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Impact of simultaneous Cd and Zn substrate amendments on metal accumulation in two Cd/ Zn hyperaccumulating *Thlaspi* species

Vpliv interakcije Cd in Zn v substratu na njuno kopičenje pri dveh hiperakumulacijskih vrstah Cd in Zn iz rodu *Thlaspi*

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Abstract: The impact of simultaneous Cd and Zn amendments in the substrate on the accumulation of Cd and Zn were studied in a recently discovered Cd/ Zn hyperaccumulating *Thlaspi praecox* (Brassicaceae) and compared to a model hyperaccumulating plant species *T. caerulescens*. The plants were grown in pots with added Cd or Zn or both for three months in a greenhouse. The addition of Zn in the substrate increased Cd extractability in the substrate significantly without a significant pH change and this increase resulted in increased concentration and content of Cd in the shoots of both species indicating that species have similar abilities to extract Cd from the substrate. In the combined treatment (Cd and Zn) an increase in shoot biomass accompanied with a decrease in Zn concentration in roots and shoots of both species was observed, while no changes in total accumulated Zn in shoots were seen. These results suggest different uptake and translocation systems for Cd and Zn in *T. praecox,* positioning this plant species in the superior Cd hyperaccumulating league of *T. caerulescens* Ganges ecotype.

Keywords: *Thlaspi caerulescens*, *Thlaspi praecox*, cadmium uptake, hyperaccumulation, zinc uptake

Izvleček: Preučevati smo vpliv sočasnega dodatka Cd in Zn v substrat na njuno akumulacijo pri nedavno odkriti hiperakumulacijski vrsti rani mošnjak (*Thlaspi praecox,* Brassicaceae) in jo primerjali z akumulacijo pri modelni hiperakumulacijski rastlini modrikasti mošnjak (*T. caerulescens*). Obe vrsti smo gojili v rastlinjaku tri mesece. Dodatek Zn v substrat je povečal dostopnost Cd v substratu, ne da bi se ob tem povečala pH vrednost substrata, posledično pa smo izmerili večje koncentracije in vsebnosti Cd v poganjkih pri obeh vrstah, kar pomeni, da imata vrsti podobno sposobnost odstranjevanja Cd iz substrata. V kombiniranem tretmaju (Cd in Zn) smo pri obeh vrstah izmerili največjo biomaso poganjkov in zmanjšano koncentracijo Zn v koreninah in poganjkih, vsebnost Zn pa se pri tem ni spremenila. Rezultati nakazujejo na ločen privzem in transport Cd in Zn pri vrsti *T. praecox*, kar jo postavlja ob bok ekotipu vrste *T. caerulescens* Ganges, za katero velja superiorna sposobnost hiperakumulacije Cd.

Ključne besede: *Thlaspi caerulescens*, *Thlaspi praecox*, privzem kadmija, hiperakumulacija, privzem cinka

Introduction

Accumulation of metals in plant shoots results from the mechanisms of both root uptake and root-to-shoot translocation. The interactions between metals in soil and in the plant itself are important factors in these processes. Soil interactions can be explained by simple ionic competition between metals for sorption sites (Christensen 1987). An increase in bioavailable metal concentration of one after the addition of the other in the soil solution is frequently observed (Ueno & al. 2004). In the plant, the competition for the metal binding sites in transport proteins normally leads to a decrease in the accumulation of one metal in the presence of other(s). Studies of these interactions are of immense importance in plants that are capable of taking up and storing high levels of metals without suffering from metal toxicity, the so-called hyperaccumulators (Baker & Brooks 1989) because of their potential application in phytoextraction technologies (Regvar 2008).

Thlaspi caerulescens J. & C. Presl (Brassicaceae) is one of the most studied hyperaccumulators which occurs on metalliferous as well as on non-metalliferous soils (Reeves & Baker 2000). Hyperaccumulation of non-essential metals Cd (>0.01% Cd in the shoot dry weight) and essential Ni (>0.1% Ni) has been reported in some populations of *T. caerulescens* and a superior ability to hyperaccumulate Cd was described in a population of *T. caerulescens* (Ganges) from Southern France (Lombi & al. 2000, Zhao & al. 2003). Recently, *T. praecox* Wulfen from a multi-metal polluted site in Žerjav (Slovenia) was reported to hyperaccumulate up to 0.6% Cd in shoots (Vogel-Mikuš & al. 2005), up to 0.07% Cd in flowering and seeding stalks (Pongrac & al. 2007), and up to 0.14% Cd in seeds (Vogel-Mikuš & al. 2007) under field conditions. Besides the *T. praecox* population from Žerjav, populations from Mežica and Lokovec in Slovenia also exhibited Cd hyperaccumulating character (Likar & al. 2009).

Hyperaccumulation of Zn (>1% Zn in the shoot dry weight) was, unlike Cd hyperaccumulation, found to be a constitutive trait in *T. caerulescens* (Escarré & al. 2000, Assunção & al. 2003). In *T. praecox* plants collected in Žerjav, up to 1.5% Zn was found in shoots (Vogel-Mikuš & al. 2005). The uptake and translocation of Cd and Zn were studied in *T. praecox* and *T. caerulescens* Ganges ecotype in a hydroponic and in a pot experiment. The hydroponic experiment using radiolabels ^{109}Cd and ^{65}Zn showed that the short-term uptake rate of Cd and Zn was higher in *T. caerulescens* than in *T. praecox*, whereas the Cd but not Zn translocation efficiency was higher in *T. praecox* (Xing & al. 2008). In the pot experiment the two species hyperaccumulated Cd in the shoots to a similar extent whereas Zn concentration in *T. praecox* shoots was lower than that in *T. caerulescens* (Pongrac & al. 2009). However, the design of these experiments did not enable conclusions on the impact of interaction between Cd and Zn on the uptake and translocation of these two metals. Therefore a long-term pot experiment was set up in which *T. praecox* and *T. caerulescens* Ganges ecotype were treated with Zn, Cd or their combination (Cd + Zn) to study their interactions and are presented in this paper.

Material and Methods

Plant material and experimental design

Seeds of Zn/ Cd hyperaccumulating population of *Thlaspi praecox* Wulfen were collected from a heavy metal polluted site in Žerjav, Slovenia and seeds of *Thlaspi caerulescens* J. & C. Presl were collected from the Ganges area (south France). The seeds were germinated on a mixture of perlite and vermiculite (1:1 v/v) moistened with deionised water. Thirty days old seedlings were transplanted to plastic pots (three per pot) filled with 500 g of commercial peat-based substrate (Damjan Čamernik s.p., Biobrazda; pH 6.9–7.2, 7.45 g N kg^{-1}, 2.64 g P_2O_5 kg^{-1}, 2.67 g K_2O kg^{-1} and 251 g kg^{-1} organic matter). The substrate was amended 3 weeks before with Cd and/or Zn (both as a sulphate salt) to obtain the following treatments: the Zn treatment contained 100 mg Zn kg^{-1}, the Cd treatment contained 50 mg Cd kg^{-1} and the combined treatment contained 100 mg Zn kg^{-1} and 50 mg Cd kg^{-1}. The control treatment did not receive the addition of Zn nor Cd. One batch of substrate was prepared per metal amendment treatment and used to fill four pots for each treatment and each plant species. Immediately before transplanting a sample of the substrate was taken from each pot to determine metal availability using the extraction method with 1 M ammonium acetate (Baker & al. 1994). The substrate pH in the water fraction was determined after diluting 1 g of dried soil in 20 ml of MiliQ water and shaking vigorously for 2 h (Öhlinger 1995). The plants were grown for three months in a growth chamber under controlled conditions with 16 h day period, light intensity of 160 μmol $m^{-2}s^{-1}$, 18°C:16°C day:night temperature and 50–60% relative humidity. Upon harvest, the plant material was carefully washed with deionised water; the shoots and roots were lyophilized and weighed (dry weight).

Cadmium and zinc determination

Subsamples (30 mg) of finely grinded plant tissue were digested with a mixture (7:1 v/v) of HNO_3 and $HClO_4$. The concentrations of Cd and Zn in the digest were determined using atomic absorption spectrometry (AAS) (Perkin Elmer AAnalyst 100) (Vogel-Mikuš & al. 2005).

Statistical analysis

The translocation factors (TF) were calculated as ratios of shoot and root concentration. The contents of Cd and Zn (μg) in the plant tissues were calculated by multiplying concentration and dry biomass. The effects of treatment on all the studied parameters were investigated using two-way analysis of variance (ANOVA) with species and treatment as independent factors (**Tab. 1**). When the within-species factor (effect of treatment) was significant, one-way ANOVA was undertaken with Tukey's honest significant difference (HSD) test to determine the significance of the differences between the treatments for both species ($p<0.05$). When the between-species factor (effect of species) was significant, differences for each treatment between *T. praecox* and *T. caerulescens* were determined separately using Student t-test at $p<0.05$. All the tests were performed using Statistica Statsoft® (version 6.0) software.

Results

The addition of Zn significantly increased Cd extractability in the substrate without a significant change in pH, whereas the addition of Cd did not influence the extractability of Zn (t-test, $p<0.05$; Tab. 2). The plant species did not differ significantly in the root nor shoot biomass; only the treatments influenced the plant biomass significantly (Tab. 1; Fig. 1A, B). The combination of Cd and Zn significantly increased the shoot biomass of both species in comparison to both control and Zn treated plants (Fig. 1B).

Overall the two species did not differ significantly in the Zn root and shoot concentration, nor in the Zn translocation factor (TF) (Tab. 1). Nevertheless, differences were observed between the species in particular treatments, e.g. *T. caerulescens* accumulated higher shoot Zn concentrations in the Zn treatment compared to *T. praecox* (t-test; $p<0.05$). In the combined treatment (Zn and Cd) decreased root and shoot Zn concentration when compared to the Zn treatment in both species was observed (Fig. 1C, D). Zinc TF was higher in the Zn and combined treatments in comparison to the control and Cd treatments (Fig. 2A). There was no significant change in the total accumulated Zn in the plant shoots between the Zn and the combined treatments (Fig. 3A).

Species, treatment and their interaction influenced the root Cd concentration and the Cd TF, but only the treatment influenced Cd concentration and content in the shoots (Tab. 1). In *T. caerulescens* higher concentrations of Cd in the roots were measured in the Cd treatment. However, in the shoots the Cd concentration was not different between the two species (Fig. 1E, F). In the combined treatment increased Cd concentrations (Fig. 1F) and content (Fig. 3B) in the shoots of both species were observed when compared to the Cd treatment. The Cd TF was higher in *T. praecox* than in *T. caerulescens* in the Cd and combined treatments (Fig. 2B).

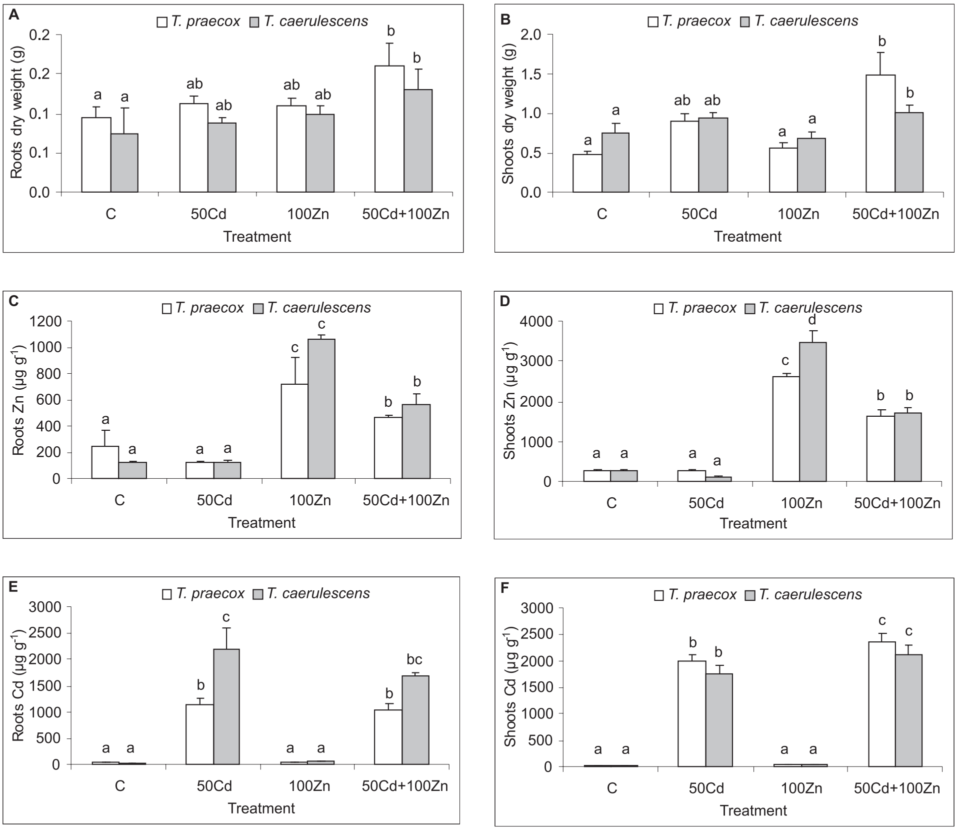

Fig. 1: Plant biomass (A and B), concentration of Zn (C and D) and Cd (E and F) in *Thlaspi praecox* and *Thlaspi caerulescens* grown in Cd and Zn amended substrates (means ± standard error, n=4), C – control treatment, 50Cd – treatment with 50 mg kg^{-1} Cd, 100Zn – treatment with 100 mg kg^{-1} Zn, 50Cd+100Zn – treatment with 50 mg kg^{-1} Cd and 100 mg kg^{-1} Zn. Different letters above the columns indicate significant statistical differences (one-way ANOVA and post-hoc Tukey HSD test; $p<0.05$).

Slika 1: Biomasa rastlin (A in B), koncentracija Zn (C in D) in Cd (E in F) pri vrstah *Thlaspi praecox* in *Thlaspi caerulescens*, ki smo ju gojili na substratu z dodanim Cd in Zn (povprečja ± standardna napaka; n=4), C – kontrola, 50Cd – tretma z 50 mg kg^{-1} Cd, 100Zn – tretma z 100 mg kg^{-1}1 Zn, 50Cd+100Zn – tretma z 50 mg kg^{-1} Cd in 100 mg kg^{-1} Zn. Različne črke nad stolpci nakazujejo statistično značilno razliko (enosmerna ANOVA in Tukeyjev HSD post hoc test, p<0,05).

Discussion

The accumulation capacity of Cd and Zn in response to the addition of Zn, Cd or their combination in the substrate was studied in Cd/ Zn hyperaccumulating species *T. praecox* from Žerjav (Slovenia) and *T. caerulescens* Ganges ecotype. The addition of Zn in the combined treatment increased the Cd extractability significantly in the substrate as previously observed (Ueno & al. 2004). In contrast, the extractability of Zn was not changed due to the presence of Cd in the combined treatment which may be contributed to a constantly high amount (> 90%) of total Zn remaining insoluble in the substrate and thus unavailable for plant uptake (Broadley & al. 2007). The concentrations of ammonium-extractable concentrations of Zn and Cd were measured three weeks after amending the substrate to ensure homogenous distribution of these two metals and

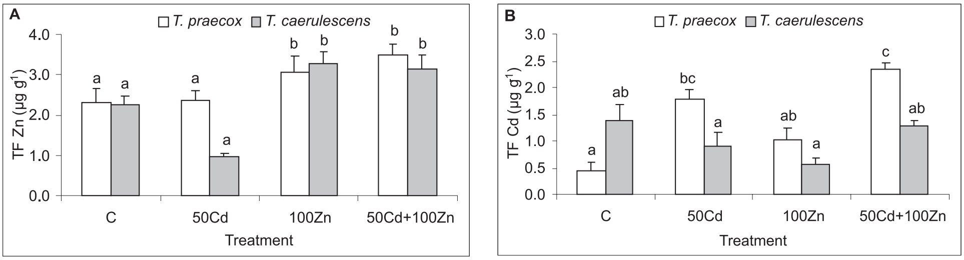

Fig. 2: Translocation factor (TF; the ratio between shoot and root concentration) for Zn (A) and Cd (B) in *Thlaspi praecox* and *Thlaspi caerulescens* grown in Cd and Zn amended substrates (means ± standard error, n=4); C – control treatment, 50Cd – treatment with 50 mg kg^{-1} Cd, 100Zn – treatment with 100 mg kg^{-1} Zn, 50Cd+100Zn – treatment with 50 mg kg^{-1} Cd and 100 mg kg^{-1} Zn. Different letters above the columns indicate significant statistical differences (one-way ANOVA and post-hoc Tukey HSD test; p<0.05).

Slika 2: Translokacijski faktor (TF, razmerje koncentracij v poganjkih in koreninah) za Zn (A) in Cd (B) pri vrstah *Thlaspi praecox* in *Thlaspi caerulescens*, ki smo ju gojili na substratu z dodanim Cd in Zn (povprečja ± standardna napaka; n=4), C – kontrola, 50Cd – tretma z 50 mg kg^{-1} Cd, 100Zn – tretma z 100 mg kg^{-1} Zn, 50Cd+100Zn – tretma z 50 mg kg^{-1} Cd in 100 mg kg^{-1} Zn. Različne črke nad stolpci nakazujejo statistično značilno razliko (enosmerna ANOVA in Tukeyjev HSD post hoc test, p<0,05).

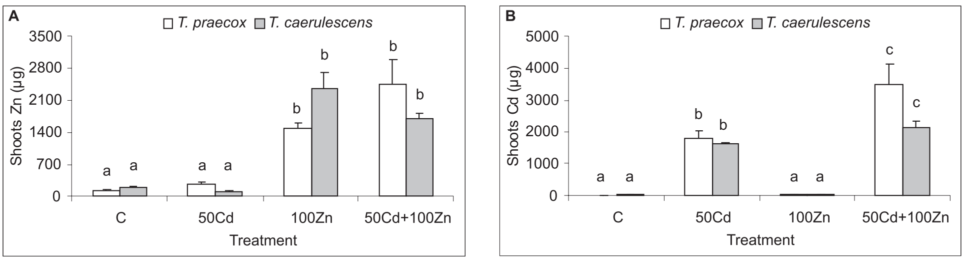

Fig. 3: Content (μg) of Zn (A) and Cd (B) in shoots of *Thlaspi praecox* and *Thlaspi caerulescens* grown in Cd and Zn amended substrates (means ± standard error, n=4); C – control treatment, 50Cd – treatment with 50 mg kg^{-1} Cd, 100Zn – treatment with 100 mg kg^{-1} Zn, 50Cd+100Zn – treatment with 50 mg kg^{-1} Cd and 100 mg kg^{-1} Zn. Different letters above the columns indicate significant statistical differences (one-way ANOVA and post-hoc Tukey HSD test; p<0.05).

Slika 3: Vsebnost (μg) Zn (A) in Cd (B) v poganjkih vrst *Thlaspi praecox* in *Thlaspi caerulescens*, ki smo ju gojili na substratu z dodanim Cd in Zn (povprečja ± standardna napaka; n=4), C – kontrola, 50Cd – tretma z 50 mg kg^{-1} Cd, 100Zn – tretma z 100 mg kg^{-1} Zn, 50Cd+100Zn – tretma z 50 mg kg^{-1} Cd in 100 mg kg^{-1} Zn. Različne črke nad stolpci nakazujejo statistično značilno razliko (enosmerna ANOVA in Tukeyjev HSD post hoc test, p<0,05).

before the substrate was used in the experiment thus the observed changes in the extractability of Cd were not a result of plant growth.

Increased shoot biomass of both species was observed in the combined treatment in comparison to only Zn treatment indicating that the combination of Cd and Zn is beneficiary to plant growth of these hyperaccumulating plant species. In our previous experiment increasing Cd in the substrate resulted in the increase of the roots and shoots biomass of the same species (Pongrac & al. 2009). However, in all the Cd treatments 100 μg Zn g^{-1} was added as *T. caerulescens* is extremely sensitive to Zn deficiency in soils (Ozturk & al. 2003) and has higher requirement for Zn (Shen & al. 1997). The growth enhancing effect of Cd in *T. caerulescens* was previously demonstrated (Escarré & al. 2000, Roosens & al. 2003, Yanai

Table 1: Two-way ANOVA table for analysed parameters with species and treatment as independent factors. Values p<0.05 are given in bold.

Tabela 1: Preglednica rezultatov statistične analize podatkov z dvosmerno ANOVA pri čemer smo kot neodvisni spremenljivki uporabili vrsto in tretma. Statistično značilne vrednosti (p<0,05) so odebeljene.

Source	**df**	**F**	**p**
Root dry weight			
Species	1	2.26	0.146
Treatment	3	3.39	**0.035**
Species × treatment	3	0.10	0.959
Error	24		
Shoot dry weight			
Species	1	0.02	0.885
Treatment	3	10.98	**<0.000**
Species × treatment	3	3.15	0.043
Error	24		
Root Zn concentration			
Species	1	1.58	0.220
Treatment	3	32.22	**<0.000**
Species × treatment	3	2.37	0.095
Error	24		
Shoot Zn concentration			
Species	1	4.05	0.056
Treatment	3	26.37	**<0.000**
Species × treatment	3	5.83	**0.004**
Error	24		
Zn translocation factor			
Species	1	3.75	0.066
Treatment	3	15.20	**<0.000**
Species × treatment	3	3.04	0.051
Error	22		
Shoot Zn content			
Species	1	0.0043	0.948
Treatment	3	39.19	**<0.000**
Species × treatment	3	3.96	0.020
Error	24		
Root Cd concentration			
Species	1	15.88	**<0.000**
Treatment	3	63.60	**<0.000**
Species × treatment	3	5.75	**0.004**
Error	24		
Shoot Cd concentration			
Species	1	2.14	0.157
Treatment	3	213.90	**<0.000**
Species × treatment	3	0.80	0.508
Error	23		
Cd translocation factor			
Species	1	7.09	**0.014**
Treatment	3	11.48	**<0.000**
Species × treatment	3	10.38	**<0.000**
Error	23		
Shoot Cd content			
Species	1	4.03	0.057
Treatment	3	50.78	**<0.000**
Species × treatment	3	2.95	0.054
Error	23		

Table 2: Ammonium acetate extractable concentrations (µg g^{-1}) of Zn and Cd and pH in the substrate before planting *Thlaspi praecox* and *Thlaspi caerulescens* in the pot experiment (means ± standard error; n=4). 50Cd – treatment with 50 mg kg^{-1} Cd, 100Zn – treatment with 100 mg kg^{-1} Zn, 50Cd^{+}100Zn – treatment with 50 mg kg^{-1} Cd and 100 mg kg^{-1} Zn. Different letters beside the numbers indicate significant statistical differences (one-way ANOVA and post-hoc Tukey HSD test; p<0.05).

Tabela 2: Koncentracija (µg g^{-1}) Zn in Cd po ekstrakciji z amonijevim acetatom in pH v substratu pred presaditvijo vrst *Thlaspi praecox* in *Thlaspi caerulescens* (povprečja ± standardna napaka; n=4). 50Cd – tretma z 50 mg kg^{-1} Cd, 100Zn – tretma z 100 mg kg^{-1} Zn, 50Cd^{+}100Zn – tretma z 50 mg kg^{-1} Cd in 100 mg kg^{-1} Zn. Različne črke ob številkah nakazujejo statistično značilno razliko (enosmerna ANOVA in Tukeyjev HSD post hoc test, p<0,05).

Treatment	*Thlaspi praecox*			*Thlaspi caerulescens*		
	Zn	Cd	pH	Zn	Cd	pH
Control	0.80 ± 0.12 a	-	7.09 ± 0.01	0.75 ± 0.12 a	-	7.05 ± 0.02
100Zn	5.83 ± 0.98 b	-	7.02 ± 0.01	5.01 ± 0.33 b	-	7.01 ± 0.02
50Cd	0.66 ± 0.05 a	6.37 ± 0.17 A	7.09 ± 0.04	0.60 ± 0.04 a	6.13 ± 0.18 A	7.06 ± 0.03
50Cd+100Zn	4.91 ± 0.33 b	7.56 ± 0.29 B	7.06 ± 0.02	4.82 ± 0.28 b	7.12 ± 0.19 B	7.04 ± 0.02

& al. 2006) and a physiological role of Cd in *T. caerulescens* was proposed (Liu & al. 2008). Our results indicate that also in *T. praecox* Cd may have a physiological role and that high Zn substrate concentrations are required for optimal growth.

The Zn hyperaccumulating concentration criterion set at 10,000 µg Zn g^{-1} in dry weight of shoots (Baker & Brooks 1989) was not reached in either of the species in this experiment which may be a result of the length of the plant growth as well as low availability of Zn for the plants. Zinc concentrations from the field exceeding this criterion were reported repeatedly for *T. caerulescens* (Baker & al. 1994, Reeves & al. 2001) and for *T. praecox* (Vogel-Mikuš & al. 2005, Likar & al. 2009). The highest concentration of Zn in shoots was measured in *T. caerulescens* in the Zn treatment which was significantly higher than that in *T. praecox* as observed in our previous experiment (Pongrac & al. 2009). However, the species did not differ in the Zn TF indicating equally efficient Zn transport in both species supporting the observation from the hydroponic experiment (Xing & al. 2008). In the Cd treatment lower Zn concentrations in the shoots as well as Zn TF were found in *T. caerulescens* indicating a decreased Zn transport in this species in the case of high Cd to Zn ratio in the substrate that may lead to Zn deficiency in plants as previously suggested (Chaney & al. 2006). A significant decrease in Zn concentration in the roots and shoots in both species was observed in the combined treatment when compared to the Zn treatment indicating a competition of Cd and Zn in the substrate that may have led to a decreased Zn uptake into the roots. This however did not result in a changed translocation of Zn within plants. High external Cd concentration was already reported to inhibit Zn accumulation in *T. caerulescens* Ganges ecotype (Lombi & al. 2001, Zhao & al. 2002, Roosens & al. 2003), but not in Prayon ecotype (Papoyan & al. 2007). Thus different uptake systems for Cd and Zn in *T. caerulescens* Ganges ecotype were proposed (Lombi & al. 2001) and based on our results may exist also in *T. praecox*. In another Cd/Zn hyperaccumulator *Sedum alfredii* H. the addition of Cd enhanced Zn translocation and partition to the shoots (Yang & al. 2004) indicating similar response to the one observed in *T. caerulescens* Prayon ecotype. On the other hand, no effect on Zn accumulation by Cd supply was observed in a Cd accumulating chamomile (*Matricaria recutita* L.) plants (Chizzola & Mitteregger 2005). Increasing Cd application to Zn-deficient durum wheat plants (*Triticum durum* Desf. cv. Cakmak) tended to decrease Zn concentrations, whereas in plants with adequate Zn supply, the concentrations of Zn were either not affected or increased by Cd (Köleli & al. 2004). It seems that the concentration range may profoundly affect metal interactions and accumulation in both metal (hyper)accumulating and non-accumulating species.

The concentration of Cd of both studied species exceeded the hyperaccumulating Cd concentration set at 100 µg Cd g^{-1} in shoot dry weight (Brooks 1998) in the Cd and in the combined treatments, thus confirming the observations from our previous pot study in which *T. praecox* matched the superior Cd hyperaccumulation ability (Pongrac & al. 2009) reported for *T. caerulescens* Ganges ecotype (Lombi & al. 2000, Zhao & al. 2003). However, higher concentrations of Cd in the roots of *T. caerulescens* were observed in the same two treatments and consequently lower Cd TFs were calculated. This supports the results from the hydroponic experiment where using radiolabels ^{109}Cd the short-term uptake rate of Cd was higher in *T. caerulescens* Ganges ecotype than in *T. praecox*, whereas the Cd translocation efficiency was higher in *T. praecox* (Xing & al. 2008). Relatively high Cd concentrations were measured also in the control and Zn treatments which may be a consequence of Cd presence in the commercial substrate or presence of Cd in the seeds of these two hyperaccumulating plants, or both. High Cd concentrations have been reported in seeds of *T. praecox* that contained on average 1,000 µg Cd g^{-1} when grown at the most polluted site in Žerjav (Vogel-Mikuš & al. 2007) and seeds of *T. caerulescens* were reported to contain on average 3,200 µg Cd g^{-1} (Kachenko & al. 2009). The concentration of Cd in the substrate of these two treatments was however below the detection limit of the method used to determine ammonium-extractable Cd concentration in soil.

The interaction of Zn and Cd in the substrate in the combined treatment significantly increased the concentration and content of Cd in the shoots of both species which is probably a result of the increased Cd extractability in the substrate that was not a result of the pH change. In contrast, the Cd accumulation was not affected by the Zn addition in *T. caerulescens* Ganges ecotype (Ueno & al. 2004) and an inhibition in the Cd accumulation was observed in *T. caerulescens* Prayon due to the Cd and Zn interaction in the substrate (Lombi & al. 2001, Zhao & al. 2002, Roosens & al. 2003). Similarly, in a Cd accumulating *M. recutita* plants the addition of Zn to the soil led to a decreased Cd accumulation, whereas further increase in the Zn supply did not further decrease the Cd concentration in shoots (Chizzola & Mitteregger 2005). Neither was additional Zn supply accompanied by a corresponding decrease in Cd shoot concentrations of Cd sensitive *T. durum* (Köleli & al. 2004). In the presence of Cd adding Mn to the solution significantly reduced the concentrations of Cd in all organs of Mn hyperaccumulator *Phytolacca americana* L. (Peng & al. 2008). These observations indicate the importance of the metal in question and its concentrations used in the experiment as well as plant species and metal (hyper)accumulation properties of the plants when studying the elemental interactions in soil and their importance for plant uptake.

In conclusion, the interactions between the metals in the substrate may significantly affect their accumulation in the aboveground plant parts that are dependent on the metal(s) in question, its concentrations, plant species and/or even population and soil properties. The metal concentrations and their mode of accumulation may already indicate the underlying metal uptake and transport mechanisms in plants. Studies of combined pollution are therefore important as they are more likely to relate to the field conditions. The two studied hyperaccumulating plants showed similar responses to the interaction of Cd and Zn in the substrate with an observed decrease in Zn but an increase in the Cd concentration and content in the shoots. The results thus suggest that different uptake systems for Cd and Zn may also exist in *T. praecox* and that the two species have similar ability to extract Cd from substrate.

Povzetek

Na območjih obremenjenih s kovinami lahko življenjski cikel zaključijo le rastline, ki so na povečane koncentracije kovin v tleh prilagojene. Ena izmed prilagoditev na tovrstni stres je razvoj razstrupljevalnih mehanizmov, ki rastlinam omogočajo, da v nadzemnih delih kopičijo zelo velike koncentracije kovin. Ta redek pojav imenujemo hiperakumulacija.

V Sloveniji je bila v okolici Žerjava (Mežiška dolina), ki je zaradi delovanja talilnice in predelovalnice Pb obremenjena s Zn, Cd in Pb, nedavno odkrita hiperakumulacijska vrsta rani mošnjak (*Thlaspi praecox* Wulfen) (Vogel-Mikuš & al. 2005). Za vrsto *T. praecox* smo že pokazali, da ima primerljivo dobre lastnosti kopičenja Cd kot modelna hiperakumulacijska vrsta za Cd modrikasti mošnjak (*T. caerulescens* J. & C. Presl) (Pongrac & al. 2009). Po do sedaj znanih podatkih ima izmed različnih ekotipov vrste *T. caerulescens*

ekotip Ganges superiorno sposobnost hiperakumulacije Cd in pri njem obstajata različna sistema za privzem in transport Cd in Zn. V pričujočem delu smo pri vrstah *T. praecox* in *T. caerulescens* ekotip Ganges preučevali vpliv interakcije med Cd in Zn v substratu na privzem in translokacijo Cd in Zn, da bi ugotovili, ali sta ta dva sistema različna tudi pri vrsti *T. praecox*.

V rastlinjaku smo tri mesece gojili obe vrsti v substratu, ki smo mu dodali Zn ali Cd ali njuno kombinacijo. Pred poskusom smo v substratu izmerili pH in dostopnost Zn in Cd po ekstrakciji z amonijevim acetatom, po poskusu pa rastlinam izmerili biomaso in koncentracije Zn in Cd s pomočjo atomskega absorpcijskega spektrometra po mineralizaciji suhega rastlinskega materiala v mešanici kislin $HClO_4$:HNO_3 (7:1 v/v).

Primerjava dostopnih koncentracij Zn in Cd v substratu posameznih tretmajev in kombiniranega tretmaja je pokazala, da je dodatek Zn v substrat povečal dostopnost Cd, ki ni bil posledica sprememb pH vrednosti, dodatek Cd pa ni povečal dostopnosti Zn. Povečanje dostopnosti Cd v substratu je vplivalo na povečano kopičenje Cd v poganjkih preučevanih vrst. Pri tretmaju s Zn smo v poganjkih vrste *T. caerulescens* izmerili večje koncentracije Zn kot v poganjkih vrste *T. praecox*. V kombiniranem tretmaju (Cd in Zn) smo pri obeh vrstah izmerili največjo biomaso hkrati pa zmanjšano koncentracijo Zn v koreninah in poganjkih v primerjavi s Zn tretmajem. Ti rezultati nakazujejo na ločen privzem in transport Cd in Zn tudi pri vrsti *T. praecox*, kar jo postavlja ob bok superiornem ekotipu vrste *T. caerulescens* (Ganges).

Poznavanje mehanizmov, ki omogočajo toleranco in kopičenje velikih koncentracij kovin je pomembno zaradi potencialne uporabe hiperakumualcijskih rastlin v fitoekstrakciji, eni izmed tehnik fitoremediacije, t.j. čiščenja okolja s pomočjo rastlin. Pri fitoekstrakciji bi z uporabo hiperakumulacijskih rastlin z veliko biomaso v relativno kratkem času odstranili presežne koncentracije kovin iz tal. S čiščenjem kmetijskih površin bi na ta sonaraven način preprečili prenos kovin naprej v prehranjevalno verigo. Izsledki naše raziskave potrjujejo, da sta vrsti *T. praecox* in *T. caerulescens* v enaki meri sposobni odstranjevanja Cd iz tal.

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