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EFFECTS OF HIGH ZINC AND CADMIUM CONCENTRATIONS ON THE METALLOPHYTE *THLASPI CAERULESCENS* J. et C. PRESL. (BRASSICACEAE)

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RESUMEN

EFECTOS DE ALTAS CONCENTRACIONES DE CINC Y CADMIO SOBRE LA PLANTA METALOFITA *THLASPI CAERULESCENS* J. et C. PRESL. (BRASSICACEAE)

Se ha estudiado la influencia de altas concentraciones de Zn y Cd sobre el crecimiento, el contenido en clorofilas y las concentraciones de Cd y Zn en ***Thlaspi caerulescens*** J. et C. Presl. Las plantas se cultivaron sobre solución nutritiva Hoagland al 10%, que contenía 1, 10 o 100 μM Zn en combinación factorial con 0, 1, 10 y 100 μM Cd. Las altas concentraciones de Cd y Zn no interfirieron con el crecimiento radicular. El crecimiento de la parte aérea sólo fué afectado por la concentración más alta de Cd (100 μM) en plantas con bajo suministro de Zn (1 μM), pero no en las con dosis mayores de Zn. La alta tolerancia de ***T. caerulescens*** frente al Zn y al Cd no fué debida a la exclusión de estos metales, ni a la inhibición de su translocación. Al contrario, se observó hiperacumulación de ambos elementos tanto en las raíces como en las partes aéreas. Se hallaron hasta 93 g Zn kg^{-1} peso seco y 11.75 g Zn kg^{-1} p.s. en raíces y partes aéreas respectivamente. Las concentraciones de Cd alcanzaron valores de hasta 75 g kg^{-1} p.s. en raíces y de hasta 13.68 g kg^{-1} p.s. en las partes aéreas.

Palabras clave: ***Thlaspi caerulescens.*** Cadmio. Zinc. Toxicidad por metales pesados. Tolerancia. Hiperacumulación.

SUMMARY

The influence of high Zn and Cd concentrations on growth, chlorophyll content and Cd and Zn concentrations in ***Thlaspi caerulescens*** J. et C. Presl was investigated. The plants were grown on 10% Hoagland nutrient solution containing 1, 10, or 100 μM Zn factorially combined with 0, 1, 10, and 100 μM Cd. The high Cd and Zn concentrations did not significantly affect root growth. Only in plants with low Zn supply (1 μM) but not in those with higher Zn doses, shoot growth was significantly inhibited by the highest Cd concentration (100 μM). High metal tolerance was due neither to

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metal exclusion nor to inhibition of metal transport. In contrast, hyperaccumulation of both elements occurred in roots as well as in shoots. Up to 93 g Zn kg^{-1} dry weight and 11.75 g Zn kg^{-1} d.w. were found in roots and shoots, respectively. Cadmium concentrations reached 75 g kg^{-1} d.w. in roots and 13.68 g kg^{-1} d.w. in shoots.

Key words: ***Thlaspi caerulescens.*** Cadmium. Zinc. Heavy metal toxicity. Tolerance. Hyperaccumulation.

INTRODUCTION

Zinc and cadmium are geochemically related elements, which frequently occur together at high concentrations in soils around Zn mining and smelting areas. In such soils total Zn and Cd concentrations may range from about 200 to 103000 mg kg^{-1} and from 0.6 to 468 mg kg^{-1}, respectively (references in Ernst, 1974 and in Kabata-Pendias and Pendias, 1984). High concentrations of both metals have been found in soil solutions (Ernst, 1974), and the high availability of ionic Zn and Cd in these soils is an important selection force for plant species or ecotypes which are tolerant to these metals (Ernst, 1990).

Zinc and Cd tolerance has been described in a number of species from different botanical families (Ernst, 1974; Baker *et al.*, 1990). Some sound examples are *Agrostis tenuis* Sibth., *Holcus lanatus* L. (Coughtrey and Martín, 1978; 1979), *Silene cucubalus* Wib. (Verkleij and Bast-Kramer, 1985; Verkleij *et al.*, 1986) and *Thlaspi caerulescens* C. et L. Presl (Hajar, 1987).

The mechanisms of Zn and Cd tolerance are not clearly established. It is generally accepted that metal tolerance is a complex syndrome (Baker and Walker, 1990), which often is determined by polygenes (MacNair,1983). Different strategies for coping with excess metal ions have been observed. 1) Metal exclusion by a) avoidance or restriction of uptake, b) restriction of transport and c) external mechanisms in the rhizosphere (Baker and Walker, 1990); 2) subcellular compartmentation and 3) biochemical compartmentation by binding. (Verkleij and Schat, 1990). Strategies 2 and 3 are also known as detoxification mechanisms.

Excluding strategy "a" does not seem to play an important role in Zn and Cd tolerance. However, restriction of transport from roots to shoots has been found in several species (Baker and Walker, 1990). This tolerance strategy must imply either inactivation of toxic levels of metal ions by subcellular compartmentation in root cells (vacuoles or cell walls), precipitation, chelation or binding, or tolerance of metabolic processes in the roots to excess metal ion levels. There is no experimental evidence which supports this latter hypothesis.

The subcellular compartmentation of Zn and Cd is not clearly established. The conflicting results reported in the literature my arise from both methodological shortcomings of the detection methods (Vázquez *et al.*, 1991; Vázquez *et al.*, 1992) and differences among plant species. Zinc has been reported to be bound in cell walls or stored in vacuoles (Peterson, 1969; Mullins *et al.*, 1985).

Recent studies indicate binding of excess Zn to phytate (Van Steveninck *et al.*, 1990b). Preferent binding of Cd in cell walls (Khan *et al.*, 1984) but also accumulation in vacuoles (Vázquez *et al.*, 1991; Vázquez *et al.*, 1992) and binding to phytochelatines have been reported (Robinson, 1990).

The present study shows first results on the effects of high Zn and Cd concentrations on *Thlaspi caerulescens*, a metallophyte which naturally occurs on zinc mine soils. In the genus *Thlaspi* several metal accumulating species have been described (Baker and Walker, 1990), but only little information on their physiology and phytochemistry is available.

MATERIALS AND METHODS

Seeds from *Thlaspi caerulescens* J. et C. Presl *(Thlaspi alpestre* L.) (Brassicaceae) collected from a Zn mine soil next to Prayon (Belgium) during summer 1990, were germinated on moisted filter paper. At day four from sowing, seedlings were transplanted to continuously aerated 10% Hoagland nutrient solution (pH 5) containing 1, 10 or 100 μM Zn as $ZnSO_4.7H_2O$ in factorial combination with 0, 1, 10 and 100 μM Cd as $CdCl_2.2.5\ H_2O$. The nutrient solution was changed every two weeks. Plants were grown in controlled-environment chamber under the following conditions: photoperiod 12 h light, 12 h darkness, day/night temperatures 25/20 °C, day/night relative humidity 40/70%. After 52 days plants were harvested. Growth was determined by the measurement of root and shoot fresh and dry weights. Given results are means of ten plants per treatment. The chlorophyll and total carotenoid contents were determined on 80% acetone extracts of leaves according to Lichtenthaler and Wellburn (1983). For the analysis of Zn and Cd concentrations in roots and leaves, the oven dried (80 °C) plant material was digested in closed vials with a mixture of $H_2O_2:HClO_4 = 3:1$. Excess H_2O_2 was eliminated under vacuum and the digests were filtered through Whatman n° 2 paper. Zinc and Cd was determined by ICP-ES (Yvon JY-VHR).

Significance of differences among treatments was determined by two-way layout ANOVA.

RESULTS

Plants from all treatments exhibited a very small growth rate. Root length and root and shoot dry weight data are shown in Tables 1, 2 and 3 respectively. High standard deviations were observed indicating genetic variability in the original population. The high Cd and Zn concen-

TABLA 1

Root length (cm) of** Thlaspi caerulescens **exposed to different Zn and Cd concentrations.

Zn in sol. (μM)	Cd concentration (μM) in solution 0	1	10	100
1	2.62a*	2.75a	2.80a	2.23a
10	2.88a	2.21a	3.28a	2.40a
100	2.84a	2.41a	3.42a	2.00a

ANOVA:	F	Probability
Columns	1.570	0.2227
Files	0.054	0.9476
Interaction	0.257	0.9516

* Values followed by the same letter are not significantly different ($p > 0.05$).

trations did not significantly affect neither root length (Table 1) nor root dry weight (Table 2). The shoot dry weight (Table 3) was significantly affected only in plants receiving low Zn (1 μM) and high Cd (100 μM) supply, but not in those with higher Zn doses. Both root and shoot dry weights were non-significantly increased by low Cd (1 μM) supply. The concentrations of chlorophyll a (Table 4), chlorophyll b (data not shown) and total carotenoids (Table 5) were significantly decreased by the 100 μM Zn treatment; lowest concentrations were found for plants

TABLE 2

Root dry weight (mg) of** Thlaspi caerulescens **grown with different Zn and Cd concentrations.

Zn in sol. (μM)	Cd concentration (μM) in solution 0	1	10	100
1	0.27a	0.37a	0.13a	0.12a
10	0.14a	0.39a	0.35a	0.20a
100	0.20a	0.18a	0.18a	0.11a

ANOVA:	F	Probability
Columns	1.122	0.3598
Files	1.807	0.1857
Interaction	1.161	0.3594

* Values followed by the same letter are not significantly different ($p > 0.05$).

TABLE 3

Shoot dry weight (mg) of Thlaspi caerulescens ***grown with different Zn and Cd concentrations.***

	Cd concentration (μM) in solution			
Zn in sol. (μM)	0	1	10	100
1	1.74ab	3.02a	1.92a	0.16b
10	1.66a	2.53a	1.63a	0.85ab
100	1.92a	2.11a	1.76a	1.56ab

ANOVA:	F	Probability
Columns	4.196	0.01560
Files	0.126	0.88121
Interaction	0.550	0.7652

* Values followed by the same letter are not significantly different ($p > 0.05$).

exposed to 100 μM Zn in the presence of 100 μM Cd. Lower Cd concentrations had no negative effect on the pigment content.

Figure 1 shows the Zn and Cd concentrations in roots and shoots of *Thlaspi caerulescens* plants exposed to different combinations of Zn and Cd. The Zn concentrations within both roots and shoots significantly increased with the Zn supply. Zinc concentrations in roots and shoot ranged from 0.1 to 93 g kg^{-1} d.w. and 0.2 to 11.7 g kg^{-1} d.w., res-

TABLE 4

Chlorophyll a concentration (mg g^{-1} fresh weight) in Thlaspi caerulescens ***grown with different Zn and Cd concentrations.***

	Cd concentration (μM) in solution			
Zn in sol. (μM)	0	1	10	100
1	0.854a*	0.988a	0.863a	0.821a
10	0.943a	0.839a	1.009a	0.825a
100	0.615b	0.768ab	0.624b	0.577b

ANOVA:	F	Probability
Columns	2.660	0.0711
Files	24.846	1.42×10^{-6}
Interaction	1.727	0.1579

* Values followed by the same letter are not significantly different ($p > 0.05$).

TABLE 5

Concentration of total carotenoides (mg. g^{-1} fresh weight) in Thlaspi caerulescens ***grown with different Zn and Cd concentrations.***

	Cd concentration (μM) in solution			
Zn in sol. (μM)	0	1	10	100
1	0.223a*	0.232a	0.239a	0.232a
10	0.247a	0.235a	0.264a	0.216ac
100	0.175b	0.215ab	0.204ab	0.195cb

ANOVA:	F	Probability
Columns	1.604	0.2147
Files	10.562	5.12×10^{-4}
Interaction	1.051	0.41

* Values followed by the same letter are not significantly different ($p > 0.05$).

pectively. Excepting plants exposed to 100 μM Zn without Cd supply, the Zn concentrations in shoots generally were higher than in roots. Only in plants receiving 100 μM Zn an inhibitory effect of Cd on Zn concentrations could be observed. But no significant correlation between Cd supply and Zn concentration could be established. In plants receiving 1 or 10 μM Cd, the Cd concentrations were within the same order in roots and shoots, ranging from 9.4 mg kg^{-1} d.w. to 2.9 g kg^{-1} d.w. In plants exposed to 100 μM Cd, up to 75 g Cd kg^{-1} d.w. were found in roots; in shoots up to 13.68 g Cd kg^{-1} d.w. were detected. Increase of Zn supply either favoured Cd uptake or did not significantly influence the Cd concentrations. No inhibitory effect of Zn on Cd accumulation was observed.

DISCUSSION

Our results indicate that the *Thlaspi caerulescens* plants used in our study exhibit considerable tolerance to high concentrations of both Zn and Cd. Root extension growth, a parameter which is generally used for tolerance measurements (Wilkins, 1978), was unaffected even by the simultaneous supply of 100 μM Zn and 100 μM Cd. Zinc and Cd sensitive crop plants, such as *Phaseolus vulgaris* L. cv Contender showed root growth inhibition with 13.5 μM Zn in nutrient solution (Ruano *et al.*, 1988) and concentrations as low as 0.5 μM Cd induced growth inhibition in beans (Vázquez *et al.*, 1992). High Zn levels are known to interfere with

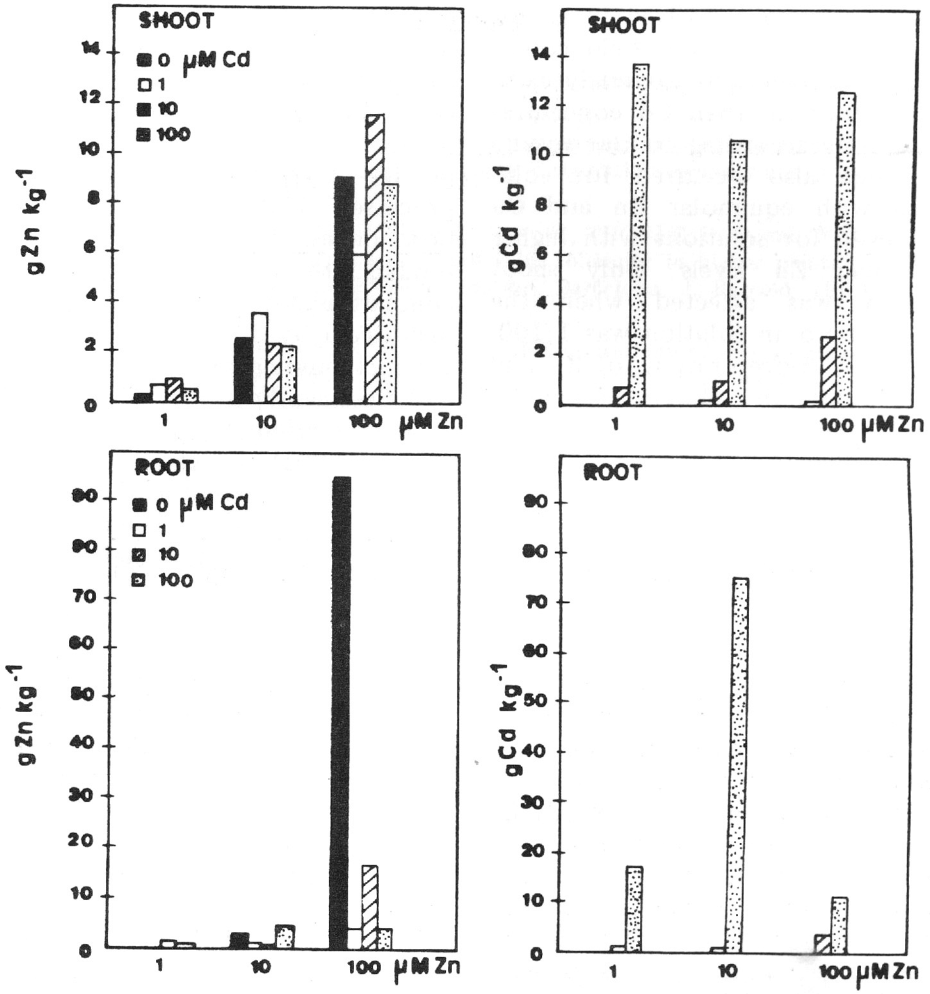

FIG. 1.–*Zinc and cadmium concentrations (g kg^{-1} dry weight) in roots and shoots of* Thlaspi caerulescens *grown with different Zn and Cd concentrations in nutrient solution.*

Fe nutrition in plants. The negative effect of high Zn supply on the chlorophyll content probably was related to a Zn-induced Fe deficiency. This may be an artefact of our experimental conditions which does not reflect the real situation in the field. Zinc mine soils frequently have a relatively high Fe availability (Ernst, 1974), while our nutrient solution contained only 20 μM Fe as Fe-EDTA.

The fact that our *T. caerulescens* plants showed tolerance to both high Zn and Cd does not imply cotolerance. Both metals are generally present in high amounts in Zn-mine soils and tolerance to both metals may have evolved in an independent way. Under field conditions, soil solutions generally contain higher Zn than Cd concentrations, but according to our results tolerance also occurred for solutions with equimolar Zn and Cd and even for solutions with higher Cd than Zn levels. Only shoot growth was affected when the Zn:Cd ratio in solution was 1:100. The high concentrations of Zn and Cd found in our experimental plants confirm earlier reports (Hajar, 1987) indicating that *T. caerulescens* is a metal hyperaccumulator plant according to the definition of both Baker (1981) and Peterson (1983). Hyperaccumulation of Zn has already been found in several species, (Baker, 1989) but, as far as we know, *T. caerulescens* is the only higher plant species in which the capacity to hyperaccumulate Cd has been described.

This species will be a specially interesting tool for studies on metal tolerance mechanisms and metal compartmentation. In this study we could not clearly observe a mutual interaction of Zn and Cd in the respective uptake and translocation processes. This could suggest that both metals do not share common binding sites and that the subcellular compartmentation of both metals may occur by different mechanisms. Distinct binding mechanisms for Zn and Cd have been recently reported in *Lemna minor* L. (Van Steveninck *et al.*, 1990a). Nevertheless, in our study, high Zn concentrations had a positive influence on shoot growth under high Cd (100 μM) supply conditions. This may indicate a Zn-induced tolerance mechanism, which may also be effective in Cd-detoxification. To proof these hypothesis, phytochemical studies and X-ray microanalytical investigations on this species are in progress in our laboratory.

CONCLUSIONS

From our study we may conclude that the *T. caerulescens* plants, rosen from seeds collected on a Zn mine soil, are highly tolerant to both Zn and Cd. Tolerance is neither due to exclusion strategies nor to inhibition of metal translocation. In contrast, hyperaccumulation of both Zn and Cd occurs within roots and shoots.

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