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Comparison of Elemental Composition and Solubility in the Zinc Hyperaccumulator *Thlaspi caerulescens* with the Non-Hyperaccumulator *Thlaspi ochroleucum*

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Recently, there has been increased interest in *Thlaspi caerulescens* J. & C. Presl., which has been identified as a Zn hyperaccumulator (Reeves and Brooks 1983), because of its high capacity to take up and accumulate Zn in the shoot (Baker et al. 1994; Brown et al. 1995; McGrath et al. 1997). Previous studies using solution culture showed that *T. caerulescens* could accumulate Zn in the shoots to 25-28 g kg^{-1} dry matter (DM) without visual symptoms of Zn toxicity or significant growth depression (Brown et al. 1995; Shen et al. 1997). Baker et al. (1994) reported that the natural populations of *T. caerulescens* in the UK contained Zn up to 21 g kg^{-1} DM in the shoots.

The fundamental mechanisms for metal hyperaccumulation and internal detoxification in plants are not fully understood. The previous experiments showed that *T. caerulescens* has greater capacity in transporting Zn from roots to shoots compared to *T. ochroleucum* (Shen et al. 1997). The restricted transport of Zn from roots to shoots might result from lower solubility of Zn in *T. ochroleucum*. Recently, Kupper et al. (1999) reported that the Zn concentrations in epidermal vacuolar sap reached an average of 385 mM in plant with 20g Zn kg^{-1} dry weight of shoots. Malate may be involved in Zn chelation in vacuoles (Mathys 1977) because of its high concentration, but it dose not explain why *T. caerulescens* can hyperaccumualate Zn in the shoots (Shen et al. 1997). In the shoots of *T. caerulescens*, the majority of intracellular Zn was found to be coordinated with citrate, whereas in the roots Zn was coordinated by histidine (Salt et al. 1999).

Zinc-phosphorus interactions have been frequently studied in some plants. It is suggested that increased P supply induces a higher physiological requirement for Zn (Millikan et al. 1968). Cakmak and Marschner (1987) postulated that formation of insoluble Zn phosphates in leaves and stems reduced the physiological availability of Zn. However, it is not clear how excessive Zn affects P nutrition in hyperaccumulator plant. Binding of Zn with myoinositol hexakisphosphate (phytate) in root cortical cell has been suggested as a detoxification mechanism (Van Steveninck et al. 1987). In the present study, solubility of Zn and P in *T. caerulescens* and a closely related species, *Thlaspi ochroleucum* Boiss. & Heldr, which is a non-hyperaccumulator but heavy metal tolerant plant (McGrath et al. 1997) was compared. In addition, mineral nutrient concentrations in both species were investigated to determine the influence of different Zn treatment on the uptake and distribution of essential nutrients by a hyperaccumulator.

MATERIALS AND METHODS

Experiments were conducted under controlled-environment conditions: 16 h day lengthen with photon flux density of 350 μmol m^{-2} s^{-1}, 20°C/16°C day/night temperature, and 60-

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70% humidity. Seeds of *T. caerulescens* and *T. ochroleucum* were sown in a nylon mesh tray containing black polyethlene beads. The tray was floated on deionized water in an incubator at 25°C for 5 d. The 5-day-old seedlings of *T. caerulescens* and *T. ochroleucum* were grown in a modified 0.2-strength Rorison nutrient solution (Hewitt 1966) containing 10 μM Zn as $ZnSO_4$ for 44d before treatments (10 and 500 μM Zn) were initiated. Other nutrients included in the solutions were (in μM): 400 $Ca(NO_3)_2$, 50 $MgSO_4$, 300 K_2HPO_4, 9.2 H_3BO_3, 1.8 $MnSO_4$ 4 H_2O, 0.21 $NaMoO_4$ $2H_2O$, 0.31$CuSO_4$ $5H_2O$ and 10.8 Fe-(III)EDDHA (ethylenediamine-di(o-hydroxyphenylacetic acid) at pH 5.7. Eight and six replicates were used for *T. caerulescens* and *T. ochroleucum*, respectively, with each vessel containing six plants. Two plants were harvested from each vessel after 16 d treatment. The replicates of each treatment were then divided into two groups, one receiving the same Zn concentrations (10/10 or 500/500 μM) and the other receiving no Zn (10/0 or 500/0). After a further 33 d of growth, the remaining four plants per vessel were harvested. Plants were washed with deionized water, and blotted dry with tissue paper. Shoots and roots were separated, and dried at 80°C for 16 h and weighted.

Plant materials were digested with a mixture of HNO_3/ $HClO_4$ (87%/13% by volume) and the total concentrations of Zn and other elements were determined using inductively coupled plasma atomic emission spectroscopy (ICP-AES; Fisons ARL Maxim III) (Zhao et al. 1994). Water-soluble Zn and other elements were extracted with 1 mM MES (2-morpholinoethanesulphonic acid) buffer at pH 6.0 (Cakmak & Marschner 1987), and the concentrations were determined by ICP-AES. Two-factor analysis of variance (ANOVA) was performed on all data sets.

RESULTS AND DISCUSSION

In all treatments, *T. caerulescens* shoots had significantly higher weight of dry matter (DM) than *T. ochroleucum* ($p<0.001$) (Fig. 1). Zinc treatment had no significant effect on *T. caerulescens* shoot DM. At the second harvest, the 500 μM Zn treatment decreased significantly *T. ochroleucum* shoot DM compared to the 10 μM Zn treatment ($p<0.01$). Withholding Zn supply (500/0) increased significantly *T. ochroleucum* shoot DM.

The shoots of *T. caerulescens* had significantly higher concentrations of total and water-soluble Zn than those of *T. ochroleucum* (Figs. 2a and 2b)($p<0.001$). Concentrations of total and water-soluble Zn in shoots of *T. caerulescens* reached 28.4 and 23.8 g kg^{-1} dry mass respectively, in the 500 μM Zn treatment at the first harvest, which were two time higher than that in *T. ochroleucum*. More than 80% of the Zn accumulated in the shoots of *T. caerulescens* was water-soluble (Fig. 2c). Shoots of *T. caerulescens* generally had higher proportion of water-soluble Zn than *T. ochroleucum* ($p <0.001$). The result is in agreement with observations of Zhao et al. (1998) and Kupper et al. (1999). Salt et al (1999) reported that the majority of intracellular Zn in roots of *T. caerulescens* was found to be coordinated with histidine, whereas in the leaves Zn was coordinated by citrate. When the supply of 10 or 500 μM Zn was withheld, the concentrations of water-soluble Zn in shoots of *T. caerulescens* and *T. ochroleucum* decreased markedly (Fig. 2b), This decrease was relatively more evident in *T. caerulescens* than in *T. ochroleucum*.

In contrast to Zn, the concentrations of total and water-soluble P in the shoots of *T. ochroleucum* were greater than those in *T. caerulescens* ($p< 0.001$) in 10 and 500 μM Zn treatments (Figs. 3a and 3b). This led to a higher Zn/P ratio in the *T. caerulescens* shoot than that in the *T. ochroleucum* (Fig. 4). Compared with the 10 μM treatment, the 500 μM Zn treatment decreased the concentration of water-soluble P in the shoots of both

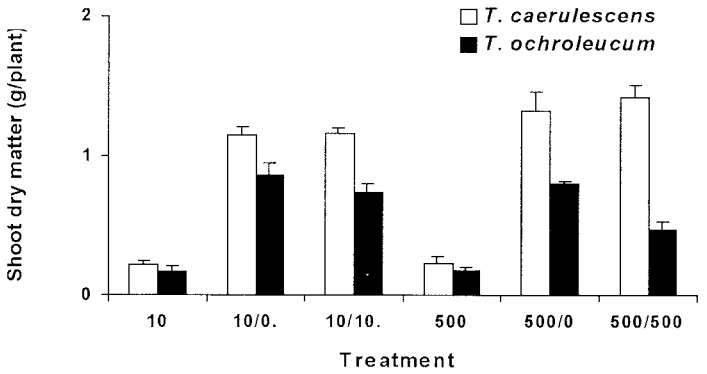

Figure 1. Effects of Zn treatments on shoot dry matter of *T. caerulescens* and *T. ochroleucum* in the first harvest (10 and 500)(16 d after Zn treatment) and in the second harvest (10/10,10/0, 500/500 and 500/0)(plants receiving the same Zn concentrations or no Zn for 33 d after the first harvest). The values are means of 4 replicates for *T. caerulescens* and 3 replicates for *T. ochroleucum*. Error bars represent the standard deviations.

species (Fig. 3b). The decrease was less evident in *T. caerulescens* than in *T. ochroleucum*. The withholding of the Zn supply had no significant effect on the concentrations of total and water-soluble P in the shoots of *T. caerulescens* compared with the continued Zn supply.

For most plants, the P requirement for optimal growth is in the ranges of 3--5 g kg^{-1} DM during the vegetative stage of growth (Marschner 1995). In the present experiment, total P concentration in the shoots of *T. caerulescens* was maintained around 2.1 g kg^{-1} DM in 500 μM Zn treatment, which was lower than the adequate range obtained for normal plants. But, no symptoms of P deficiency were observed in any treatment of the present experiments. The concentrations of total and water-soluble P in the shoots of *T. caerulescens* were always lower than those in *T. ochroleucum* (Fig. 3). Moreover, previous studies showed that Zn deficiency led to an increase in tissue P and accelerated rates of P absorption (Loneragan et al. 1982; Webb and Loneragan 1988). However, the withdrawal of Zn supply did not result in elevated P concentrations in the shoots of both species in this study. In another experiment, the activity of free Zn^{2+} in nutrient solution decreased from $10^{-5.13}$ to $10^{-9.83}$ M by EDTA, which had no significant effect on P concentrations in the shoots of *T. caerulescens* (Shen et al. 1998). Even when the activity of free Zn^{2+} decreased to $10^{-11.0}$ M by DTPA, the shoot P concentration was 3.76 g kg^{-1} DM in *T. caerulescens*, which was much lower than toxic levels obtained for other plants. It has been reported that tissue P concentration ranging from 8 to 30 g kg^{-1} in whole shoots, and from 12 to 45 g kg^{-1} in leaves were toxic to normal plants (Loneragan et al. 1982). The present results suggest that *T. caerulescens* as an hyperaccumulator plant has a lower optimal tissue P concentration than non-hyperaccumulator species and possesses mechanisms to maintain their P nutrition regardless of external or internal Zn levels.

Generally, *T. ochroleucum* shoots had higher total and water-soluble K, Ca and Mg concentrations than *T. caerulescens* (Tables 1 and 2). At the first harvest, Zn treatment

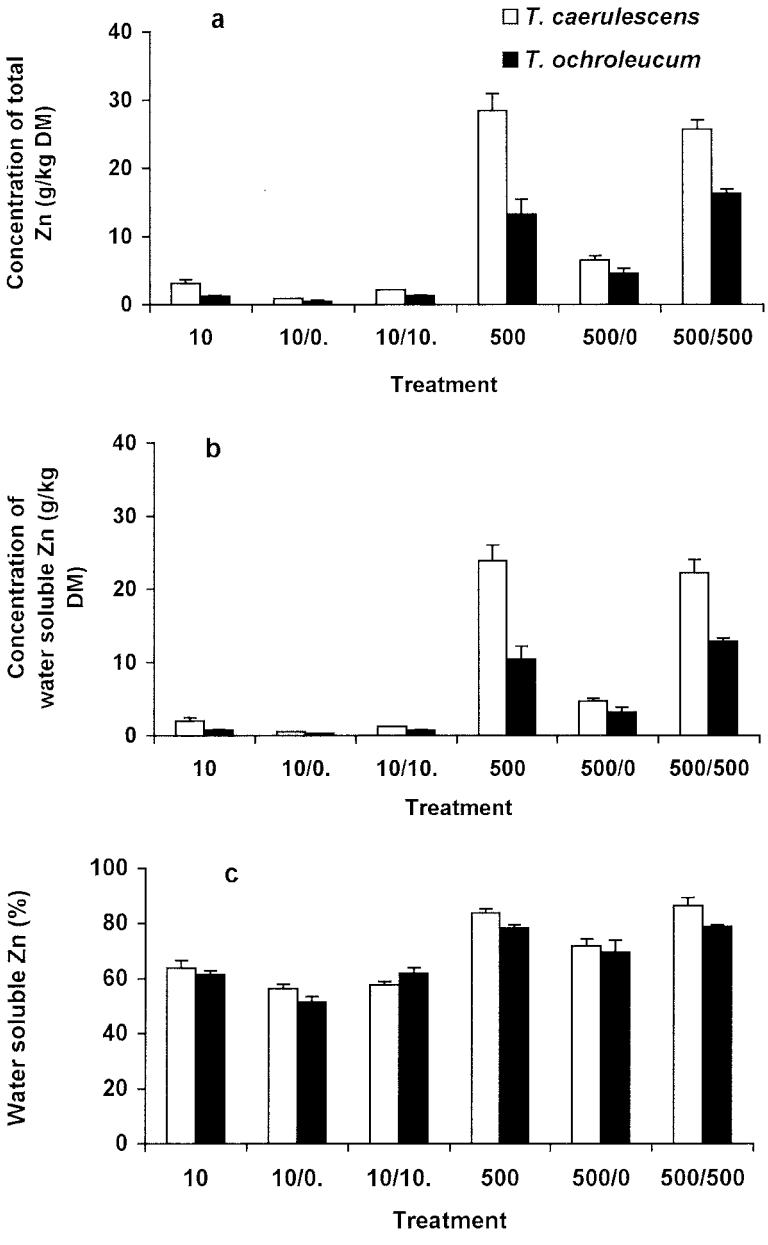

Figure 2. Concentrations of total (a) and water-soluble Zn (b) and the percentage of water-soluble Zn (c) in shoots of *T. caerulescens* and *T. ochroleucum*.
Error bars represent the standard deviations.

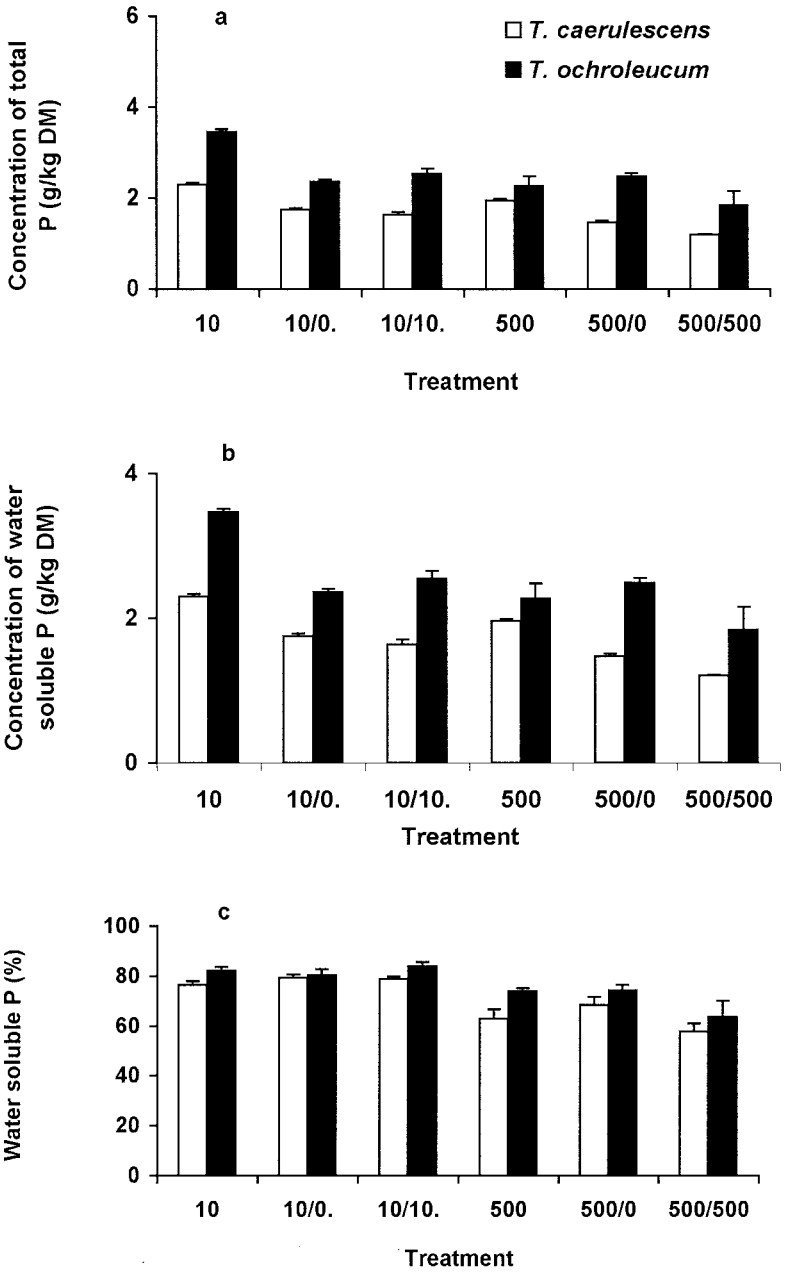

Figure 3. Concentrations of total (a) and water-soluble P (b) and the percentage of water-soluble P (c) in shoots of *T. caerulescens* and *T. ochroleucum*. Error bars represent the standard deviations.

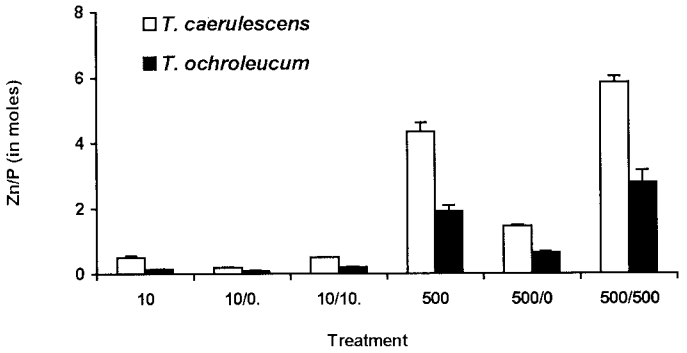

Figure 4. The ratio of Zn/P in the shoots of *T. caerulescens* and *T. ochroleucum*. Error bars represent the standard deviations.

Table 1. Effects of Zn supply on the concentrations of total K, Ca, Mg, and Fe in the shoots of *T. caerulescens* and *T. ochroleucum* (g kg $^{-1}$ dry mass)

mmol	1st harvest				mmol	2nd harvest			
m^{-3}Zn	K	Ca	Mg	Fe	m^{-3}Zn	K	Ca	Mg	Fe
T. caerulescens									
10	49.0	16.3	2.76	0.064	10/10	33.5	9.24	1.05	0.050
					10/0	36.6	10.1	1.08	0.061
500	50.4	14.3	4.38	0.062	500/500	28.4	9.77	2.68	0.048
					500/0	31.7	10.6	1.59	0.049
T. ochroleucum									
10	51.3	21.6	4.19	0.071	10/10	46.8	19.0	3.97	0.052
					10/0	39.1	19.1	3.48	0.049
500	62.1	13.0	3.01	0.040	500/500	39.8	12.0	2.70	0.031
					500/0	50.3	19.4	3.74	0.068
ANOVA *F* ratio									
Species	6.6*	7.7*	0.02 ^{NS}	2.4 ^{NS}		62.0***	370***	705***	0.4 ^{NS}
Zn	5.0 ^{NS}	52.0***	0.8 ^{NS}	12.1**		4.1*	19.1***	13.1***	4.8*
Species x Zn	2.9 ^{NS}	20.5***	33.2**	8.6*		5.3**	21.7***	81.9***	5.4**

1st harvest: plants were harvested 16 d after Zn treatment; 2nd harvest: plants receiving the same Zn concentrations or no Zn for 33 d after the first harvest were harvested. The values are means of 4 replicates for *T. caerulescens* and 3 replicates for *T. ochroleucum*. *P< 0.05; ** P< 0.01; *** P< 0.001; NS: not significant.

had little effect on the concentrations of total and water-soluble K and Mg in the shoots of both species. However at the second harvest the concentrations of total and water-soluble K, Ca and Mg in *T. ochroleucum* shoots were significantly lower in 500μM Zn treatment than in 10 μM Zn treatment (p<0.05). For *T. caerulescens*, increasing Zn level in the solution increased the Mg concentrations and had no significant effect on the Ca concentrations. Withholding 500 μM Zn supply significantly (p < 0.05) increased the concentrations of total and water-soluble K, Ca and Mg in *T. ochroleucum* shoots and had little effect on those in *T. caerulescens* shoots. Compared with 10 μM Zn treatment, 500μM Zn treatment decreased significantly the concentrations of total Fe in shoots of *T. ochroleucum*, and increased water-soluble Fe in shoots of *T. caerulescens* (p<0.05)

Table 2. Effects of Zn supply on the concentrations of water-soluble K, Ca, Mg and Fe in the shoots of *T. caerulescens* and *T. ochroleucum* (g kg^{-1} dry mass)

mmol m^{-3}Zn	1st harvest K	Ca	Mg	Fe	mmol m^{-3}Zn	2nd harvest K	Ca	Mg	Fe
T. caerulescens									
10	44.2	12.0	2.31	0.016	10/10	33.4	6.03	0.81	0.010
					10/0	36.4	6.57	0.82	0.012
500	39.2	11.6	3.94	0.028	500/500	23.7	8.08	2.36	0.031
					500/0	26.4	7.55	1.25	0.020
T. ochroleucum									
10	50.7	16.6	3.67	0.018	10/10	43.6	14.4	3.43	0.016
					10/0	35.9	12.1	2.86	0.014
500	49.4	10.8	2.70	0.017	500/500	35.4	10.3	2.51	0.023
					500/0	43.1	14.0	3.09	0.030
ANOVA *F* ratio									
Species	20.3*	8.9*	0.1^{NS}	15.4**		50.0***	352***	658***	1.8^{NS}
Zn	2.9^{NS}	24.9**	2.2^{NS}	25.2***		8.9***	4.54*	22.3***	13.1***
Species x Zn	1.0^{NS}	18.6**	34.5*	36.0***		7.2**	18.5***	66.5***	3.5*

*P< 0.05; ** P< 0.01; *** P< 0.001; NS: not significant.

(Tables 1 and 2). The concentrations of total Fe in shoots of *T. caerulescens* were at or below deficiency levels for conventional plant species (50 mg kg^{-1} DM) (Marschner 1995) in all Zn treatments (Table 1). But no symptoms of Fe deficiency were observed in *T. caerulescens* received 500μM Zn. In contrast, *T. ochroleucum* showed clear foliar chlorosis 7 d after received the 500 μM Zn treatment, and withholding Zn supply (500/0 treatment) resulted in foliar re-greening and normal growth of the plants. Moreover, shoots of hyperaccumulator *T. caerulescens* had always lower concentrations of total and water-soluble K, Ca and Mg than non-hyperaccumulator *T. ochroleucum* (Tables 1 and 2). Although concentrations and distribution of these elements within the plants varied with Zn treatment, the total and water-soluble concentrations of the elements in *T. caerulescens* shoots were less affected by Zn treatment than those in *T. ochroleucum*. In the pot experiment, it was also found that the concentrations of K, P and Fe in shoots of *T. caerulescens* were much lower than in *T. ochroleucum*, and there was no significant difference between the two species in the concentrations of Ca, Mg and Mn (unpublished data).

From the present study, it can be concluded that the majority of Zn in shoots of hyperaccumulator *T. caerulescens* is present in water soluble forms, and Zn-hyperaccumulation in *T. caerulescens* is accompanied by the ability to maintain the essential nutrients within adequate range for optimum growth.

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