Effects of co-cropping on bioaccumulation of trace elements in *Thlaspi caerulescens* and *Salix dasyclados*

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ABSTRACT

Most phytoremediation technologies are based on the use of selected plant species cropped as in monoculture. Separated (monoculture) and combined (simultaneous) cropping of hyperaccumulator *Thlaspi caerulescens* and accumulator tree *Salix dasyclados* were tested in our experiment. We used moderately and highly contaminated soil. Extremely contaminated soil caused progressive mortality of willows planted separately. Combined cropping with *T. caerulescens* enabled willows to survive. Generally, we determined decreased bioaccumulation of As, Cd, and Pb in both tested species and Zn in willow plants. Combined cropping enhanced bioaccumulation of Zn in *T. caerulescens* shoots. The remediation efficiency of the individual species in the co-cropping system did not differ from those obtained in separate cropping mode. For As and Pb the negligible effectiveness of phytoextraction was confirmed for both separate and combined cropping of the tested plant species.

Keywords: phytoextraction; cadmium; zinc; bioaccumulation; combined cropping

Recent remediation technologies prefer environmentally friendly methods. Phytoextraction belongs to the most advanced strategy. It uses plants to extract potentially toxic trace elements (or other contaminants) from contaminated soil and accumulate them in the harvestable above-ground biomass. The total amount of elements extracted from soil by plants is affected by factors such as the total concentration of trace element in soil, its bioavailable fraction, plant biomass production, tolerance and accumulation ability of used plant species, and others (Alloway 1990, Jungk 1996, Echevarria et al. 1998, Schmidt 2003). Yield of harvestable biomass can be affected by a number of factors including agronomic practices such as irrigation, application of fertilizers and weed and pest control (McGrath et al. 2006). Uptake of elements depends on root architecture and its activity (Marschner and Römheld 1996).

Most hyperaccumulator species produce only small harvestable biomass which causes problems with crop management. Cropping of species with lower accumulation capacity and higher yield production seems to be a solution. The use of trees with promising accumulation capability was tested recently for members of the Salicaceae family (Saxena et al. 1999). Selected clones of willows (e.g. *Salix dasyclados*) and poplars show good capability of bioaccumulation. Their accumulation capacity could be comparable to that of hyperaccumulators (e.g. *T. caerulescens*) (Robinson et al. 2000, Pulford and Watson 2002, Fischerová et al. 2006). Plants of *Salix* spp. accumulate in above-ground biomass more Cd and Zn than As and Pb and the capacity for trace elements accumulation depends on the clone used (Rosselli et al. 2003, Vysloužilová et al. 2003a). We can find species with favourable phytoextraction capacity such as *S. viminalis*, *S. dasyclados*, *S. caprea*, *S. smithiana*, and others (Greger and Landberg 2001, Vysloužilová et al. 2003b, Meers et al. 2005, Dos Santos Utmazian et al. 2007) with respect to soil characteristics and actual contamination.

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Phytoextraction experiments generally employ cropping of selected species as a monoculture. We tested growth and phytoextraction capability in two plant species with differential accumulation capacity at separated and simultaneous cropping on two soils with different level of contamination. *Thlaspi caerulescens* was deemed to change conditions and bioavailability of trace elements in the rhizosphere shared with Cd/Zn accumulator tree *Salix dasyclados* and thus enhance metal removal by this high biomass species.

MATERIAL AND METHODS

Vegetation experiment. The efficiency of trace elements uptake by hyperaccumulator and accumulator plant species in different cropping systems was tested in a two-year pot experiment. Pots were placed in vegetation hall and were partially covered to protect them from rainfall. We used two different anthropogenically contaminated soils from the Příbram area (Central Bohemia, Czech Republic): moderately contaminated Cambisol (total concentrations of metals: 5.46 mg/kg of Cd, 956 mg/kg of Pb, and 279 mg/kg of Zn; pH 5.9) and highly contaminated Fluvisol (total content – 60.0 mg/kg of Cd, 4626 mg/kg of Pb, and 5919 mg/kg of Zn; pH 5.7). All treatments involved five replicates. Pots were filled with five kilograms of air-dried and homogenised topsoil fertilized with 0.5 g N, 0.16 g P, and 0.4 g K. Pots were kept in a controlled outdoor vegetation hall and plants were watered with demineralised water as required to maintain soil moisture at 60% of its maximal water holding capacity.

Thlaspi caerulescens J. et C. Presl, a herbaceous hyperaccumulator of Cd and Zn, and *Salix dasyclados* Vimm., a metal accumulator tree with a high biomass production were used in this experiment. The seeds of *T. caerulescens* originated from the Ganges area (France) and clones of *S. dasyclados* were cultivated in Silva Tarouca Research Institute for Landscape and Ornamental Gardening in Průhonice (Czech Republic) (Weger and Havlíčková 2002).

The pot experiment consisted of three treatments: separately planted penny-cress (treatment *Thlaspi*), separately planted willow (*Salix*) and co-cropping (or intercropping) of both species (*Thlaspi* plus *Salix*). In both years, above-ground biomass was harvested and separated in different plant organs (leaves of *T. caerulescens*, leaves and twigs of *S. dasyclados*). Total concentration of elements in above-ground biomass was calculated as weighted mean of all their concentration in the individual organs.

Laboratory procedure. After harvest, plants were washed manually with deionised water, dried at 65°C, homogenised, and digested using a modified dry ashing procedure and the mixture of oxidising gases (O_2 + O_3 + NO_x) in the Apion Dry Mode Mineralizer (Tessek, Czech Republic) at 400°C for 10 h. The ash was dissolved in 20 ml 1.5% HNO_3 (Miholová et al. 1993). Concentrations of trace elements (As, Cd, Pb, and Zn) were determined by inductively coupled plasma optical emission spectrometry (ICP-OES, Varian Vista Pro, Varian, Australia). Certified reference material RM NCS DC 73350 Poplar leaves was used to assess quality of analytical data.

A bioaccumulation factor (FB) was calculated from concentrations of studied elements in plant biomass and total concentration in soil. A factor of remediation (FR) was obtained as the percentage of metal removed from a defined volume of soil during one vegetation period. Statistical analyses were made using the Statgraphics Plus v. 5.0 software with multi-factorial ANOVA analysis followed by a Tukey HSD test (α = 0.05).

RESULTS AND DISCUSSION

Above-ground biomass production in separate and co-cropping treatments. Generally, we present cumulative biomass data for the whole experimental period since the variation in biomass production was comparably small between the two vegetation periods. Combined cropping induced a significant decrease in total above-ground biomass of *T. caerulescens* grown on moderately contaminated soil whereas no significant effect could be observed on the highly contaminated soil (Table 1). Similarly, Wieshammer et al. (2007) reported clear annual increases of leaf biomass production of the willows but marginal changes for the herbaceous *A. halleri*. *Salix dasyclados* co-cropped with *T. caerulescens* was able to grow in the highly contaminated soil but showed significant yield reduction and other toxicity symptoms such as wilting and strong necrosis (Wieshammer et al. 2007).

Hernández-Allica et al. (2006) found similar total shoot production of *T. caerulescens* plants grown on moderately versus highly contaminated soils (Cd, Pb, and Zn) whereas Whiting et al. (2001a) observed increased production of total above-

Table 1. Average concentration of trace elements in aboveground biomass (mg/kg); the total concentration was calculated as weighed mean of all their concentration in the individual organs

Treatment	As			Cd			Pb			Zn		
	total	leaves	twigs	total	leaves	twigs	total	leaves	twigs	total	leaves	twigs
Moderately contaminated soil												
Thlaspi	5.30^{a}	5.30	–	271^{a}	271	–	57.6^{a}	57.6	–	1501^{a}	1501	–
Thlaspi plus	0.880^{b}	0.88	–	237^{a}	237	–	7.33^{b}	7.33	–	1894^{b}	1894	–
Salix	0.964^{b}	1.20	0.77	41.1^{b}	57.5	28.7	10.9^{b}	5.67	15.4	591^{c}	900	362
Salix plus	1.14^{b}	1.39	0.91	34.3^{b}	48.3	23.2	9.00^{b}	6.46	12.4	621^{c}	905	350
Highly contaminated soil												
Thlaspi	21.2^{a}	21.2	–	870^{a}	870	–	18.3^{a}	18.3	–	3890^{a}	3890	–
Thlaspi plus	14.0^{a}	14.0	–	623^{b}	623	–	5.90^{a}	5.90	–	4089^{a}	4089	–
Salix	18.6^{a}	17.4	14.2	60.6^{c}	60.3	47.9	157^{b}	165	92.9	4547^{a}	4972	3890
Salix plus	1.23^{b}	1.46	1.04	29.5^{c}	34.4	22.5	2.08^{a}	1.13	3.54	1604^{b}	2146	1076

ground biomass grown on soils with higher bioavailable zinc concentrations. We also determined a significant increase of shoot biomass yield on the highly contaminated soil compared to the moderately contaminated treatment.

None of the two plant species in our experiment showed visible symptoms of toxicity when grown on the moderately contaminated soil. *T. carulescens* grew vigorously also on the highly contaminated soil, which is in accordance with previous findings demonstrating the high metal tolerance of this metal hyperaccumulator species (Baker and Brooks 1989, Baker et al. 2000). In contrast, *S. dasyclados* planted separately on highly contaminated soil gradually died during the first vegetation period. Combined cropping improved the survival rate

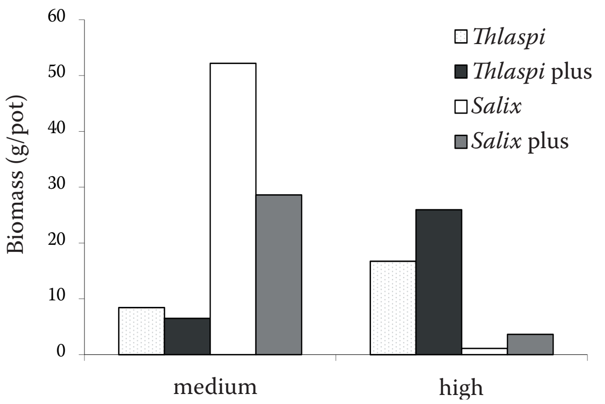

Figure 1. Average yield (g/pot) of total above-ground biomass of tested plant species

of *S. dasyclados* even though severe symptoms of toxicity such as strong necrosis, chlorosis and reduced biomass production were observed. In our study, this appears to be the most important benefit of combined cropping.

Metal concentrations in above-ground biomass. Mean concentrations of As, Cd, Pb, and Zn in total above-ground biomass were calculated. Combined cropping of both species caused decreased As, Cd, and Pb concentrations in *T. caerulescens* shoots compared to separately planted treatment on both soils (Table 1). From the phytoextraction angle, As and Pb contents in both *T. caerulescens* and *S. dasyclados* above-ground biomass are negligible confirming thus our previous results (Fischerová et al. 2006, Tlustoš et al. 2007). In *T. caerulescens* shoots, total concentration of Zn increased significantly in the combined treatment on moderately contaminated soil (Table 1) whereas no significant changes in Zn concentrations in total above-ground biomass could be observed for *S. dasyclados*. On the contrary, the Cd concentration in *T. caerulescens* tended to decrease, suggesting antagonistic pattern of Cd and Zn uptake (Smilde et al. 1992).

Differences among different plant species even within one plant genus are already described and well known. Limited information was published concerning the behavior of intercropped plant species with different metal accumulation ability. No significant change of Zn concentrations in leaves of *T. arvense* cropped together with *T. caerulescens* was observed by Whiting et al. (2001b). Decreased trace Cd and Zn concentrations in the aerial bio-

Table 2. Average factor of bioaccumulation according to experimental treatments

Treatment	As			Cd			Pb			Zn		
	total	leaves	twigs	total	leaves	twigs	total	leaves	twigs	total	leaves	twigs
Moderately contaminated soil												
Thlaspi	0.189^{a}	0.189	–	49.7^{a}	49.7	–	0.060^{a}	0.060	–	5.38^{a}	5.38	–
Thlaspi plus	0.031^{b}	0.031	–	43.5^{a}	43.5	–	0.008^{b}	0.008	–	6.79^{b}	6.79	–
Salix	0.034^{b}	0.043	0.028	7.53^{b}	10.5	5.25	0.011^{b}	0.006	0.016	2.12^{c}	3.22	1.30
Salix plus	0.041^{b}	0.050	0.032	6.28^{b}	8.85	4.25	0.009^{b}	0.007	0.013	2.23^{c}	3.24	1.26
Highly contaminated soil												
Thlaspi	0.088^{a}	0.088	–	14.5^{a}	14.5	–	0.004^{a}	0.004	–	0.657^{a}	0.657	–
Thlaspi plus	0.058^{a}	0.058	–	10.4^{b}	10.4	–	0.001^{a}	0.001	–	0.691^{a}	0.691	–
Salix	0.077^{a}	0.072	0.059	1.01^{c}	1.01	0.799	0.034^{b}	0.036	0.020	0.768^{a}	0.840	0.657
Salix plus	0.005^{b}	0.006	0.004	0.492^{c}	0.574	0.374	0.0004^{a}	0.0002	0.001	0.271^{b}	0.363	0.182

mass of co-cropped *A. halleri* and *S. caprea* were also reported by Wieshammer et al. (2007). In contrast to these results, combined cropping of *T. caerulescens* and *Hordeum vulgare* caused a significant increase in Cd accumulation and a decrease in Zn concentration in barley biomass and higher concentration of heavy metals (Cd, Pb, and Zn) in the biomass of *T. caerulescens* (Gove et al. 2002).

We observed no significant effect of co-cropping on the overall Cd and Zn content in *T. caerulescens* and *S. dasyclados* aerial biomass on the moderately contaminated soil. On the highly contaminated soil, surviving willows in the combined cropping treatment showed significantly lower metal concentrations in twigs and leaves compared to the separate treatments. Decreased metal accumulation in *S. dasyclados* biomass can be explained by removal of toxic metals from by the hyperaccumulator, resulting in lower, less toxic metal concentrations in the shared rhizosphere. Evidently, the cropping system (separated or combined) significantly affected the uptake behaviour of the two species. Combined cropping apparently induced competition for phytoavailable nutrients and pollutants in shared rhizospheres with stronger effect on the hyperaccumulator plant when grown on moderately contaminated soil. Excess uptake of toxic metals in the hyperaccumulator probably caused a significant decrease of phytoavailable metal concentrations in shared rhizosphere when plants were grown in extremely contaminated soil, which resulted in a better survival of the co-cropped willow.

Factor of bioaccumulation and remediation efficiency of combined cropping. The factor of bioaccumulation (FB) is usually calculated as a ratio of element concentration in plant biomass and total concentration in tested soil (Baker 1987, McGrath and Zhao 2003, Zhao et al. 2003). The FB indicates the plant's ability to uptake and accumulate an element in its total above-ground biomass in relation to the tested medium and typically varies between 0.01 to 0.1 for As, Pb, and Cr, between 0.1 to 1.0 for Cu and Ni, and between 1.0 to 10 for Cd and Zn for plants growing in soil containing background concentrations of these elements (Sauerbeck 1985, Brandstetter et al. 2000, Adriano 2001). In our case the changes in FBs of investigated treatments almost reflected differences in biomass concentration of elements (Table 2). We noted a statistically significant decrease of As and Pb transfer in leaves of *T. caerulescens* on moderately contaminated soil and Cd on highly contaminated soil. On the other side, we calculated significantly increased bioaccumulation of Zn in leaves of *T. caerulescens* that were co-cropped with *S. dasyclados* in moderately contaminated soil compared to separately grown plants. Furthermore, we determined significantly lower transport and accumulation of all studied elements in leaves and twigs of *S. dasyclados* co-cropped with *T. caerulescens* relative to the separate treatment.

The FBs were significantly lower in highly contaminated soil compared to moderately contaminated treatment although no symptoms of phytotoxicity were observed for *T. caerulescens* plants. We expected enhanced hyperaccumulation capacity in soil with higher contamination based on our previous investigation (Hernández-Allica

Table 3. Average factor of remediation (percentage per year) according to experimental treatments

Treatment	As	Cd	Pb	Zn
Moderately contaminated soil				
Thlaspi	0.028[a]	7.55[ab]	0.010[ab]	0.963[a]
Thlaspi plus	0.004[b]	5.02[ab]	0.001[c]	0.880[a]
Salix	0.033[a]	8.10[a]	0.012[a]	2.24[b]
Salix plus	0.023[a]	3.63[b]	0.005[bc]	1.25[a]
Combined	0.026[a]	8.65[a]	0.006[abc]	2.13[b]
Highly contaminated soil				
Thlaspi	0.037[a]	4.22[a]	0.001[a]	0.217[a]
Thlaspi plus	0.028[a]	4.88[a]	0.0007[a]	0.336[b]
Salix	0.001[b]	0.015[b]	0.0005[ab]	0.013[c]
Salix plus	0.0004[b]	0.032[b]	0.00003[b]	0.017[c]
Combined	0.028[a]	4.91[a]	0.0007[a]	0.353[b]

et al. 2006, McGrath et al. 2006). Although *Thlaspi* plants accumulated significantly more As, Cd and Zn in highly contaminated soil, they showed significantly lower BFs compared to plants grown in moderately contaminated soil. This is in line with the hyperaccumulator concept of Baker and Brooks suggesting a steep increase of metal accumulation in the lower range of soil concentrations levelling of when the concentration in soil becomes very high. The investigated *T. caerulescens* provenience from Ganges is known to have a larger phytoextraction potential for Cd than for Zn (Keller et al. 2003, Hernández-Allica et al. 2006, McGrath et al. 2006). However, *T. caerulescens* accumulated As and Pb only to levels commonly found in non-accumulator species.

The efficiency of the phytoextraction process was calculated as factor of remediation (FR) from the amount of metal removed by the plant and the total content in the rooted soil volume as described by Vysloužilová et al. (2003a) and Zhao et al. (2003). Co-cropping decreased the remediation efficiency of the individual species except for Cd and Zn extraction from the highly-contaminated soil (Table 3). On the moderately contaminated soil, the phytoextraction efficiency of the overall co-cropping system (i.e., of both species together) was comparable to the individual treatments. Separately planted willows gradually died on the highly contaminated soil whereas removal of toxic metals by *Thlaspi* roots probably decreased their phytoavailable concentration in the shared rhizosphere and thus improved *Salix* growth and survival. In the soil-plant system used in our experiment, the decreased metal toxicity in the rhizosphere of *S. dasyclados* may be considered as the main benefit of co-cropping for enhancing the phytoextraction process.

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