

# Root exudates of the hyperaccumulator *Thlaspi caerulescens* do not enhance metal mobilization

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## Summary

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- To examine whether root exudates of the Zn/Cd hyperaccumulator *Thlaspi caerulescens* play a role in metal hyperaccumulation, we compared the metal mobilization capacity of root exudates collected from two ecotypes of *T. caerulescens*, and from the nonaccumulators wheat (*Triticum aestivum*) and canola (*Brassica napus*).
- Plants were grown hydroponically and three treatments (control, -Fe and -Zn) were later imposed for 2 wk before collection of root exudates.
- On a basis of root d. wt, the total soluble organic C in the root exudates of *T. caerulescens* was similar to that of wheat, and significantly higher than that of canola. In all treatment, the root exudates of *T. caerulescens* and canola mobilized little Cu and Zn from Cu- or Zn-loaded resins, and little Zn, Cd, Cu or Fe from a contaminated calcareous soil. By contrast, the root exudates of wheat generally mobilized more metals from both resin and soil. In particular, the -Fe treatment, and to a lesser extent the -Zn treatment, elicited large increases in the metal mobilization capacity of the root exudates from wheat.
- We conclude that root exudates from *T. caerulescens* do not significantly enhance mobilization of Zn and Cd, and therefore are not involved in Zn and Cd hyperaccumulation.

**Key words:** *Thlaspi caerulescens*, cadmium, zinc, root exudates, hyperaccumulation, canola, wheat, phytoremediation.

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## Introduction

Plants that can hyperaccumulate heavy metals in the shoots have received increased attention in recent years, due to the potential of using these plants for phytoremediation or phytomining (Brooks, 1998). Metal hyperaccumulator plants also provide important germplasm resources for comparative studies on the mechanisms of uptake, accumulation, and tolerance of trace elements.

So far, 11 species have been reported as Zn hyperaccumulators, which are defined as being able to accumulate > 10 000 mg kg<sup>-1</sup> Zn in the shoot dry matter (Baker *et al.*, 2000). *Thlaspi caerulescens* is the best known example of a Zn hyperaccumulator. This plant can tolerate up to 30 000 mg kg<sup>-1</sup> Zn in the shoot dry matter without suffering from Zn toxicity (Baker *et al.*, 1994; Brown *et al.*, 1995; Shen

*et al.*, 1997). The extraordinary internal tolerance to Zn is achieved through cellular compartmentation and vacuolar sequestration (Vázquez *et al.*, 1994; Küpper *et al.*, 1999). Organic acids, particularly citric acid, may play a role in Zn sequestration in vacuoles (Salt *et al.*, 1999). Tolerance to Zn and Zn hyperaccumulation are, however, independent genetic traits (Macnair *et al.*, 1999). Tolerance alone is not enough to explain Zn hyperaccumulation in *T. caerulescens*. Lasat *et al.* (1996) showed that Zn influx into the root cells of *T. caerulescens* was at a much higher rate than that of the non-accumulator *Thlaspi arvense*. Furthermore, the gene encoding a Zn transporter, *ZNT1*, was highly expressed in the roots of *T. caerulescens* even when the plants had been supplied with relatively high concentrations of Zn, whereas *ZNT1* was expressed in *T. arvense* roots only when Zn was deficient (Pence *et al.*, 2000).

*T. caerulescens* is also a Cd hyperaccumulator. However, the ability to accumulate Cd was found to vary greatly among different ecotypes (Lombi *et al.*, 2000). In a recent study, Lombi *et al.* (2001) showed that the  $V_{\max}$  for Cd influx was fivefold higher in the high Cd ecotype from Ganges in southern France than in the low Cd ecotype from Prayon in Belgium. In addition, the concentration of Cd in the xylem sap was at least fivefold higher in the Ganges than in the Prayon ecotypes. These differences in the Cd uptake were not related to the characteristics of Zn uptake, because both ecotypes exhibited similar Zn influx kinetics and hyperaccumulation ability.

Plants can modify the rhizosphere to enhance acquisition of nutrients, particularly ions for which diffusion is important for transport to the root surface (e.g. Fe, Zn and P) and under nutrient limiting conditions (Marschner, 1995). Rhizosphere acidification and release of root exudates are two common mechanisms employed. However, it is not clear whether metal hyperaccumulators employ rhizosphere related processes to enhance metal accumulation. If so, then understanding the traits involved could lead to development of strategies to enhance both phytoremediation and Zn uptake efficiency by crops growing in Zn limiting soils. Previous studies using *T. caerulescens* have ruled out the role of rhizosphere acidification in metal accumulation (Knight *et al.*, 1997; McGrath *et al.*, 1997). One possibility is that hyperaccumulators such as *T. caerulescens* may release chelating compounds to the rhizosphere to mobilize heavy metals. In the nonhyperaccumulators *Nicotiana tabacum*, *Nicotiana rustica* and *Zea mays*, Mench & Martin (1991) observed that uptake of Cd from soils by these species followed the same order as the extent of Cd extraction by their root exudates. These authors suggest that root exudates of the *Nicotiana* spp. may play an important role in Cd accumulation. In wheat, genotypic variation in Zn efficiency may also be related to the release of phytosiderophores (Cakmak *et al.*, 1996b; Rengel *et al.*, 1998). However, the role of root exudates in metal hyperaccumulation has been little researched. The objective of this study was to investigate if root exudates play a role in Zn/Cd hyperaccumulation by *T. caerulescens*. To answer this question, we compared metal mobilization capacity of root exudates collected from two contrasting ecotypes (Ganges and Prayon) of *T. caerulescens*, and from the nonaccumulating crop species wheat (*Triticum aestivum*) and canola (*Brassica napus*).

## Materials and Methods

### Plant culture

Seeds of the Prayon (Belgium) and Ganges (southern France) ecotypes of *T. caerulescens* J. & C. Presl (Brassicaceae) were sown in trays containing a mixture of vermiculite and perlite, which was moistened with deionized water. After germination

in the dark (*c.* 1 wk), seedlings were given full nutrient solution with the following composition: 3.55 mM  $\text{Ca}(\text{NO}_3)_2$ , 1.2 mM  $\text{KNO}_3$ , 0.075 mM  $\text{K}_2\text{HPO}_4$ , 1.45 mM  $\text{MgSO}_4$ , 75  $\mu\text{M}$  Fe-HBED (di-(hydroxybenzoyl)-ethylenediamine-diacetic acid), 5  $\mu\text{M}$   $\text{ZnSO}_4$ , 10  $\mu\text{M}$   $\text{MnCl}_2$ , 0.2  $\mu\text{M}$   $\text{CuSO}_4$ , 10  $\mu\text{M}$   $\text{HBO}_3$ , 0.2  $\mu\text{M}$   $\text{Na}_2\text{MoO}_4$ , 0.5  $\mu\text{M}$   $\text{NiCl}_2$  and 10  $\mu\text{M}$  NaCl. Solution pH was buffered at  $6.0 \pm 0.2$  with 2 mM MES (2-morpholinoethanesulphonic acid). After germination (3 wk), vermiculite and perlite were washed from the roots, and three seedlings were transferred to a 250-ml pot wrapped with aluminium foil. Twelve pots were prepared for each ecotype. The nutrient solution was topped up every day, completely renewed every week, and aerated continuously.

Wheat (*Triticum aestivum*, cv Frame) and canola (*Brassica napus*, cv Karoo) were germinated in the dark on filter paper moistened with saturated  $\text{CaSO}_4$ . After germination (5 d), three seedlings were transferred to each 250-ml pot filled with nutrient solutions. The composition of nutrient solutions was the same as above, except that  $\text{K}_2\text{HPO}_4$  was 0.15 mM and  $\text{ZnSO}_4$  1  $\mu\text{M}$  for both plant species. Iron was supplied as Fe-EDTA at 10  $\mu\text{M}$  for wheat and as Fe-HBED at 25  $\mu\text{M}$  for canola. These differences were introduced because *T. caerulescens* has a higher requirement for Zn (Shen *et al.*, 1997) and Fe (McLaughlin & Henderson, 1999) than normal crop species, whereas P was given to wheat and canola at a higher concentration to compensate for their higher growth rates. Fe(III)-HBED was prepared as described by Chaney (1988), such that all HBED is saturated with Fe. Fe(III)-EDTA was prepared from an analytical reagent (BDH, Poole, England). All four plant species/ecotypes showed normal growth without any signs of nutrient deficiency or toxicity.

The plants were grown in a controlled environment growth cabinet (day/night period 14/10 h, day/night temperatures  $22^\circ\text{C}/16^\circ\text{C}$ , and a light intensity of  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ ).

### Collection of root exudates

Three treatments were imposed 40 d after seedlings were transferred to nutrient solution for *T. caerulescens*, and 16 d for wheat and canola, respectively. The treatments were control (full nutrient composition as above), -Fe or -Zn. In the -Fe and -Zn treatments, Fe (and the associated chelates) or Zn was left out of the nutrient solution, respectively. Each treatment was replicated four times. Before the treatments were imposed, roots were rinsed thoroughly with deionized water. The treatments were imposed for 2 wk, during which period nutrient solution was renewed twice weekly.

The procedure for the collection and preparation of root exudates was modified from that described by Cakmak *et al.* (1996b). Roots were washed thoroughly with deionized water, and placed in 220 ml autoclaved 0.1 mM  $\text{CaCl}_2$  solution 3 h after the onset of the light period. The solution was aerated continuously during the 3-h period of exudate

collection. To remove microorganisms, the exudate solutions were filtered immediately after collection through a sterile 0.2 µm filter into an autoclaved glass bottle. The filtration was carried out inside a laminar flow cabinet using standard axenic techniques. The filtered solutions were later plated onto nutrient agar plates and incubated at 25°C for 3 d. No microorganisms were observed on any plates. The exudate solutions were concentrated at 50°C, under vacuum, to 10 ml using a rotary evaporator. Further plating onto nutrient agar plates confirmed that sterility was maintained in the concentrated exudate solutions. The exudate solutions were stored at -19°C until use.

After collection of exudates, plants were washed thoroughly with deionized water, separated into roots and shoots, and dried at 60°C for 48 h before the dry weights (d. wt) were determined.

### Metal mobilization tests

Mobilization of Cu and Zn from Cu- and Zn-loaded resins by root exudates was quantified according to Cakmak *et al.* (1996b). Five g of the Chelex 100 resin (100–200 mesh, Na form, Bio-Rad Laboratories, Richmond, CA, USA) were equilibrated with 500 ml of either 50 mM CuSO<sub>4</sub> or 50 mM ZnSO<sub>4</sub> for 30 min. Excess Cu and Zn were leached with deionized water until Cu or Zn in the leachates was below detection limits. The Cu- and Zn-loaded resins were suspended in 500 ml 10 mM MES (pH 5.0). To determine Cu or Zn mobilization, 2 ml resin suspension, 2 ml concentrated root exudates and 3 ml deionized water were shaken in an end-over-end shaker for 45 mins, and then filtered through a 0.45-µm filter. The concentrations of Cu, Zn and Ca were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES; Spectro Instruments). Because the concentrated root exudates contained c. 1–2 mM Ca and this could also exchange Cu or Zn from the resin, a series of CaCl<sub>2</sub> solutions with increasing concentrations were prepared, and their capacities to mobilize Cu and Zn from the resins were determined as above. Within the concentration range of Ca found in the concentrated root exudate samples, increasing Ca did not increase mobilization of Cu from the Cu-loaded resin. By contrast, because the selectivity of Chelex 100 for Zn over Ca is lower than that for Cu over Ca, increasing Ca concentration resulted in increased Zn mobilization from the resin. The effect of Ca on Zn mobilization followed a well-defined pattern (slightly curvilinear), and this was corrected for in the mobilization results presented.

Mobilization of metals was also determined using a calcareous soil contaminated with Cd and Zn. The soil contained 6.3% (w/w) CaCO<sub>3</sub>, 8% (w/w) clay, 1.6% (w/w) organic C, 5.5 mg kg<sup>-1</sup> total Cd, 1228 mg kg<sup>-1</sup> total Zn, 49 mg kg<sup>-1</sup> total Cu, 10 684 mg kg<sup>-1</sup> total Fe, and had a pH of 8.2. The soil was finely ground and shaken (1 g) with 2 ml concentrated root

exudates and 3 ml deionized water for 1 h. The suspension was then centrifuged at 1820 g for 10 min and filtered through a 0.45-µm filter. The concentrations of metals mobilized were determined by ICP-AES. The soil was also extracted with 1 mM CaCl<sub>2</sub> in the same way, and this extraction served as the blank that was subtracted from the mobilization results of the root exudates.

### Plant and exudate analyses

Plant samples were ground to < 0.5 mm, and digested with hot concentrated HNO<sub>3</sub>. Elemental concentrations in the digests were determined by ICP-AES.

The concentration of dissolved organic C in root exudates was determined by a Shimadzu TOC analyser (model 5000A).

### Statistical analysis

ANOVA was performed on all data sets, and Tukey's range test was used to compare treatment means. The data for metal mobilization differed by more than 100-fold between the plant species. To stabilize variance, these data were log transformed before ANOVA. In some cases subtraction of the blanks (see above) returned a small negative value of metal mobilization by root exudates. These were taken to indicate zero mobilization of metals by the root exudates. A small positive value (one tenth of the observed minimum) was added to the zero values in order to perform log transformations and ANOVA.

## Results

### Plant growth and nutrient concentrations

All plants appeared normal and healthy when grown in the full nutrient solutions. In the -Fe treatment, young leaves of all three plant species were chlorotic, showing clear symptoms of Fe deficiency. By contrast, there were no visible symptoms in the -Zn treatment. This is not surprising, because previous studies have shown that it is not easy to induce Zn deficiency in hydroponically grown plants without using a chelate buffering system to control free Zn<sup>2+</sup> activity (Parker *et al.*, 1995).

Shoot and root d. wt of wheat and canola were significantly higher than those of the *T. caerulea* plants (Table 1), despite a longer pretreatment growing period for the latter. This reflects the slower growth rate of *T. caerulea* than the domesticated wheat or canola. The other interesting difference is a much smaller root to shoot ratio in *T. caerulea* (0.23 for both ecotypes) than in wheat (0.49) or canola (0.32). This suggests that metal hyperaccumulation in the shoots of *T. caerulea* is not a result of a large root : shoot ratio, at least under hydroponic conditions. Withholding Fe supply for 2 wk decreased shoot and root d. wt significantly

**Table 1** Effects of -Fe and -Zn treatments on d. wt and Zn and Fe concentrations of shoots and roots of *Thlaspi caerulescens*, wheat and canola

Plant species/ecotype	Treatment	D. wt (g pot <sup>-1</sup> )		Zn concentration		Fe concentration	
		Shoot	Root	Shoot (mg kg <sup>-1</sup> )	Root (mg kg <sup>-1</sup> )	Shoot (mg kg <sup>-1</sup> )	Root (mg kg <sup>-1</sup> )
<i>Thlaspi caerulescens</i> Prayon	Control	1.55	0.39	381.4	124.5	73.8	941.4
	-Fe	1.15	0.28	440.6	212.3	31.6	453.8
	-Zn	1.41	0.35	191.8	49.8	87.9	608.0
<i>Thlaspi caerulescens</i> Ganges	Control	1.32	0.33	485.0	246.0	68.7	722.6
	-Fe	1.03	0.25	476.8	555.3	42.6	619.3
	-Zn	0.95	0.21	291.9	107.1	71.4	683.4
Wheat	Control	1.93	0.87	43.6	38.6	60.5	139.9
	-Fe	1.39	0.75	65.3	38.0	28.7	58.3
	-Zn	2.05	0.99	12.4	13.1	59.8	159.6
Canola	Control	2.85	0.90	29.2	37.1	41.5	113.2
	-Fe	1.73	0.54	47.4	59.5	15.3	61.9
	-Zn	2.76	0.94	8.8	16.7	40.8	85.7
ANOVA	Species	***	***	***	***	***	***
	Treatment	***	***	***	***	***	***
	Species × treatment	*	*	NS	***	NS	***

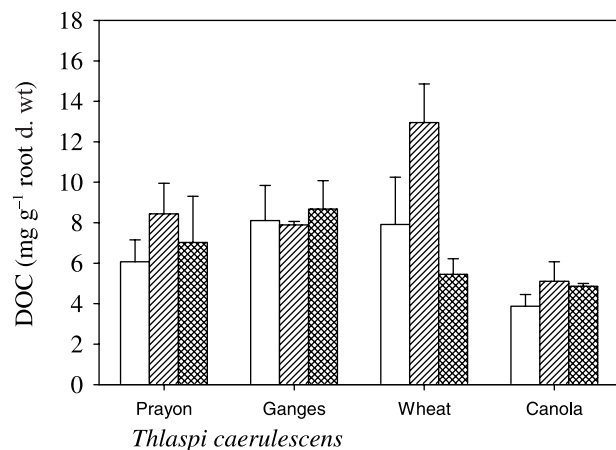
\*\*\*,  $P < 0.001$ ; \*\*,  $P < 0.01$ ; \*,  $P < 0.05$ ; NS, not significant.

in all plants, whereas the -Zn treatment had no significant effect (Table 1). One exception is the smaller shoot and root weights in the -Zn treated Ganges ecotype of *T. caerulescens* compared with the control treatment. However, the reduced growth may not be a true effect of the -Zn treatment, because the plants did not show any Zn deficiency symptoms and two of the four replicates produced similar plant d. wt to the control.

The concentrations of Zn and Fe in plants are shown in Table 1. The necessity to supply different concentrations of Zn, and different concentrations and chelate forms of Fe to the three plant species makes direct comparisons between species difficult. However, both -Zn and -Fe treatments led to significantly decreased concentrations of the respective nutrients in shoots and roots. The concentrations of Zn in the shoots of the -Zn treated wheat and canola were in the borderline range of Zn deficiency (Marschner, 1995). Although the shoot Zn concentrations in the -Zn treated *T. caerulescens* were much higher than in wheat and canola, it is likely that the critical concentration of Zn deficiency in *T. caerulescens* is also much higher than in the nonaccumulating crop species (Shen *et al.*, 1997).

#### Dissolved organic C (DOC) in the root exudates

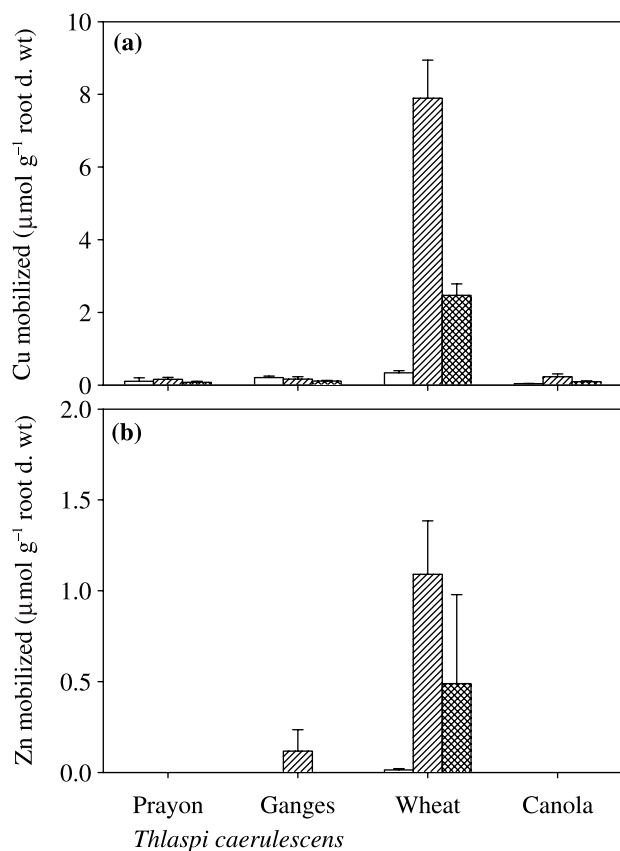
When expressed on a root d. wt basis, the mean DOC in the root exudates collected over the 3-h period were similar between the Prayon and Ganges ecotype of *T. caerulescens* and wheat, and significantly ( $P < 0.05$ ) higher than canola (Fig. 1). The -Fe treatment led to an increase in DOC released by wheat, but not by other plant species. The -Zn treatment had no significant effect on the amount of DOC released by any species.



**Fig. 1** Amounts of dissolved organic C in the root exudates collected from *Thlaspi caerulescens*, wheat and canola. Error bars indicate SEs. Control, open columns; -Fe, hatched columns; -Zn, dotted columns.

#### Mobilization of metals from resin by root exudates

Root exudates from the two ecotypes of *T. caerulescens* and canola mobilized negligible amounts of Cu from the Cu-loaded resins (Fig. 2a). Furthermore, the -Fe or -Zn treatments had little effect on the Cu mobilization by the exudates collected from these two species. In the control treatment, Cu mobilization by the root exudates from wheat was significantly ( $P < 0.05$ ) higher than the other two species. However, the most striking difference between wheat and *T. caerulescens* or canola was the response to the -Fe and -Zn treatments. The -Fe treatment elicited a 23-fold increase



**Fig. 2** Mobilization of Cu (a) and Zn (b) from Cu- or Zn-loaded resin by root exudates collected from *Thlaspi caerulescens*, wheat and canola. Error bars indicate SEs. Control, open columns; -Fe, hatched columns; -Zn, dotted columns.

( $P < 0.01$ ) in Cu mobilization by the root exudates collected from wheat, and the -Zn treatment produced a sevenfold increase ( $P < 0.01$ ). The pattern of Zn mobilization from the Zn-loaded resin (Fig. 2b) was similar to that of Cu mobilization. Again, the -Fe and -Zn treatments resulted in much higher mobilization capacity for Zn in the wheat exudates, whereas the root exudates from *T. caerulescens* and canola in all three treatments showed little mobilization of Zn.

#### Mobilization of metals by root exudates from a contaminated soil

The amounts of Zn, Cd, Cu and Fe mobilized by different root exudates are shown in Fig. 3. In general, wheat exudates mobilized more metals from soil than *T. caerulescens* and canola. In particular, metal mobilization capacity of wheat exudates was greatly enhanced by the -Fe treatment, and to a smaller extent, by the -Zn treatment. By contrast, the exudates of *T. caerulescens* and canola mobilized either negligible or very small amounts of Zn, Cd, Cu and Fe, and the mobilization showed little or inconsistent response to the -Fe and -Zn treatments. The Ganges ecotype of

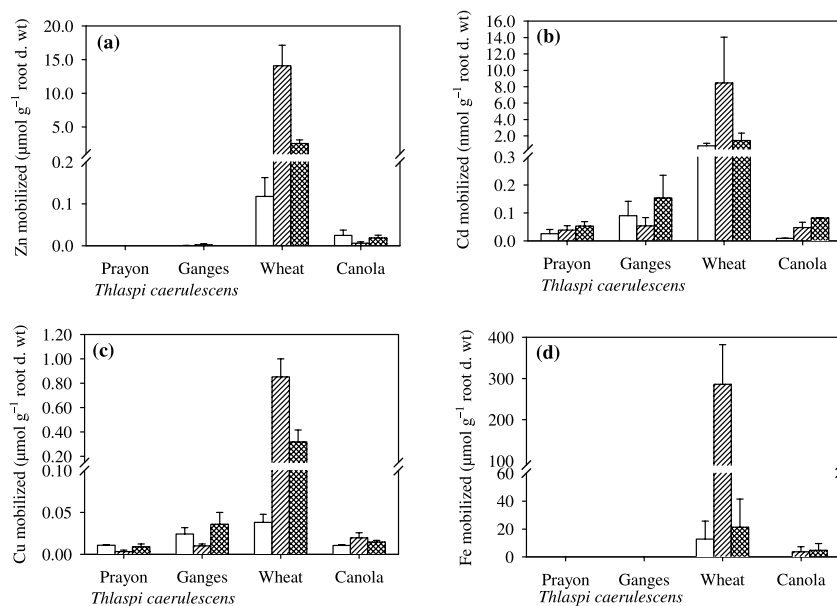
*T. caerulescens* has been shown to accumulate much more Cd in the shoots than the Prayon ecotype, although the two ecotypes accumulate Zn similarly (Lombi *et al.*, 2000; Lombi *et al.*, 2001). However, there was no significant difference between the two ecotypes in the amounts of Cd mobilized from the soil (Fig. 3b).

The results of metal mobilization presented in Figs 2 and 3 are expressed on a basis of root d. wt. A similar pattern is apparent when results are expressed on the basis of the DOC in root exudates (data not shown), except that the difference in metal mobilization between the root exudates collected from the -Zn and -Fe treated wheat was smaller (soil Zn and Fe) or nonsignificant (resin Cu and Zn, and soil Cd and Cu).

#### Discussion

The question addressed in this study was whether the Zn/Cd hyperaccumulator *T. caerulescens* employs a root exudate related mechanism to enhance Zn and Cd availability in soils, as a part of the metal hyperaccumulation strategy. For comparison, we chose canola (*Brassica napus*), which is in the same Brassicaceae family as *T. caerulescens*, and wheat, which adopts a different mechanism of Fe acquisition to dicotyledonous species. Based on the results obtained from this study, the answer to the question is negative. The two ecotypes of *T. caerulescens* did not release significantly greater quantities of exudates (in terms of DOC) than the other species. Also, the exudates released by both *T. caerulescens* and canola mobilized little Cu and Zn from the metal-loaded resin, and little Zn, Cd, Cu and Fe from a contaminated soil. This was true regardless of whether the results were expressed on a basis of root d. wt or of the DOC produced. By contrast, the exudates released by wheat were more effective at mobilizing the metals. Furthermore, withdrawing the supply of Fe or Zn from the nutrient solution for 2 wk did not enhance the metal mobilization capacity of the root exudates from either *T. caerulescens* or canola. The results indicate that the root exudates of the Zn/Cd hyperaccumulator *T. caerulescens* contained no significant amounts of chelating compounds with a high affinity for metals. These results are consistent with the findings of Zhang *et al.* (1991), who studied nonhyperaccumulator species. They showed that although Zn deficiency increased root exudation of amino acids, sugars and phenolics by several dicotyledonous species, including apple, bean, cotton, sunflower and tomato, the root exudates did not enhance Zn mobilization from a synthetic resin or from a calcareous soil. In a study with the Ni hyperaccumulator *Thlaspi goesingense*, Salt *et al.* (2000) found no evidence of the presence of any high-affinity Ni-chelating compounds in the root exudates.

By contrast to *T. caerulescens* and canola, Fe deficiency in wheat led to an increase in the total amount of dissolved organic C released by the roots, compared with the control,



**Fig. 3** Mobilization of Zn (a), Cd (b), Cu (c) and Fe (d) from a contaminated calcareous soil by root exudates collected from *Thlaspi caerulescens*, wheat and canola. Error bars indicate SEs. Control, open columns; -Fe, hatched columns; -Zn, dotted columns.

indicating that a larger proportion of C was diverted to root exudates under the Fe deficiency conditions. Further, -Fe treatment dramatically increased the capacity of the root exudates collected from wheat to mobilize Cu and Zn from the metal loaded resin, and of Fe, Cu, Zn and Cd from a calcareous soil. Even when Fe was sufficient (the control treatment), metal mobilization capacity of the root exudates from wheat was generally higher than that of the exudates of *T. caerulescens* and canola. It is well-known that graminaceous plants respond to Fe deficiency by increasing markedly the release to the rhizosphere of phytosiderophores, predominantly 2'-deoxymugineic acid in the case of wheat (Marschner, 1995; Ma & Nomoto, 1996). Phytosiderophores are capable of chelating not only Fe, but also Cu, Zn and Mn, thus mobilizing these metals from soils (Treeby *et al.*, 1989). Because the stability constants of mugineic acid for Fe(III) ( $\log K = 18.1$ ) and Cu ( $\log K = 18.3$ ) are very similar (Ma & Nomoto, 1996), mobilization of Cu from a Cu-loaded resin has often been used as an indirect method to estimate the quantity of phytosiderophores in root exudates (Cakmak *et al.*, 1996b). The root exudates of wheat mobilized more Cu than Zn from the Cu or Zn loaded resin, probably because mugineic acid has a much higher affinity for Cu ( $\log K = 18.3$ ) than for Zn ( $\log K = 10.7$ ). The order of metal mobilization from the soil by the root exudates from the -Fe treated wheat was Fe > Zn > Cu > Cd; but this order probably reflects more the concentrations of these metals in the soil than the affinity of the exudates for the metals.

Whether Zn deficiency enhances the release of phytosiderophores in wheat appears to be controversial. Zhang *et al.* (1989) and Cakmak *et al.* (1996a) found that Zn deficiency increased substantially the release of phytosiderophores in wheat and in three other wild grass species. It has been

suggested that, in wheat, genotypic differences in Zn efficiency are causally related to phytosiderophore release (Cakmak *et al.*, 1996b; Rengel *et al.*, 1998). By contrast, Gries *et al.* (1995) and Pedler *et al.* (2000) found that the effect of Zn deficiency on the release of phytosiderophores in barley and wheat was small and inconsistent. It has been suggested that the observed effect of Zn deficiency may be due to an impaired translocation of Fe from roots to shoots (Walter *et al.*, 1994). Our experiment was not designed to address these issues. Nevertheless, our results clearly show that, despite there being no increase in the total amount of carbon released by wheat as exudates, the -Zn treatment significantly increased the mobilization by the exudates of metals from both the metal loaded resin and the contaminated soil, compared with the control. The results are consistent with the previous finding that Zn deficiency in wheat enhances the release of phytosiderophores, even though no visible symptoms or growth reduction were observed at the time of the exudate collection. The -Zn treatment also did not affect the concentrations of Fe in the roots and shoots of wheat.

Returning to the question of the mechanisms of Zn and Cd hyperaccumulation by *T. caerulescens*, evidence so far points to two most likely possibilities. First, the plasma membranes of root cells of *T. caerulescens* have a higher density of the Zn transporters. This is shown by a 4.5-fold higher  $V_{\max}$  for Zn influx in *T. caerulescens* than in the nonaccumulator *T. arvense* (Lasat *et al.*, 1996), and by a higher expression of the Zn transporter gene, *ZNT1*, in the roots of *T. caerulescens* (Pence *et al.*, 2000). In the case of Cd, a fivefold higher  $V_{\max}$  for Cd influx was found in the high Cd accumulating ecotype (Ganges) than in the low Cd accumulating ecotype (Prayon) (Lombi *et al.*, 2001). Second, roots of *T. caerulescens* appear to be able to forage actively Zn and Cd in soil by proliferating

root branches in the Zn/Cd rich patches (Schwartz *et al.*, 1999; Whiting *et al.*, 2000). These two mechanisms can be seen as a powerful and complementary combination, the first increasing the root uptake and the second locating the micro-zones of high metal supply in soil. This combination would offset the constraint of diffusional limitation of Zn and Cd in the rhizosphere created by the enhanced uptake. The lack of a direct involvement of root exudates in metal hyperaccumulation by *T. caerulescens*, as shown in this study, agrees with the observation that *T. caerulescens* and several nonaccumulator plants accessed a similar pool of metals in soils (Hamon & McLaughlin, 1999; Gérard *et al.*, 2000; Hutchinson *et al.*, 2000). It also implies that hyperaccumulators are no more efficient in extracting metals from the nonlabile pools in soils than nonaccumulator plants.

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