mediate Zn transport across plasma or tonoplast membranes, although their exact functions have not been established.

The Zn hyperaccumulators *Thlaspi caerulescens* and *Sedum alfredii* are also able to hyperaccumulate Cd (Baker *et al.*, 1994; Yang *et al.*, 2004), suggesting that Zn and Cd hyperaccumulation may share a similar pathway. However, recent studies have shown a large variation among different *T. caerulescens* populations in the ratio of Cd to Zn concentrations in the shoots (Lombi *et al.*, 2000; Roosens *et al.*, 2003). The status of *A. halleri* as a Cd hyperaccumulator is less certain. Bert *et al.* (2002) surveyed 33 populations of *A. halleri* in Germany, Czech Republic, Slovakia and Poland and found only two containing > 100 mg Cd kg^{-1} DW in the aerial parts, the threshold value commonly used to define Cd hyperaccumulation in the natural habitat (Baker *et al.*, 2000). Wenzel & Jockwer (1999) showed that *A. halleri* growing on the metalliferous sites in the Austrian Alps contained less than 100 mg Cd kg^{-1} DW, whereas Dahmani-Muller *et al.* (2000) reported up to 280 mg Cd kg^{-1} DW in *A. halleri* shoots growing on a heavily contaminated site near a smelter. The relatively few cases of shoot Cd exceeding 100 mg Cd kg^{-1} DW observed in field samples may be the result of a low bioavailability of Cd in soil rather than a low ability for accumulation.

Although significant progress has been made in the understanding of the physiology (Bert *et al.*, 2000; Zhao *et al.*, 2000), genetics (Macnair *et al.*, 1999; Macnair, 2002; Bert *et al.*, 2003) and molecular mechanisms (Becher *et al.*, 2004; Dräger *et al.*, 2004; Weber *et al.*, 2004) of Zn hyperaccumulation and detoxification in *A. halleri*, the physiological aspects of Cd accumulation by this species are still poorly understood. The objectives of our study were to investigate Cd accumulation and tolerance in *A. halleri*, and the competitive effect of Zn on Cd uptake and root-to-shoot translocation. We focused on the comparison between Cd and Zn, because of the chemical similarity between the two metals and the fact that the Zn hyperaccumulation trait in this plant species is better understood.

Materials and Methods

Plant culture

Seeds of *Arabidopsis halleri* (L) O'Kane & Al-Shehbaz (Brassicaceae; formerly known as *Cardaminopsis halleri* (L.) Hayek) from a metallicolous population in Blankenrode, Germany, were sown in a plastic seed tray filled with a general-purpose compost. Four weeks after germination, seedlings were transferred to hydroponic culture after roots had been washed carefully with deionized water. The basal nutrient solution contained 1 mM $Ca(NO_3)_2$, 0.5 mM $MgSO_4$, 0.25 mM K_2HPO_4, 50 μM KCl, 10 μM H_3BO_3, 1.8 μM $MnSO_4$, 0.2 μM Na_2MoO_4, 0.31 μM $CuSO_4$, 0.5 μM $NiSO_4$, 50 μM Fe(III)-EDDHA (ethylenediamine-di(o-hydroxyphenylacetic acid)), and 1 μM $ZnSO_4$. Solution pH was maintained at around 6.0 with 2 mM MES (2-morpholinoethanesulphonic acid, 50% as potassium salt) (Zhao *et al.*, 2000). All experiments were carried out in a controlled environment growth room with the following conditions: 12 h photo period with a light intensity of 350 μmol photons m^{-2} s^{-1} supplied by sodium vapour lamps; 20 : 16°C day : night temperature; and 70 : 80% day : night relative humidity.

Accumulation of Cd and Zn

Seedlings were transferred to 1 l plastic vessels (two seedlings per vessel) and grown for 1 wk with the basal nutrient solution. Plants were then divided into two groups of 20 vessels for two experiments. In experiment 1, plants were exposed to 0, 5, 15, 50 or 100 μM Cd (as $CdSO_4$) and a constant 5 μM Zn (as $ZnSO_4$). In experiment 2, plants were treated with 1, 5, 50 or 500 μM Zn and a constant 5 μM Cd, plus an additional treatment of 5 μM Zn and 0 μM Cd as the control. Each treatment was replicated in four vessels. Nutrient solutions were aerated continuously and renewed once every week. Plants were harvested after 3 wk in the treatments. Shoots and roots were separated, washed with deionized water, blotted dry with tissue paper, and dried at 60°C for 48 h. Dry weights of shoots and roots were recorded. Dried plant materials were ground using a ball mill. Plant materials were digested with $HNO_3/HClO_4$ (87/13 v/v) and the total concentrations of Zn and Cd were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES; Fisons ARL Accuris, Ecublens, Switzerland).

Effect of Zn on ^{109}Cd uptake kinetics

The kinetics of ^{109}Cd uptake were determined using intact seedlings of *A. halleri* according to the method described by Lasat *et al.* (1996) and Lombi *et al.* (2001). Four- week-old seedlings were transferred to 55 ml plastic pots (one seedling per pot) and grown for 10 d in basal nutrient solution, which was renewed once every 2 d. The nutrient solution was then replaced with a pretreatment solution containing 2 mM MES (pH adjusted to 6.0 with KOH) and 0.5 mM $CaCl_2$. After 24 h pretreatment, the seedlings were exposed to seven concentrations of $CdSO_4$ (0.2–10 μM) labelled with 2 kBq ^{109}Cd per 55 ml pot, with or without 10 μM $ZnSO_4$. The uptake solutions also contained 0.5 mM $CaCl_2$ and 2 mM MES (pH 6.0), and were aerated vigorously during the experiment. Each concentration was replicated five times.

After 20 min uptake, the seedlings were quickly rinsed with the unlabelled pretreatment solution, and then transferred to pots containing an ice-cold desorption solution (2 mM MES, 5 mM $CaCl_2$, and 50 μM $CdSO_4$) for 15 min. After desorption, the seedlings were separated into roots and shoots, blotted dry and weighed. The radioactivities of ^{109}Cd in the samples were assayed by gamma spectroscopy (Wallac Wizard 1470, PerkinElmer Life and Analytical Sciences, Boston, MA, USA). Radioactivities were found to be negligible in the shoots.

Effect of plant Zn and Fe status on ^{109}Cd and ^{65}Zn uptake

Four-week-old seedlings were transferred to 55 ml plastic pots (one seedling per pot) and precultured with basal nutrient solution for 10 d. Plants were divided into three groups of six pots, each receiving three different pretreatments: full nutrients (control), Zn supply withheld (–Zn) and Fe supply withheld (–Fe). Plants were grown in the pretreatments for 7 d. Before the ^{109}Cd and ^{65}Zn uptake assay, roots were rinsed briefly with a solution containing 0.5 mM $CaCl_2$ and 2 mM MES (pH 6.0). The uptake solution contained 4 μM $CdSO_4$ and 4 μM $ZnSO_4$, labelled with 2 kBq ^{109}Cd and 4 kBq ^{65}Zn, as well as 0.5 mM $CaCl_2$ and 2 mM MES (pH 6.0). After 20 min, the roots were rinsed and desorbed as described above, except that the desorption solution also contained 50 μM $ZnSO_4$. Radioactivities of ^{109}Cd and ^{65}Zn in the roots were determined by gamma counting.

Effect of Zn on the translocation of ^{109}Cd from roots to shoots

Four-week-old seedlings were transferred to 55 ml plastic pots (one seedling in each pot) and precultured for 1 wk. Plants in 35 pots were then exposed to 1 μM $CdSO_4$ labelled with 2 kBq ^{109}Cd per pot for 24 h, in the basal nutrient solution. At the end of the ^{109}Cd labelling period, roots were rinsed with the basal nutrient solution for 10 min to remove ^{109}Cd from the root surfaces. Five replicates were harvested. The remaining 30 pots were divided into two groups: one supplied with 1 μM $ZnSO_4$ and the other 10 μM $ZnSO_4$, both in the basal nutrient solution. Five replicates per treatment were harvested 1, 2 and 4 d after the ^{109}Cd labelling period. The radioactivities of ^{109}Cd in roots and shoots were determined.

Effect of abscisic acid treatment on the translocation of ^{109}Cd and ^{65}Zn from roots to shoots

Thirty seedlings were transferred to 55 ml pots (one plant per pot). After preculture for 1 wk, half of the plants were treated with 100 μM abscisic acid (ABA) for 1 d (Salt *et al.*, 1995). ABA was dissolved in methanol, and 1 ml of this solution was added to 54 ml basal nutrient solution. The control treatment received 1 ml methanol only. After 1 d, plant roots were rinsed for 10 min with the basal nutrient solution, and transferred to a nutrient solution labelled with 1 kBq ^{109}Cd and 2 kBq ^{65}Zn per pot. The concentrations of both $CdSO_4$ and $ZnSO_4$ were 1 μM. After 24 h, roots were rinsed with the basal nutrient solution for 10 min, and the nutrient solution was replaced with fresh solution without Zn and Cd. Five replicates per treatment were harvested immediately after radioactive labelling, or 2 and 3 d later. Plant roots and shoots were separated, and the radioactivities of ^{109}Cd and ^{65}Zn were determined.

Statistical analysis

Analysis of variance (ANOVA) was performed on all data sets. Where necessary, data were transformed logarithmically before ANOVA to stabilize the variance. Tukey's HSD test was used to compare treatment means. The relationship between shoot biomass and shoot Cd concentration in the experiments 1 and 2 were fitted with a logistic curve to estimate the Cd concentration that caused 10 and 50% reduction in shoot biomass. The software Genstat® (VSN International, Hemel Hempstead, UK) was used.

The concentrations of free Cd^{2+} and Zn^{2+} in the nutrient or uptake solutions were computed using GEOCHEM-PC (Parker *et al.*, 1995). In the absence of Zn in the uptake solution, the ^{109}Cd uptake kinetics showed a saturable (hyperbolic) component and a nonsaturable linear component. Therefore, the data were fitted to a model that includes both the Michaelis-Menten model and a linear component (Eqn 1), as described by Lasat *et al.* (1996):

$$V = (V_{max} C/(K_m + C)) + \alpha C \quad \text{Eqn 1}$$

(C, concentration of free Cd^{2+}; V_{max} and K_m, Michaelis-Menten parameters; α, slope of the linear component). This approach was used by Lasat *et al.* (1996) and Lombi *et al.* (2001) to estimate V_{max} and K_m of Zn^{2+} or Cd^{2+} uptake in the hyperaccumulator *T. caerulescens*. Curve-fitting was performed with the software SIGMAPLOT (Systat Software, Inc., Point Richmond, CA, USA).

Results

Accumulation of Cd and Zn and tolerance to Cd

The concentrations of free Cd^{2+} and Zn^{2+} in the nutrient solutions in experiments 1 and 2 are shown in Table 1. Precipitation of cadmium phosphate was predicted for the treatments 3–5 in experiment 1, and of zinc phosphate in treatment 5 in experiment 2, even though phosphate concentration in the nutrient solution was lowered to a quarter of that in the Hoagland solution. This explains why the concentrations of free Cd^{2+} and free Zn^{2+} did not increase

Table 1 Concentrations of total and free cadmium (Cd) and zinc (Zn) in the nutrient solutions in experiments 1 and 2

		Cd (μM)		Zn (μM)	
Experiment	Treatment	Total	Free	Total	Free
1	1	0	0.0	5	4.6
	2	5	4.6	5	4.6
	3	15	8.9	5	4.6
	4	50	9.3	5	4.6
	5	100	10.5	5	4.6
2	1	0	0.0	5	4.6
	2	5	4.6	1	0.9
	3	5	4.6	5	4.6
	4	5	4.6	50	45.7
	5	5	4.4	500	195.0

Free metal concentrations were computed using GEOCHEM-PC.

proportionally with the total concentrations in the nutrient solution. The speciation of Fe in the nutrient solution was not affected by Cd or Zn treatments (data not shown).

In experiment 1, shoot growth was more sensitive to Cd exposure than root growth (Fig. 1a); the lowest Cd concentration (5 μM) decreased shoot growth by 45% ($P < 0.05$), whereas 5–50 μM Cd had no significant effect on root biomass. At the highest Cd concentration (100 μM, 10.5 μM free Cd^{2+}), both shoot and root growth were inhibited by 82 and 74%, respectively ($P < 0.01$ compared with the control). Leaves were chlorotic in the treatments with 15 μM or more Cd. In experiment 2 with the presence of 5 μM Cd, increasing Zn concentration from 1 to 5–500 μM increased shoot growth of *A. halleri* significantly ($P < 0.05$), whereas root biomass was increased significantly ($P < 0.05$) by the 50 and 500 μM Zn treatments (Fig. 1b).

In experiment 1, shoot Cd concentration reached > 1000 mg kg^{-1} DW in the 5 μM Cd treatment (Fig. 2a). Shoot Cd concentration showed a pattern of saturation in relation to solution Cd concentration, reaching a maximum of approximately 4000 mg kg^{-1} DW in the 50 μM Cd treatment (Fig. 2a). This pattern is not surprising, considering

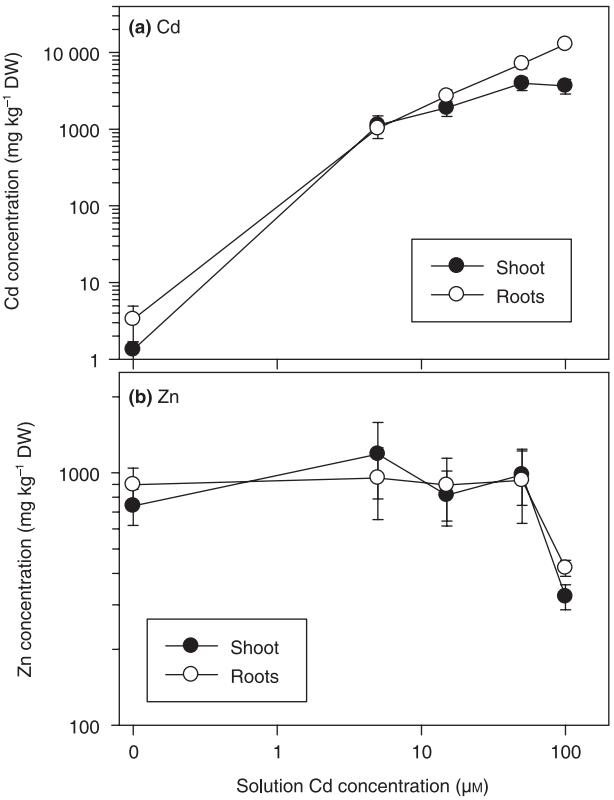

Fig. 2 Effect of solution cadmium (Cd) on the concentrations of Cd (a) and zinc (Zn) (b) in roots and shoots of *Arabidopsis halleri* in experiment 1. Bars represent ± SE ($n = 4$).

that the concentration of free Cd^{2+} increased only slightly in the last three treatments (Table 1). In contrast, root Cd concentration increased with increasing Cd concentration in the solution, reaching 12 800 mg kg^{-1} DW in the 100 μM Cd treatment (Fig. 2a). The concentrations of Zn in roots and shoots were similar. Root Zn concentration was not significantly affected by the Cd treatments, whereas shoot Zn concentration was decreased significantly ($P < 0.05$ based on log-transformed data) only by the highest Cd treatment (Fig. 2b), in which the plants were suffering from severe toxicity of Cd.

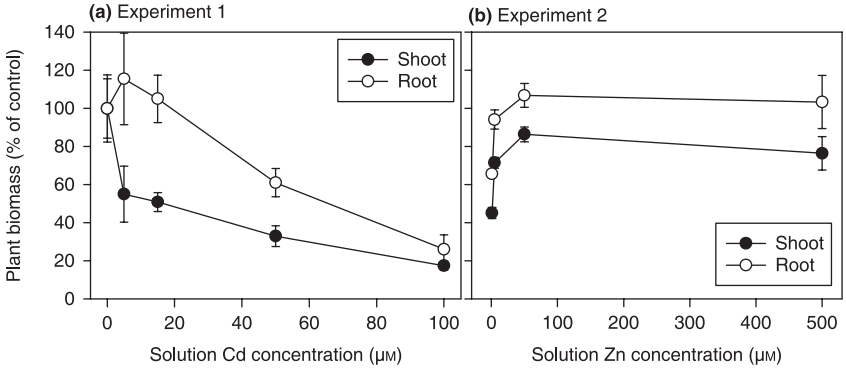

Fig. 1 Effect of solution cadmium (Cd) (a) and zinc (Zn) (b) on shoot and root biomass of *Arabidopsis halleri*. Biomass is expressed as a percentage of the no Cd control. The concentration of Zn was 5 μM in experiment 1 (a), and the concentration of Cd was 5 μM in experiment 2 (b). Bars represent ± SE ($n = 4$).

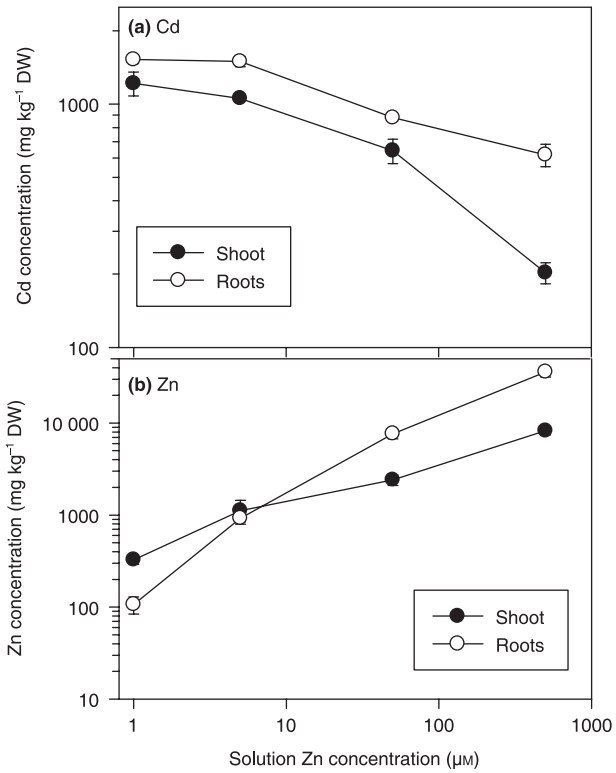

Fig. 3 Effect of solution zinc (Zn) on the concentrations of cadmium (Cd) (a) and Zn (b) in roots and shoots of *Arabidopsis halleri* in experiment 2. Bars represent ± SE ($n = 4$).

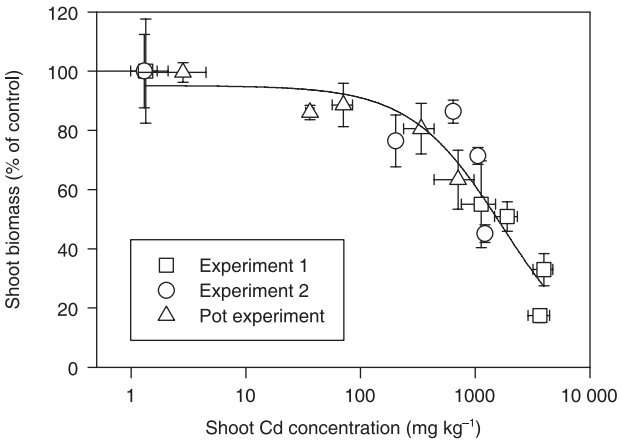

Fig. 4 Relationship between shoot cadmium (Cd) concentration and shoot biomass expressed as a percentage of the no Cd control. Data are from experiments 1 and 2, as well as an additional pot experiment. In the pot experiment, *Arabidopsis halleri* was grown for 6 wk in a general-purpose compost amended with 0, 5, 10, 25 and 50 mg Cd kg^{-1} (as $CdSO_4$), and a constant 250 mg Zn kg^{-1} (as $ZnSO_4$), with four replicates for each treatment. Bars represent ± SE ($n = 4$).

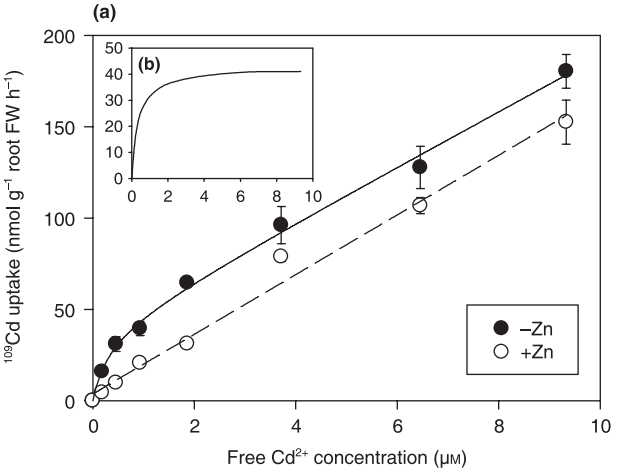

Fig. 5 Concentration-dependent uptake kinetics of ^{109}Cd with or without the presence of 10 μM Zn. The curve in (b) represents the Michaelis-Menten model for the saturable component in the treatment without Zn. Bars represent ± SE ($n = 5$).

In experiment 2, the concentrations of Cd in both shoots and roots were decreased significantly ($P < 0.01$) by increasing Zn in the solution (Fig. 3a), with the effect being greater on shoot Cd than on root Cd concentration. The concentrations of Zn in shoots and roots increased with increasing Zn in the solution (Fig. 3b). The concentrations of Zn in shoots and roots were similar in the low Zn treatments, whereas in the high Zn treatments (50 and 500 μM Zn), root Zn concentration was three- to fourfold higher than shoot Zn concentration.

Figure 4 shows the relationship between shoot growth, expressed as a percentage of the no Cd control, and shoot Cd concentration in both experiments. Figure 4 also includes additional data from a pot experiment (see Fig. 4 legend for more details) to fill the gap of shoot Cd concentration in the range 1–100 mg kg^{-1}. A log-logistic dose–response curve can be fitted to the relationship between shoot Cd concentration and biomass, from which the concentrations of Cd in the shoot that caused either 10 or 50% reduction in shoot growth were estimated to be 228 ± 108 and 1720 ± 295 mg kg^{-1} DW, respectively.

Effect of Zn on ^{109}Cd uptake kinetics

The kinetics of ^{109}Cd uptake showed a curvilinear pattern when Zn was not present in the uptake solution (Fig. 5a). A similar pattern has been observed for ^{65}Zn and ^{109}Cd uptake in *T. caerulescens* (Lasat *et al.*, 1996; Lombi *et al.*, 2001) and wheat (Hart *et al.*, 2002). The uptake data could be resolved into a Michaelis-Menten saturable component and a linear component ($R^2 = 0.997$). From the fitted model, V_{max} and K_m for the saturable component (Fig. 5b) were estimated to be 39.7 ± 7.8 nmol g^{-1} root FW h^{-1} and 0.35 ± 0.19 μM, respectively, and the slope for the linear component was 15.0 ± 1.0 nmol g^{-1} root FW h^{-1}. Addition of 10 μM Zn suppressed ^{109}Cd uptake (Fig. 5a). The saturable component at the low Cd concentration range appeared to be largely abolished in the presence of Zn, and ^{109}Cd uptake was linear

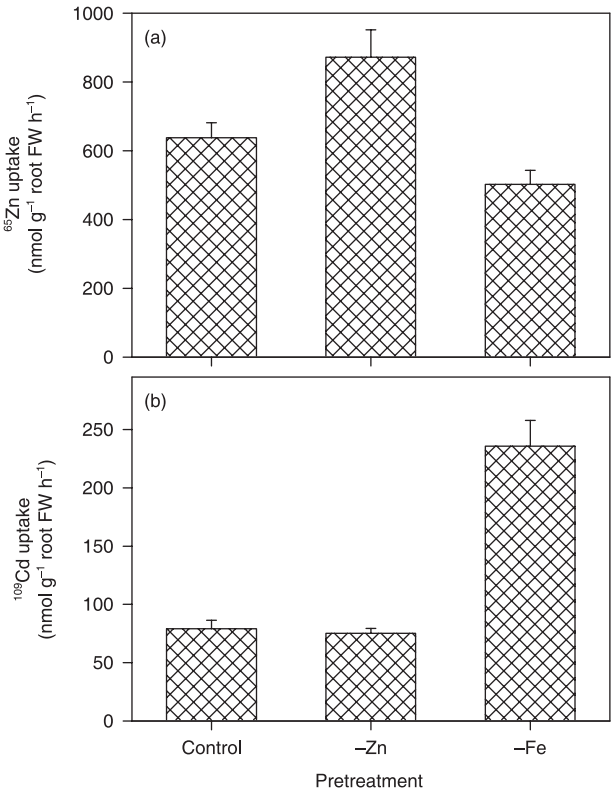

Fig. 6 Effects of –Zn or –Fe pretreatments for 7 d on short-term (20 min) uptake of ^{65}Zn (a) and ^{109}Cd (b). Bars represent ± SE (n = 6).

with respect to the free Cd^{2+} concentration in the solution. A simple linear equation described the ^{109}Cd uptake data well (R^2 = 0.988), yielding a slope of 16.3 ± 0.7, which was similar to that of the linear component of ^{109}Cd uptake in the absence of Zn.

Effects of plant Zn and Fe status on ^{65}Zn and ^{65}Cd uptake

One week after Fe was withheld from the nutrient solution, *A. halleri* plants showed typical symptoms of Fe deficiency (chlorosis of young leaves). In contrast, no symptoms were visible in the plants subject to the –Zn pretreatment. Uptake of ^{65}Zn was significantly (P < 0.05) increased by the –Zn pretreatment, by 37% compared with the control, but was not significantly affected by the –Fe pretreatment (Fig. 6a). In contrast, uptake of ^{109}Cd was not significantly affected by the –Zn pretreatment, but was increased threefold (P < 0.01) by the –Fe pretreatment (Fig. 6b).

Effect of Zn on the distribution of ^{109}Cd between roots and shoots

At the end of the 24 h labelling period, 56% of the ^{109}Cd taken up by *A. halleri* was distributed to the shoots (Fig. 7).

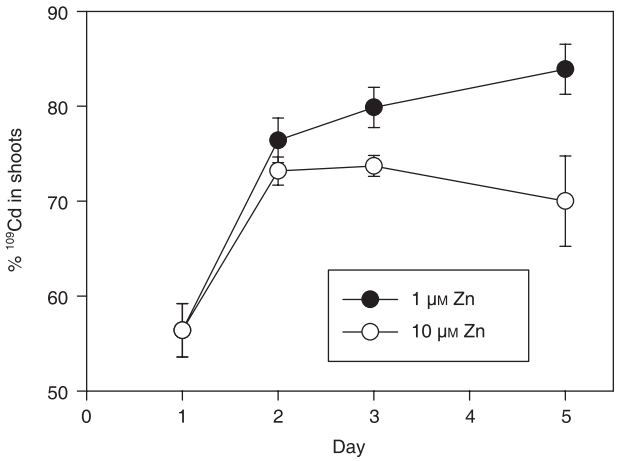

Fig. 7 Effect of solution zinc (Zn) on the percentage distribution of ^{109}Cd to shoots of *Arabidopsis halleri*. Plants were exposed to 1 µM $CdSO_4$ labelled with ^{109}Cd for 1 d, followed by two different Zn concentrations (1 and 10 µM $ZnSO_4$). Bars represent ± SE (n = 5).

In the following 4 d without further supply of either radioactive or stable Cd isotopes, the percentage of ^{109}Cd in the shoots increased gradually to 84% in the 1 µM Zn treatment. In contrast, with 10 µM Zn in the solution, the percentage of ^{109}Cd in the shoots increased only in day 2 and remained at around 70% thereafter (Fig. 7). The difference between the two Zn treatments was significant (P < 0.01) on days 3 and 5. The results suggest that Zn inhibited Cd translocation from roots to shoots.

Effect of ABA treatment on the translocation of ^{109}Cd and ^{65}Zn from roots to shoots

The distribution patterns between roots and shoots were similar for ^{109}Cd and ^{65}Zn (Fig. 8). Pre-treatment with ABA significantly (P < 0.01) decreased the proportion of ^{109}Cd and ^{65}Zn distributed to the shoots. For example, at the end of the radioisotope labelling (day 1), < 5% of the ^{109}Cd and ^{65}Zn taken up was distributed to the shoots in the plants with ABA pretreatment, compared with 18% in those not treated with ABA. The percentage of ^{109}Cd or ^{65}Zn distributed to the shoots increased with time over the following 3 d with or without ABA pretreatment, but the difference between the treatments was maintained. The effect of the ABA pretreatment was not caused by an effect on uptake, as total uptake of ^{109}Cd and ^{65}Zn was not significantly influenced by the ABA pretreatment (data not shown). In the absence of ABA pretreatment, ^{65}Zn concentrations in the shoots were 2.3- to 3.8-fold higher than those of ^{109}Cd (data not shown).

Discussion

Arabidopsis halleri accumulated large concentrations of Cd in the shoots (> 1000 mg kg^{-1} DW) when grown hydroponically

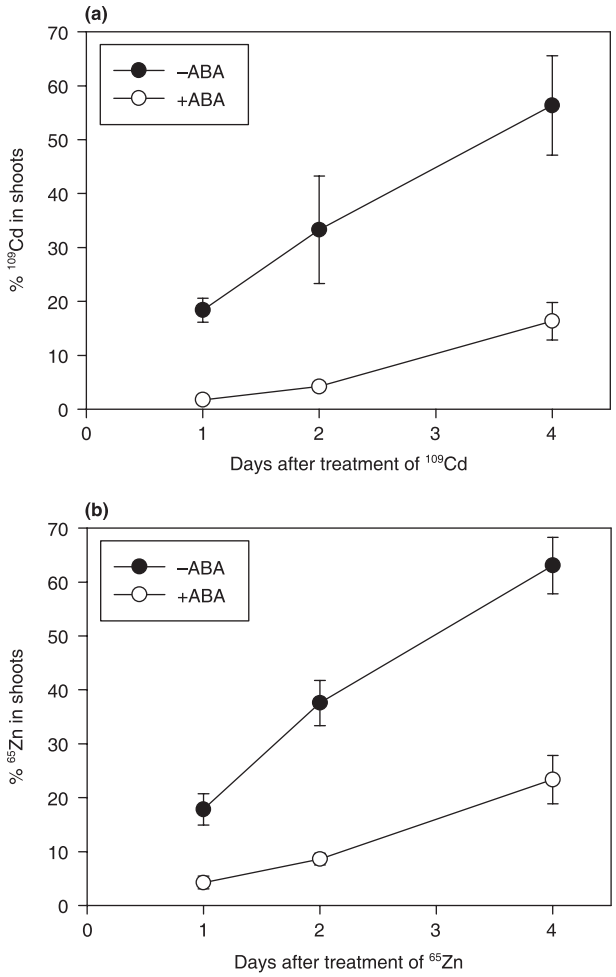

Fig. 8 Effect of abscisic acid (ABA) pretreatment on the percentage distribution of ^{109}Cd (a) and ^{65}Zn (b) to shoots of *Arabidopsis halleri*. Bars represent ± SE ($n = 5$).

for 3 wk with a solution Cd concentration of 5 μM or above (Fig. 2). Both root and shoot Cd : Zn molar ratios were close to the initial ratios of free ionic Cd^{2+} and Zn^{2+} in the nutrient solutions in experiments 1 and 2. However, the ratio of free ionic Cd^{2+} and Zn^{2+} in the nutrient solutions would not remain constant if Cd and Zn were taken up at different rates. In the short-term (20 min) uptake experiment with an equal concentration of Zn and Cd (4 μM), uptake of ^{65}Zn by nutrient-replete plants was eightfold larger than that of ^{109}Cd, although the difference narrowed to approximately twofold in the Fe-deficient plants (Fig. 6). The results indicate that, although *A. halleri* can hyperaccumulate Cd, the rate of Cd uptake is considerably slower than that of Zn uptake.

Evidence from the present study also suggests that Cd and Zn partially share the same transport pathway in *A. halleri*. The presence of Zn inhibited short-term ^{109}Cd uptake, abolishing the saturable component of ^{109}Cd uptake in the low concentration range (Fig. 5). Michaelis-Menten kinetic parameters (V_{max} and K_m) for ^{109}Cd uptake could be obtained in the absence of Zn, but not in the presence of Zn because of the dominance of the linear component. Thus, it is difficult to ascertain the nature of the competition of Zn on Cd uptake, although a competitive inhibition is plausible and has been shown in wheat (Hart *et al.*, 2002). With or without Zn, ^{109}Cd uptake showed a similar linear component. This has been interpreted as the cell-wall-bound ^{109}Cd that was not completely removed by desorption (Lasat *et al.*, 1996; Hart *et al.*, 2002). In the long-term (3 wk) hydroponic experiments, increasing external Zn concentration decreased accumulation of Cd in roots and shoots (Fig. 3), and alleviated Cd toxicity (Fig. 1b). Furthermore, increasing external Zn decreased the translocation of ^{109}Cd from roots to shoots (Fig. 7). Consistent with the physiological data presented here, Bert *et al.* (2003) showed evidence of a genetic correlation between Zn and Cd accumulation in *A. halleri* shoots. They found a significant positive correlation ($r = 0.50$, $n = 29$, $P < 0.01$) between the concentrations of Zn and Cd in the shoots in the backcross progeny derived from a cross between *A. halleri* and a nonaccumulating species *A. lyrata* ssp. *petraea*. They suggested that Cd and Zn accumulation in shoots are genetically correlated, implying that the two metals may be transported, at least partly, by the same transporter(s). Competition between Zn and Cd during root uptake have been reported in nonhyperaccumulators such as wheat (Hart *et al.*, 2002) and soybean (Cataldo *et al.*, 1983), and in a low Cd-accumulating ecotype (Prayon) of *T. caerulescens* (Lombi *et al.*, 2001; Zhao *et al.*, 2002). In *T. caerulescens* (Prayon), it has been shown that ZNT1, a metal transporter in the ZIP family, can mediate high-affinity uptake of Zn as well as low-affinity uptake of Cd, when the gene was expressed in yeast (Pence *et al.*, 2000). Functional characterization of different ZIP genes in *A. halleri* has yet to be performed.

There were clear differences in the regulation of Cd and Zn uptake by plant Zn and Fe status. Uptake of ^{65}Zn was up-regulated by a low Zn status, whereas ^{109}Cd uptake only responded to Fe deficiency (Fig. 6). The results are consistent with an up-regulation of the expression of Zn transporter genes by a low Zn status (Pence *et al.*, 2000), which would lead to an enhanced ^{65}Zn uptake. The fact that ^{109}Cd uptake was not enhanced by a low Zn status was probably because of the presence of equal molar concentration of Zn in the solution, which would out-compete Cd for the transporters that were up-regulated. In contrast, Fe deficiency leads to over-expression of Fe transporter genes (such as *IRT1*), which can also mediate high-affinity uptake of Cd (Eide *et al.*, 1996; Korshunova *et al.*, 1999; Connolly *et al.*, 2002; Lombi *et al.*, 2002; Vert *et al.*, 2002).

A key trait of metal hyperaccumulators is the efficient metal transport from roots to shoots, characterized by shoot-to-root concentration ratios of metals being greater than one. In experiments 1 and 2, the shoot-to-root ratio of Zn and Cd was around or greater than one only in the low metal treatments (5 μM) and well below one in other treatments.

Similarly, Bert *et al.* (2003) obtained a shoot-to-root concentration ratio of Cd of only 0.23 in a hydroponic experiment with 10 μM Cd, whilst Küpper *et al.* (2000) reported a ratio of < 0.2 for both Cd and Zn in treatments with 100 μM Cd and 500 μM Zn. High concentrations of Zn and Cd in roots, and consequently small shoot-to-root ratios, in the high metal treatments were probably a result of a precipitation of zinc or cadmium phosphate (Table 1), which was difficult to avoid in hydroponic culture with the need to supply a relatively high phosphate concentration to compensate for the lack of buffering capacity. Küpper *et al.* (2000) and Sarret *et al.* (2002) showed clear evidence of zinc phosphate precipitation on the root surface of *A. halleri* grown in hydroponic solutions. In contrast, Zn was predominantly coordinated with malate in *A. halleri* roots growing on a Zn-contaminated soil, with zinc phosphate representing only a minor proportion of the total Zn in roots (Sarret *et al.*, 2002). In the short-term experiments with ^{109}Cd and ^{65}Zn labelling, where precipitation with phosphate was not predicted, both metals were transported to shoots efficiently and in a similar fashion (Figs 7, 8). Pretreatment with ABA was found to dramatically decrease the translocation of ^{109}Cd and ^{65}Zn to shoots (Fig. 8). Salt *et al.* (1995) reported a similar effect of ABA on Cd distribution in the nonhyperaccumulator *Brassica juncea*. They suggested that the root-to-shoot transport of Cd was driven mainly by transpiration, because an ABA pretreatment would cause stomatal closure. Alternatively, ABA has been shown to inhibit ion channel activities in the stele of maize (*Zea mays*) roots (Roberts, 1998; Gilliham & Tester, 2005), and the expression of a gene encoding a stelar K^+ outward rectifying channel in *A. thaliana* roots that is probably involved in the xylem loading of K^+ (Gaymard *et al.*, 1998).

Although *A. halleri* is able to accumulate large concentrations of Cd in shoots, its tolerance to Cd is much lower than that to Zn. Accumulation of Zn in shoots to 32 000 mg kg^{-1} DW (= 490 mmol kg^{-1}) did not cause any phytotoxicity (Zhao *et al.*, 2000). In contrast, shoot Cd concentrations of 228 mg kg^{-1} DW (= 2 mmol kg^{-1}) and 1720 mg kg^{-1} DW (= 15 mmol kg^{-1}), respectively, were associated with 10 and 50% reductions in the shoot biomass in the present study (Fig. 4). These results indicate that Cd is much more toxic to *A. halleri* than Zn, and/or that *A. halleri* is less able to detoxify Cd than Zn. For comparison, the Ganges ecotype of *T. caerulescens* from southern France is able to tolerate more than 5000 mg Cd kg^{-1} DW in shoots without growth reduction (Lombi *et al.*, 2001; Assunção *et al.*, 2003a; Roosens *et al.*, 2003). There is strong evidence that vacuolar sequestration via the tonoplast transporter MTP1 (Dräger *et al.*, 2004) and a constitutively high expression of nicotianamine synthase genes (Becher *et al.*, 2004; Weber *et al.*, 2004) play a key role in the detoxification of Zn in *A. halleri*. However, there is so far little evidence that implies a role of either the MTP1 transporter or nicotianamine synthesis in Cd detoxification. Bert *et al.* (2003) showed a significant correlation ($r = 0.55$, $n = 66$, $P < 0.001$) between Zn and Cd tolerance in the backcross progeny from the cross between *A. halleri* and *A. lyrata*, suggesting a pleiotropic genetic control over the two characters. However, in their study, tolerance was assessed according to the resistance to external Zn and Cd, which is likely to be different from the tolerance to internal metal concentrations. Resistance to metals in the external medium may be achieved by decreased uptake or root-to-shoot translocation, whereas tolerance to internal metal concentrations has to be realized through cellular detoxification. Bert *et al.* (2003) also suggested that Cd tolerance in *A. halleri* is a more complex character than Zn and might be governed by more than one single major gene.

In conclusion, results from the present study indicate that *A. halleri* can hyperaccumulate Cd, although short-term uptake of Cd was at a lower rate than that of Zn. Both uptake and root-to-shoot translocation of Cd were inhibited by Zn, suggesting that Cd enters *A. halleri* cells partly through the Zn transport pathway. However, there were also significant differences between the two metals in their response to the status of Zn or Fe in plants, suggesting differences in regulation and/or that multiple transporters differing in the affinities for Zn and Cd ions are involved. Tolerance of the Cd accumulated in shoots was much lower than that for Zn, suggesting that the mechanisms responsible for the high degree of detoxification of Zn in *A. halleri* could not detoxify Cd effectively. Detoxification of Cd may involve mechanisms distinct from those responsible for Zn detoxification. The population of *A. halleri* studied in the present work was from a metalliferous site. Variation in Cd accumulation and tolerance among different populations may be expected (Bert *et al.*, 2002), although comparisons under identical conditions have yet to been performed.

Acknowledgements

RFJ was funded by a Rothamsted International Fellowship. We thank Jianping Xing for assistance with growing plants, and Adrian Crosland for elemental analysis. Rothamsted Research receives grant-aided support from the Biotechnology and Biological Sciences Research Council of the UK.

References

Assunção AGL, Bookum WM, Nelissen HJM, Vooijs R, Schat H, Ernst WHO. **2003a.** Differential metal-specific tolerance and accumulation patterns among *Thlaspi caerulescens* populations originating from different soil types. *New Phytologist* **159**: 411–419.

Assunção AGL, Schat H, Aarts MGM. 2003b. *Thlaspi caerulescens,* an attractive model species to study heavy metal hyperaccumulation in plants. *New Phytologist* **159**: 351–360.

Baker AJM, McGrath SP, Reeves RD, Smith JAC. 2000. Metal hyperaccumulator plants: A review of the ecology and physiology of a biochemical resource for phytoremediation of metal-polluted soils. In: Terry N, Bañuelos G, eds. *Phytoremediation of contaminated soil and water.* Boca Raton, FL, USA: Lewis Publishers, 85–107.

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.

Becher M, Talke IN, Krall L, Kramer U. 2004. Cross-species microarray transcript profiling reveals high constitutive expression of metal homeostasis genes in shoots of the zinc hyperaccumulator *Arabidopsis halleri*. *Plant Journal* **37**: 251–268.

Bert V, Bonnin I, Saumitou-Laprade P, de Laguerie P, Petit D. 2002. Do *Arabidopsis halleri* from nonmetallicolous populations accumulate zinc and cadmium more effectively than those from metallicolous populations? *New Phytologist* **155**: 47–57.

Bert V, Macnair MR, DeLaguerie P, Saumitou-Laprade P, Petit D. 2000. Zinc tolerance and accumulation in metallicolous and nonmetallicolous populations of *Arabidopsis halleri* (Brassicaceae). *New Phytologist* **146**: 225–233.

Bert V, Meerts P, Saumitou-Laprade P, Salis P, Gruber W, Verbruggen N. 2003. Genetic basis of Cd tolerance and hyperaccumulation in *Arabidopsis halleri*. *Plant and Soil* **249**: 9–18.

Cataldo DA, Garland TR, Wildung RE. 1983. Cadmium uptake kinetics in intact soybean plants. *Plant Physiology* **73**: 844–848.

Clemens S, Palmgren MG, Krämer U. 2002. A long way ahead: understanding and engineering plant metal accumulation. *Trends in Plant Science* **7**: 309–315.

Connolly EL, Fett JP, Guerinot ML. 2002. Expression of the IRT1 metal transporter is controlled by metals at the levels of transcript and protein accumulation. *Plant Cell* **14**: 1347–1357.

Dahmani-Muller H, van Oort F, Gelie B, Balabane M. 2000. Strategies of heavy metal uptake by three plant species growing near a metal smelter. *Environmental Pollution* **109**: 231–238.

Dräger DB, Desbrosses-Fonrouge AG, Krach C, Chardonnens AN, Meyer RC, Saumitou-Laprade P, Krämer U. 2004. Two genes encoding *Arabidopsis halleri* MTP1 metal transport proteins co-segregate with zinc tolerance and account for high *MTP1* transcript levels. *Plant Journal* **39**: 425–439.

Eide D, Broderius M, Fett J, Guerinot ML. 1996. A novel iron-regulated metal transporter from plants identified by functional expression in yeast. *Proceedings of the National Academy of Sciences, USA* **93**: 5624–5628.

Ernst WHO. 1974. *Schwermetallvegetation der Erde*. Stuttgart, Germany: G. Fischer Verlag.

Gaymard F, Pilot G, Lacombe B, Bouchez D, Bruneau D, Boucherez J, Michaux-Ferriere N, Thibaud JB, Sentenac H. 1998. Identification and disruption of a plant shaker-like outward channel involved in K^+ release into the xylem sap. *Cell* **94**: 647–655.

Gilliham M, Tester M. 2005. The regulation of anion loading to the maize root xylem. *Plant Physiology* **137**: 819–828.

Hart JJ, Welch RM, Norvell WA, Kochian LV. 2002. Transport interactions between cadmium and zinc in roots of bread and durum wheat seedlings. *Physiologia Plantarum* **116**: 73–78.

Korshunova YO, Eide D, Clark WG, Guerinot ML, Pakrasi HB. 1999. The IRT1 protein from *Arabidopsis thaliana* is a metal transporter with a broad substrate range. *Plant Molecular Biology* **40**: 37–44.

Küpper H, Lombi E, Zhao FJ, McGrath SP. 2000. Cellular compartmentation of cadmium and zinc in relation to other elements in the hyperaccumulator *Arabidopsis halleri*. *Planta* **212**: 75–84.

Lasat MM, Baker AJM, Kochian LV. 1996. Physiological characterization of root Zn^{2+} absorption and translocation to shoots in Zn hyperaccumulator and nonaccumulator species of *Thlaspi*. *Plant Physiology* **112**: 1715–1722.

Lombi E, Tearall KL, Howarth JR, Zhao FJ, Hawkesford MJ, McGrath SP. 2002. Influence of iron status on cadmium and zinc uptake by different ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *Plant Physiology* **128**: 1359–1367.

Lombi E, Zhao FJ, Dunham SJ, McGrath SP. 2000. Cadmium accumulation in populations of *Thlaspi caerulescens* and *Thlaspi goesingense*. *New Phytologist* **145**: 11–20.

Lombi E, Zhao FJ, McGrath SP, Young SD, Sacchi GA. 2001. Physiological evidence for a high-affinity cadmium transporter highly expressed in a *Thlaspi caerulescens* ecotype. *New Phytologist* **149**: 53–60.

Macnair MR. 2002. Within and between population genetic variation for zinc accumulation in *Arabidopsis halleri*. *New Phytologist* **155**: 59–66.

Macnair MR, Bert V, Huitson SB, Saumitou-Laprade P, Petit D. 1999. Zinc tolerance and hyperaccumulation are genetically independent characters. *Proceedings of the Royal Society of London Series B – Biology Sciences* **266**: 2175–2179.

McGrath SP, Zhao FJ. 2003. Phytoextraction of metals and metalloids from contaminated soils. *Current Opinion in Biotechnology* **14**: 277–282.

Parker DR, Norvell WA, Chaney RL. 1995. GEOCHEM-PC – A chemical speciation program for IBM and compatible personal computers. In: Loeppert RH, ed. *Chemical equilibrium and reaction models*. Madison, WI, USA: Soil Science Society of America, American Society of Agronomy. 253–269.

Pence NS, Larsen PB, Ebbs SD, Letham DLD, Lasat MM, Garvin DF, Eide D, Kochian LV. 2000. The molecular physiology of heavy metal transport in the Zn/Cd hyperaccumulator *Thlaspi caerulescens*. *Proceedings of the National Academy of Sciences, USA* **97**: 4956–4960.

Roberts SK. 1998. Regulation of K^+ channels in maize roots by water stress and abscisic acid. *Plant Physiology* **116**: 145–153.

Roosens N, Verbruggen N, Meerts P, Ximenez-Embun P, Smith JAC. 2003. Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. *Plant, Cell & Environment* **26**: 1657–1672.

Salt DE, Prince RC, Pickering IJ, Raskin I. 1995. Mechanisms of cadmium mobility and accumulation in Indian mustard. *Plant Physiology* **109**: 1427–1433.

Salt DE, Smith RD, Raskin I. 1998. Phytoremediation. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**: 643–668.

Sarret G, Saumitou-Laprade P, Bert V, Proux O, Hazemann JL, Traverse AS, Marcus MA, Manceau A. 2002. Forms of zinc accumulated in the hyperaccumulator *Arabidopsis halleri*. *Plant Physiology* **130**: 1815–1826.

Vert G, Grotz N, Dedaldechamp F, Gaymard F, Guerinot ML, Briata JF, Curie C. 2002. IRT1, an Arabidopsis transporter essential for iron uptake from the soil and for plant growth. *Plant Cell* **14**: 1223–1233.

Weber M, Harada E, Vess C, von Roepenack-Lahaye E, Clemens S. 2004. Comparative microarray analysis of *Arabidopsis thaliana* and *Arabidopsis halleri* roots identifies nicotianamine synthase, a ZIP transporter and other genes as potential metal hyperaccumulation factors. *Plant Journal* **37**: 269–281.

Wenzel WW, Jockwer F. 1999. Accumulation of heavy metals in plants grown on mineralised soils of the Austrian Alps. *Environmental Pollution* **104**: 145–155.

Yang XE, Long XX, Ye HB, He ZL, Calvert DV, Stoffella PJ. 2004. Cadmium tolerance and hyperaccumulation in a new Zn- hyperaccumulating plant species (*Sedum alfredii* Hance). *Plant and Soil* **259**: 181–189.

Zhao FJ, Hamon RE, Lombi E, McLaughlin MJ, McGrath SP. 2002. Characteristics of cadmium uptake in two contrasting ecotypes of the hyperaccumulator *Thlaspi caerulescens*. *Journal of Experimental Botany* **53**: 535–543.

Zhao FJ, Lombi E, Breedon T, McGrath SP. 2000. Zinc hyperaccumulation and cellular distribution in *Arabidopsis halleri*. *Plant, Cell & Environment* **23**: 507–514.

