# Removal of As, Cd, Pb, and Zn from contaminated soil by high biomass producing plants

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**ABSTRACT**

The uptake of As, Cd, Pb, and Zn and potential phytoremediation efficiency of five high biomass producing crops, white sweetclover (*Melilotus alba* L.), red clover (*Trifolium pratense* L.), curled mallow (*Malva verticillata* L.), safflower (*Carthamus tinctorius* L.) and hemp (*Cannabis sativa* L.) commonly used as grazing and/or energy crops was evaluated in both pot and field experiments at soils with different level of element contamination. In pot experiment the highest phytoremediation efficiency was demonstrated by *C. tinctorius* where 4.8% of Cd and 1.1% of Zn were removed from the moderately contaminated soil in one vegetation period when repeated harvest of aboveground biomass was performed. The removal of As and Pb was negligible for all the investigated plant species. At the highest element content in soil inhibition of plant growth due to the element phytotoxicity to plants was reported in most of cases. In the precise field experiment lower phytoremediation efficiency (biennial phytoremediation factors did not exceed 0.2% for Pb and Zn and 0.3% for Cd for *C. tinctorius*) was determined but yield suppress was not observed. Thus, free space for manipulation with element mobility in soil to increase element uptake by plants remains for further research.

**Keywords:** high biomass producing crops; arsenic; cadmium; lead; zinc; uptake; contaminated soil

Natural sensitivity or tolerance of plants to accumulate metals is substantially affected by plant species and genotypes. In this context, plants can be divided into three groups. (i) Excluders – plants insensitive for uptake and accumulation of potentially toxic elements. Mainly monocotyledon grasses belong into this group (sudangrass, fescue) (ii) Indicators – majority of agricultural plants whose content of elements more or less linearly responds to increasing available content of trace elements in soil (wheat, oat, maize). (iii) Accumulators – plants accumulating higher contents of elements in their tissues according to their increase in the soil. Accumulators include many species of *Brassicaceae* (mustard) and *Compositae* (lettuce, spinach) families. Hyperaccumulators are plants commonly grown on metalliferous soils and able to complete their life cycle without any symptoms of metal phytotoxicity (Baker et al. 2000). These plants can even prosper on contaminated soils and accumulate extremely high contents of trace elements in aboveground biomass (Baker 1987).

Phytoremediation, the use of plants to remedy contaminated soils, is an emerging technology requiring a greater understanding of the underlying mechanisms for its optimization (McGrath and Zhao 2003). Number of plant species was already tested because of their ability to accumulate potentially toxic elements to high extent in the above ground biomass. Recently, two strategies have been tested within the phytoremediation technologies. The application of hyperaccumulating plants (such as *Thlaspi caerulescens* or *Alyssum bertolonii*) producing a relatively low amount of aboveground biomass but accumulating high amounts of one

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or more elements in this biomass represents the first approach. The alternative approach comprises an application of high biomass producing plants characterized by lower ability to accumulate risk elements where total uptake of elements is comparable to hyperaccumulating plants due to high yield of aboveground biomass. In this context, zinc accumulating *Brassica* spp. seems to be more effective for removing this element from the polluted soil compared to zinc hyperaccumulator *Thlaspi caerulescens* producing one order lower amount of shoot biomass (Ebbs et al. 1997). The plant species tolerant to high element contents in soil followed by an intensive uptake of these elements belong in most cases into families *Caryophyllaceae*, *Brassicaceae*, *Cyperaceae*, *Poaceae*, *Fabaceae,* and *Chenopodiaceae* (Kabata-Pendias and Pendias 2001). Also EPA recommendations (EPA 2000) include metal accumulator plants such as maize (*Zea mays*), sorghum (*Sorghum bicolor*), and lucerne (*Medicago sativa*) among plants able to remove a greater mass of metals but more research is necessary to verify it.

The present results suggest that fast-growing trees and especially willows have a very promising potential for phytoremediation use because of their large biomass and good ability to accumulate risk elements, especially cadmium and zinc (Pulford and Watson 2002). Among herbaceous species, tobacco (*Nicotiana tabacum* L.) accumulating predominantly Cd and Cu, and maize (*Zea mays* L.) are discussed as effective plants because of high production of aboveground biomass with a relatively high content of elements. Comparing to *N. tabacum*, *Z. mays* is able to remove more Zn (Wenger et al. 2002). For phytoextraction purpose, however, the effectiveness of maize still seems to be insufficient (Keller et al. 2003, Schmidt 2003). Cadmium and lead are retained predominantly in roots of maize demonstrating limited mobility of elements within plant (Bricker et al. 2001). Relatively high contents of Pb in aboveground biomass showed Indian mustard [*Brassica juncea* (L.) Czern.], rye grass (*Lolium perene* L.), sunflower (*Helianthus anuus* L.) or smallwing sedge (*Carex microptera* Mack.) (Klassen et al. 2000). Moreover, sunflower demonstrated a good ability for phytoextraction of copper. Relatively high contents of As and Zn were determined in biomass of amaranth (*Amaranthus hybridus* L.) accumulating the elements in order leaves > stems > roots; this plant however seems to be insufficient and questionable for practical use for phytoremediation as well (Jonnalagadda and Nenzou 1997). The phytoremediation efficiency of some plant species, such as *Brassica juncea* for removal of Pb, *Thlaspi caerulescens* for Cd and Zn, *Amaranthus retroflexus* for Cs, and *Helianthus annus* for Cs and Sr, was also tested in field conditions (Saxena et al. 1999).

In our investigation, the uptake of As, Cd, Pb, and Zn by five high biomass producing crops (coming from the families *Asteraceae, Fabaceae, Malvaceae*, and *Cannabaceae*) commonly used as grazing and/or energy crops was determined and evaluated in both pot and field experiments at soils with different level of element contamination to assess the potential suitability of these plants for phytoremediation purpose.

## MATERIAL AND METHODS

### Pot experiments

White sweetclover (*Melilotus alba* L. – var. Krajová), red clover (*Trifolium pratense* L. – var. Beskyd), curled mallow (*Malva verticillata* L. – var. Dolina), and safflower (*Carthamus tinctorius* L. – var. Sabina) were cultivated in a preliminary pot experiment for one vegetation period in three soils differing in total content of risk elements and physicochemical characteristics (Table 1). Cambisol (Soil B) with moderate contamination mainly caused by the atmospheric emissions from the smelter, and unpolluted Chernozem (Soil A) were used. An extremely contaminated soil C was soil A spiked by 100 mg/kg As, 40 mg/kg Cd, and 2000 mg/kg Pb as aqueous solutions of $Na_2HAsO_4$, $CdCl_2$, and $Pb(CH_3COO)_2$, respectively. The vicinity of the smelter belongs to the most damaged areas in the Czech Republic. The main source of Pb contamination of soil B was the atmospheric deposition of trace elements by galenite mining followed by ore smelting and lead processing. Mining and metallurgical activities in this area led to the enhancement of As, Cd and Zn contents in soil, due to the high content of trace elements in parent rock. The accumulation of trace elements in the soil and plants has a negative influence on the quality of agricultural production (Šichorová et al. 2004). Subsequently, *C. tinctorius* and *T. pratense* were planted at the abovementioned soils in the next vegetation period (pot experiment I) and in the third vegetation period (pot experiment II) *C. tinctorius* followed *T. pratense* and *M. alba* followed *C. tinctorius* from the previous vegetation period.

Table 1. Basic characteristics and nutrient status of experimental soils, and total element contents in experimental soils used in pot experiments

| Soil | Soil type | pH | $C_{org}$ (%) | Ca* | K* | Mg* | P* | As | Cd | Pb | Zn |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | mg/kg | | | | | | | |
| A | Chernozem | 6.8 | 1.8 | 5717 | 219 | 202 | 132 | 18.0 | 0.416 | 29.3 | 87.1 |
| B | Cambisol | 6.1 | 2.1 | 2242 | 67.9 | 42.1 | 36.8 | 37.5 | 4.73 | 1158 | 180 |
| C | Chernozem | 6.8 | 1.8 | 5717 | 219 | 202 | 132 | 118 | 40.4 | 2029 | 87.1 |

*mobile portions of nutrients determined in extracts obtained by Mehlich III extraction procedure (Zbíral 2000)

Plants were cultivated in 6-litre plastic pots with 5 kg of air-dried soil and there were made three replicates for each treatment in the case of preliminary experiment and experiment II and six replicates in the case of experiment I. Soil moisture was regularly controlled and kept at 60% of MWHC. The aboveground biomass of plants was harvested in the end of vegetation period in the case of preliminary pot experiment and experiment II, while *C. tinctorius* was harvested twice and *T. pratense* even three times during the vegetation period of pot experiment I and II, where total element uptake was calculated for an evaluation of the experiments. After harvest, the aboveground biomass was gently washed with deionised water, checked for fresh and dry biomass, ground and analyzed.

## Field experiment

The phytoremediation efficiency of hemp (*Cannabis sativa* L. – var. Juso 11), curled mallow (*M. verticillata)*, white sweetclover (*M. alba*), and safflower (*C. tinctorius*) were investigated in precise field experiment (plot size 5 $m^2$) during two vegetation periods. The element contents in the soil were modified by addition of aqueous solutions of following salts: $Cd(NO_3)_2.4\ H_2O$, $Pb(CH_3COO)_2.2\ H_2O$, $Zn(NO_3)_2.6\ H_2O$, and $Zn(CHCOO)_2.2\ H_2O$ before sowing in amounts leading to three levels of element contents in soils:

Level 1 – control without addition of elements resulting in total contents 1.6 mg/kg Cd, 60 mg/kg Pb, and 95 mg/kg Zn.

Level 2 –final element content in soil resulting in total contents 10 mg/kg Cd, 1000 mg/kg Pb, and 400 mg/kg Zn.

Level 3 – final element content in soil resulting in total contents 40 mg/kg Cd, 4000 mg/kg Pb, and 1000 mg/kg Zn.

Four samples of aboveground biomass representing plant material from 0.25 $m^2$ were collected from each plot, gently washed by deionised water, checked for fresh and dry biomass, ground and analyzed.

## Analytical procedures

The plant samples were decomposed by a dry ashing procedure as follows: An aliquot (~1 g) of the dried and powdered plant biomass was weighed to 1 mg into a borosilicate glass test-tube and decomposed in a mixture of oxidizing gases ($O_2$ + $O_3$ + $NO_x$) at 400°C for 10 hours in Dry Mode Mineralizer Apion (Tessek, Czech Republic). The ash was dissolved in 20 ml of 1.5% $HNO_3$ (electronic grade purity, Analytika Ltd., Czech Republic) and kept in glass tubes until the analysis (Miholová et al. 1993). Soil samples were taken before the start of the experiment from the bulk, air dried at 20°C, ground in a mortar and passed through a 2-mm plastic sieve. Total element concentrations in soil were determined in digests obtained by two-step decomposition as follows: 0.5 of sample was decomposed by dry ashing in a mixture of oxidizing gases ($O_2$ + $O_3$ + $NO_x$) in Apion Dry Mode Mineralizer (Tessek, CZ) at 400°C for 10 h; the ash was then decomposed in a mixture of $HNO_3$ + HF, evaporated to dryness at 160°C and dissolved in diluted a*qua regia* (Száková et al. 1999).

Element concentrations in the digests were determined by atomic absorption spectrometry (AAS, VARIAN SpectrAA-400, Varian, Australia) in flame (Cd, Pb, Zn), and flameless (Pb) measurement modes, and by inductively coupled plasma optical emission spectrometry (ICP-OES, VARIAN VistaPro, Varian, Australia). Arsenic was determined by a continuous hydride generation technique using a Varian SpectrAA-300 (Australia) atomic absorption spectrometer equipped by

Table 2. Element contents in aboveground biomass of the individual plant species according to the level of total element contents in soils – preliminary pot experiment; $n = 3$, the data marked by the same letter did not significantly differ at $\alpha = 0.05$ within individual columns of data and individual soil

| Soil | Species | As | Cd | Pb | Zn |
|---|---|---|---|---|---|
| | | mg/kg | | | |
| A | *Carthamus tinctorius* | 0.599 ± 0.058$^{c}$ | 0.551 ± 0.068$^{c}$ | 0.492 ± 0.049$^{a, b}$ | 29.8 ± 3.1$^{b}$ |
| | *Melilotus alba* | 0.057 ± 0.007$^{a}$ | 0.152 ± 0.036$^{a, b}$ | 0.257 ± 0.072$^{a}$ | 11.9 ± 1.4$^{a}$ |
| | *Malva verticillata* | 0.192 ± 0.028$^{b}$ | 0.368 ± 0.179$^{b, c}$ | 0.883 ± 0.190$^{b}$ | 38.9 ± 5.7$^{c}$ |
| | *Trifolium pratense* | 0.166 ± 0.022$^{b}$ | 0.084 ± 0.026$^{a}$ | 0.870 ± 0.290$^{b}$ | 33.9 ± 4.3$^{b, c}$ |
| B | *Carthamus tinctorius* | 1.30 ± 0.05$^{c}$ | 13.9 ± 2.7$^{c}$ | 12.2 ± 3.6$^{a}$ | 65.2 ± 2.4$^{c}$ |
| | *Melilotus alba* | 0.529 ± 0.015$^{a}$ | 3.62 ± 0.54$^{a, b}$ | 23.4 ± 0.3$^{b}$ | 26.4 ± 0.7$^{a}$ |
| | *Malva verticillata* | 0.920 ± 0.099$^{b}$ | 5.07 ± 0.88$^{b}$ | 13.2 ± 1.1$^{a}$ | 43.8 ± 4.9$^{b}$ |
| | *Trifolium pratense* | 0.774 ± 0.194$^{a, b}$ | 0.766 ± 0.056$^{a}$ | 9.62 ± 1.02$^{a}$ | 38.7 ± 9.7$^{a, b}$ |
| C | *Carthamus tinctorius* | 0.986 ± 0.116$^{b}$ | 23.9 ± 2.1$^{d}$ | 15.8 ± 1.8$^{b, c}$ | 49.2 ± 5.7$^{b}$ |
| | *Melilotus alba* | 0.282 ± 0.036$^{a}$ | 4.54 ± 0.68$^{b}$ | 10.1 ± 1.3$^{a, b}$ | 14.1 ± 1.8$^{a}$ |
| | *Malva verticillata* | 1.41 ± 0.31$^{c}$ | 13.9 ± 1.5$^{c}$ | 26.6 ± 9.1$^{c}$ | 70.3 ± 15.8$^{c}$ |
| | *Trifolium pratense* | 0.572 ± 0.101$^{a}$ | 0.948 ± 0.177$^{a}$ | 4.60 ± 0.99$^{a}$ | 26.2 ± 2.2$^{a}$ |

hydride generator VGA-76. A mixture of potassium iodide and ascorbic acid was used for pre – reduction of the sample and the extract was acidified by HCl before measurement. Certified reference materials RM NCS DC 73350 Poplar leaves and RM 7001 Light Sandy Soil were applied for quality assurance of analytical data. Statgraphics Plus 5.0 for Windows (Manugistics 1997) was used to perform statistical evaluation of the data where analysis of variance was applied for the evaluation of the data.

## RESULTS AND DISCUSSION

The yield of dry aboveground biomass of experimental plants in **preliminary pot experiment** showed the effect of soil properties as well as of the contamination level. At soil A the yield varied between 17.3 g (*M. verticillata*) and 32.6 g (*M. alba*), at soil B between 22.7 g (*M. verticillata*) and 118 g (*M. alba*), and at soil C between 9.7 g (*M. verticillata*) and 52.4 g (*M. alba*). Except *M. alba* the results showed comparable biomass yield at uncontaminated soil A and moderately contaminated soil B, and a significant suppress of biomass yield at contaminated soil C. Comparing the yields of plant biomass in the pot experiment II (see below) the yields of *M. alba* at the soils B and C were surprisingly high in the preliminary pot experiment where the suppress of plant yield was observed at soil C compared to soil B, as well. Within the preliminary pot experiment, the results concerning *M. alba* cannot be compared among individual soils. The element contents in plant biomass (Table 2) showed the lowest values of As, Pb, and Zn in *M. alba,* and Cd in *T. pratense* at uncontaminated soil A. Also Gray et al. (1999) confirmed a relatively low capability of *T. repens* compared to other crops such as carrot (*Daucus carota*), lettuce (*Lactuca sativa*), or lucerne (*Medicago sativa*) to accumulate elements. At soils B and C the lowest ability of *T. pratense* to accumulate cadmium, and of *M. alba* to accumulate arsenic and zinc was confirmed but in the case of lead the response of individual plant species was different in different soils. Expectably, the element contents in plants differed according to the contamination level in the soil but the differences between the moderately contaminated soil B and extremely contaminated soil C were not significant; the values were even lower at soil C in some cases such as As, Pb, and Zn in *M. alba*. Among the plant species, only cadmium contents showed higher differences representing more than one order of magnitude at one soil. The total element uptake by plants reflecting differences in plant yield (Table 3) confirmed the importance of high production of aboveground biomass for an effective removal of elements from the soil. The highest element uptake was observed at the moderately contaminated soil B where the high contents of element

Table 3. Total element uptake by aboveground biomass of the individual plant species from the pot according to the level of total element contents in soils – preliminary pot experiment; $n = 3$, the data marked by the same letter did not significantly differ at $\alpha = 0.05$ within individual columns of data and individual soil

| Soil | Species | As | Cd | Pb | Zn |
|---|---|---|---|---|---|
| | | μg | | | |
| A | *Carthamus tinctorius* | 14.4 ± 2$^{c}$ | 13.1 ± 0.6$^{c}$ | 12.0 ± 2.6$^{a}$ | 719 ± 99$^{b}$ |
| | *Melilotus alba* | 1.8 ± 0.1$^{a}$ | 5.0 ± 1.6$^{b}$ | 8.1 ± 1.7$^{a}$ | 371 ± 16$^{a}$ |
| | *Malva verticillata* | 3.2 ± 0.6$^{a, b}$ | 5.5 ± 0.7$^{b}$ | 14.5 ± 2.9$^{a}$ | 604 ± 102$^{b}$ |
| | *Trifolium pratense* | 5.4 ± 0.4$^{b}$ | 2.7 ± 0.7$^{a}$ | 27.7 ± 7.3$^{b}$ | 1100 ± 70$^{c}$ |
| B | *Carthamus tinctorius* | 42.7 ± 2.1$^{b}$ | 460 ± 105$^{b}$ | 403 ± 135$^{a}$ | 2200 ± 30$^{b}$ |
| | *Melilotus alba* | 62.6 ± 2.2$^{c}$ | 428 ± 66$^{b}$ | 2760 ± 50$^{b}$ | 3200 ± 70$^{c}$ |
| | *Malva verticillata* | 20.9 ± 2.9$^{a}$ | 116 ± 27$^{a}$ | 299 ± 39$^{a}$ | 924 ± 64$^{a}$ |
| | *Trifolium pratense* | 23.9 ± 6.9$^{a}$ | 23.3 ± 0.6$^{a}$ | 296 ± 45$^{a}$ | 1370 ± 390$^{a}$ |
| C | *Carthamus tinctorius* | 9.1 ± 1.8$^{a}$ | 222 ± 52$^{c}$ | 155 ± 64$^{a, b}$ | 434 ± 97$^{a, b}$ |
| | *Melilotus alba* | 15.2 ± 1.7$^{b}$ | 245 ± 34$^{c}$ | 543 ± 60$^{c}$ | 712 ± 21$^{c}$ |
| | *Malva verticillata* | 13.3 ± 2.1$^{b}$ | 132 ± 7$^{b}$ | 246 ± 66$^{b}$ | 546 ± 63$^{b}$ |
| | *Trifolium pratense* | 7.1 ± 1.0$^{a}$ | 11.8 ± 1.7$^{a}$ | 57.2 ± 10.0$^{a}$ | 351 ± 19$^{a}$ |

were combined with high yield of plant biomass, unaffected by elevated total content of elements in soil. Remediation factors given as percentage of element content removed by plants from defined amount of soil did not exceed 0.1% in the case of arsenic and lead and 0.5% in the case of zinc. Therefore, phytoremediation efficiency of investigated plants was negligible. Slightly higher removal was observed in the case of cadmium where the highest remediation factor (1.9%) was calculated for *C. tinctorius* at soil B. Evidently, the plants tested in our experiment confirmed a response of element contents in plant biomass on increasing element content in soil without symptoms of element phytotoxicity in the case of moderately contaminated soil. Differences in behaviour of individual plant species are also evident. However, the phytoremediation efficiency of these plants was not sufficient for practical use of these plants in phytoremediation technologies.

In the **pot experiment I**, the possible increase of element removal by aboveground biomass of *C. tinctorius* and *T. pratense* from the experimental soils by repeated harvest of plant biomass during one vegetation period was investigated; *C. tinctorius* was harvested twice and *T. pratense* three times. While total yield of dry biomass of *T. pratense* was very similar regardless of soil type and/or soil contamination level and varied between 60.1 g and 63.4 g, suggesting thus a relative resistance of *T. pratense* to high contents of elements in soil, the yield of *C. tinctorius* was 29.4 g, 44.4 g, and 11.3 g at soils A, B, and C, respectively, confirming the negative effect of high level of soil contamination. Weighed means of element contents in plant biomass are summarized in Table 4. Cadmium contents were higher in *C. tinctorius*; on the contrary, lead contents were higher in *T. pratense* in all three experimental soils. Concerning arsenic and zinc, different pattern was observed according to individual soil types. At soil B no differences were observed between element contents in both experimental plants while at soils A and C lower As and higher Zn contents were determined in *T. pratense*. The total element uptake by experimental plants (Table 5) was affected by lower yield of *C. tinctorius*, especially at the soil C. Figure 1 presents percentage of elements removed from soil by individual harvests of plant biomass. These results suggested that *T. pratense* is able to accumulate elements more intensively at the beginning of the vegetation period and earlier harvest of the biomass is reasonable. In the case of *C. tinctorius* the elements were distributed almost equally between both harvests of biomass. The remediation factors of As and Pb did not exceed 0.2% and confirmed the poor ability of tested plants to take up these elements. In the case of zinc the remediation factors varied between 0.12 (soil C) and 0.66% (soil B) and between 1.1 (soil B) and 1.6% (soil A) for *C. tinctorius* and *T. pratense*, respectively, demonstrating the importance of biomass

Table 4. Element contents in aboveground biomass of the individual plant species according to the level of total element contents in soils – pot experiments I and II; $n = 6$ (experiment I), $n = 3$ (experiment II), the data marked by the same letter did not significantly differ at $\alpha = 0.05$ within individual columns of data and individual soil

| Soil | Species | As | Cd | Pb | Zn |
|---|---|---|---|---|---|
| | | mg/kg | | | |
| **Pot experiment I** | | | | | |
| A | *Carthamus tinctorius* | 1.79 ± 0.45$^b$ | 1.06 ± 0.21$^b$ | 1.38 ± 0.58$^a$ | 58.3 ± 23.9$^a$ |
| | *Trifolium pratense* | 0.755 ± 0.224$^a$ | 0.293 ± 0.037$^a$ | 3.09 ± 0.95$^b$ | 108 ± 5$^b$ |
| B | *Carthamus tinctorius* | 1.77 ± 0.63$^a$ | 25.4 ± 5.9$^b$ | 42.1 ± 11.5$^b$ | 158 ± 6$^a$ |
| | *Trifolium pratense* | 1.96 ± 0.42$^a$ | 4.15 ± 0.51$^a$ | 26.9 ± 2.5$^a$ | 157 ± 10$^a$ |
| C | *Carthamus tinctorius* | 17.7 ± 4.4$^a$ | 42.5 ± 5.5$^b$ | 44.4 ± 12.8$^a$ | 44.7 ± 4.2$^a$ |
| | *Trifolium pratense* | 10.4 ± 1.7$^a$ | 6.94 ± 0.34$^a$ | 28.2 ± 3.5$^a$ | 101 ± 6.0$^b$ |
| **Pot experiment II** | | | | | |
| A | *Carthamus tinctorius* | 0.458 ± 0.122$^a$ | 1.33 ± 0.69$^b$ | 2.96 ± 1.67$^a$ | 51.3 ± 7.8$^b$ |
| | *Melilotus alba* | 0.321 ± 0.079$^a$ | 0.226 ± 0.019$^a$ | 1.52 ± 0.16$^a$ | 17.2 ± 0.8$^a$ |
| B | *Carthamus tinctorius* | 1.05 ± 0.11$^a$ | 7.54 ± 1.06$^a$ | 15.2 ± 2.7$^a$ | 59.8 ± 3.8$^a$ |
| | *Melilotus alba* | 3.82 ± 0.42$^b$ | 10.4 ± 1.7$^a$ | 77.3 ± 10.9$^b$ | 67.5 ± 8.9$^a$ |
| C | *Carthamus tinctorius* | 3.57 ± 0.24$^a$ | 67.5 ± 10.6$^b$ | 48.1 ± 3.8$^b$ | 125 ± 26$^b$ |
| | *Melilotus alba* | 6.50 ± 0.69$^a$ | 6.06 ± 0.04$^a$ | 22.6 ± 0.4$^a$ | 17.6 ± 2.5$^a$ |

yield for final efficiency of phytoremediation procedure. The ability of *C. tinctorius* to accumulate cadmium in aboveground biomass resulted in the highest phytoremediation factors 1.5% at soil A and 4.8% at soil B while phytoremediation factors of *T. pratense* were 0.9 and 1.1% at soils A and B,

Table 5. Total element uptake by aboveground biomass of the individual plant species from the pot according to the level of total element contents in soils – pot experiments I and II; $n = 6$ (experiment I), $n = 3$ (experiment II), the data marked by the same letter did not significantly differ at $\alpha = 0.05$ within individual columns of data and individual soil

| Soil | Species | As | Cd | Pb | Zn |
|---|---|---|---|---|---|
| | | μg | | | |
| **Pot experiment I** | | | | | |
| A | *Carthamus tinctorius* | 52.0 ± 11.0$^a$ | 30.9 ± 5.3$^b$ | 40.3 ± 15.7$^a$ | 1620 ± 460$^a$ |
| | *Trifolium pratense* | 47.2 ± 12.9$^a$ | 18.7 ± 2.7$^a$ | 194 ± 52$^b$ | 6810 ± 600$^b$ |
| B | *Carthamus tinctorius* | 80.5 ± 32.2$^a$ | 1140 ± 310$^b$ | 1870 ± 580$^a$ | 5950 ± 2180$^a$ |
| | *Trifolium pratense* | 126 ± 31$^a$ | 258 ± 22$^a$ | 1680 ± 170$^a$ | 9870 ± 930$^b$ |
| C | *Carthamus tinctorius* | 165 ± 22$^a$ | 456 ± 85$^a$ | 433 ± 108$^a$ | 526 ± 211$^a$ |
| | *Trifolium pratense* | 623 ± 79$^b$ | 415 ± 28$^a$ | 1710 ± 270$^b$ | 6050 ± 120$^b$ |
| **Pot experiment II** | | | | | |
| A | *Carthamus tinctorius* | 15.1 ± 6.2$^a$ | 39.6 ± 14.3$^b$ | 71.0 ± 31.8$^a$ | 1610 ± 40$^b$ |
| | *Melilotus alba* | 7.7 ± 1.3$^a$ | 2.9 ± 0.3$^a$ | 17.3 ± 2.8$^a$ | 4260 ± 78$^a$ |
| B | *Carthamus tinctorius* | 44.5 ± 2.7$^a$ | 322 ± 51$^b$ | 560 ± 45$^a$ | 2550 ± 190$^b$ |
| | *Melilotus alba* | 69.9 ± 11.9$^b$ | 187 ± 16$^a$ | 1530 ± 70$^b$ | 1220 ± 90$^a$ |
| C | *Carthamus tinctorius* | 68.6 ± 12.7$^a$ | 1270 ± 120$^b$ | 1010 ± 250$^a$ | 2310 ± 110$^b$ |
| | *Melilotus alba* | 110 ± 8$^a$ | 102 ± 2$^a$ | 375 ± 11$^a$ | 295 ± 33$^a$ |

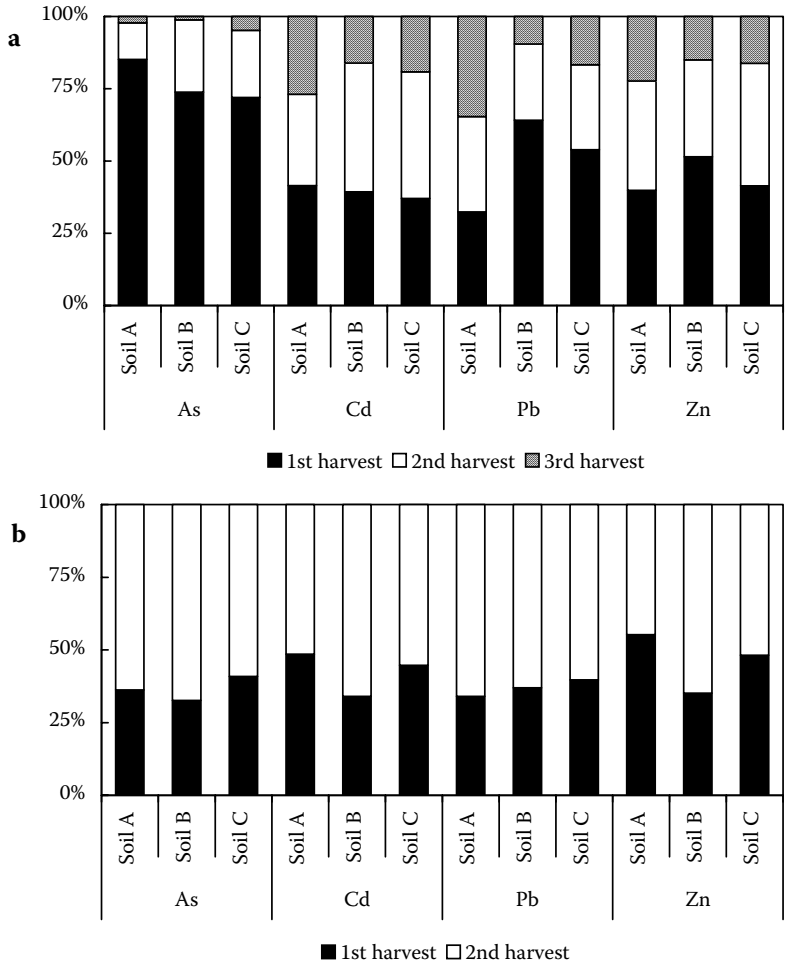


Figure 1. The distribution of uptake of investigated elements among individual harvests of aboveground biomass of *T. pratense* (a) and *C. tinctorius* (b) during one vegetation period – pot experiment I

respectively. At soil C where biomass yield was suppressed by the high element content in soil the phytoremediation factors were 0.2% for both plants. Evidently, the application of plants able to offer more than one harvest during vegetation period together with their ability to accumulate high element contents in aboveground biomass can lead to an increase of phytoremediation efficiency. In our case, however, the effectiveness of element removal from contaminated soil occurred only in the case of cadmium.

The subsequent effect on phytoremediation efficiency of high biomass producing plants was evaluated in **pot experiment II**. In this case, *C. tinctorius* followed *T. pratense* and *M. alba* followed *C. tinctorius* in the pots from the pot experiment I. The element contents in plant biomass are summarized in Table 4 and total element uptake by plant biomass in Table 5. Although some of the results differed from the experiment I, the data did not show an unambiguous trend to different pattern compared to the previous experiments. Higher ability of *C. tinctorius* to accumulate the investigated elements compared to *M. alba* was also confirmed as in the preliminary pot experiment in most of cases. The remediation factors of *M. alba* did not exceed 0.2% of zinc and in the case of cadmium varied between 0.1% (soil C) and 0.7% (soil B). *C. tinctorius* removed from 0.3 to 0.5% of zinc and from 0.6 to 1.9% of cadmium from experimental pots. The results suggested a low effect of plantation of high biomass producing crops on subsequent vegetation period of these plants where neither element contents nor phytoremediation efficiency were substantially changed.

The phytoremediation efficiency of investigated plants can be compared with the efficiency of willows cultivated in the same soils. Willows are generally characterized by high production of aboveground biomass and an intensive uptake of cadmium and zinc (Pulford and Watson 2002, Klang-Westin and Eriksson 2003). In our inves-

Table 6. The average yield of aboveground biomass and element contents in individual plant species according to the level of total element contents in soils – biennial field experiment; $n = 4$, the data marked by the same letter did not significantly differ at $\alpha = 0.05$ within individual columns of data and individual element levels in soil

| Soil | Species | Yield (kg/m$^2$) | Cd | Pb | Zn |
|---|---|---|---|---|---|
| | | | mg/kg | | |
| Level 1 | *Cannabis sativa* | 2.22 ± 0.61$^{b}$ | 0.113 ± 0.019$^{a}$ | 2.55 ± 0.75$^{a}$ | 20.0 ± 2.9$^{b}$ |
| | *Malva verticillata* | 1.41 ± 0.10$^{a}$ | 0.667 ± 0.134$^{c}$ | 5.85 ± 1.07$^{b}$ | 23.8 ± 3.5$^{b}$ |
| | *Melilotus alba* | 2.02 ± 0.29$^{a, b}$ | 0.129 ± 0.016$^{a}$ | 4.50 ± 0.45$^{a, b}$ | 14.8 ± 0.2$^{a}$ |
| | *Carthamus tinctorius* | 2.51 ± 0.52$^{b}$ | 0.481 ± 0.088$^{b}$ | 8.15 ± 2.18$^{c}$ | 22.2 ± 2.9$^{b}$ |
| Level 2 | *Cannabis sativa* | 2.52 ± 0.39$^{c}$ | 0.254 ± 0.019$^{a}$ | 6.71 ± 0.86$^{a}$ | 23.5 ± 0.8$^{b}$ |
| | *Malva verticillata* | 1.15 ± 0.04$^{a}$ | 1.79 ± 0.37$^{c}$ | 20.1 ± 5.0$^{c}$ | 36.3 ± 4.1$^{c}$ |
| | *Melilotus alba* | 1.46 ± 0.34$^{a}$ | 0.388 ± 0.047$^{a}$ | 7.12 ± 1.67$^{a}$ | 17.3 ± 2.4$^{a}$ |
| | *Carthamus tinctorius* | 1.93 ± 0.30$^{b}$ | 1.17 ± 0.23$^{b}$ | 14.6 ± 1.5$^{b}$ | 27.6 ± 2.8$^{b}$ |
| Level 3 | *Cannabis sativa* | 1.97 ± 0.25$^{a, b}$ | 0.547 ± 0.028$^{a}$ | 8.70 ± 0.62$^{a}$ | 24.0 ± 2.1$^{b}$ |
| | *Malva verticillata* | 1.38 ± 0.21$^{a}$ | 2.77 ± 0.40$^{c}$ | 18.2 ± 3.8$^{b}$ | 35.5 ± 5.3$^{c}$ |
| | *Melilotus alba* | 2.14 ± 0.54$^{b}$ | 0.402 ± 0.143$^{a}$ | 8.68 ± 2.36$^{a}$ | 17.2 ± 3.9$^{a}$ |
| | *Carthamus tinctorius* | 3.38 ± 0.62$^{c}$ | 2.03 ± 0.27$^{b}$ | 36.4 ± 8.4$^{c}$ | 31.1 ± 4.0$^{c}$ |

tigation, the cadmium concentrations in leaves and twigs of willows after two vegetation periods as dependent on willow clone varied between 9.6 and 113 mg/kg in leaves, and 11.4 and 50.2 mg/kg in twigs and the zinc contents between 117 mg/kg and 222 mg/kg in leaves and 99 mg/kg and 222 mg/kg in twigs at soil B. Comparing the remediation factors, a reasonable phytoextraction potential of willows was obtained for cadmium and zinc at this soil after three vegetation periods where aboveground biomass released about 30% and 5% of total element content, respectively. Among the investigated clones, S-150 *S.* × *smithiana* and S-391 *S.* × *rubens* demonstrated the highest phytoextraction effect for Cd and Zn (Vysloužilová et al. 2003, Száková et al. 2004). Hyperaccumulators *Arabidopsis halleri* and *Thlaspi caerulescens* were able to accumulate 82.3 and 271 mg/kg Cd and 2746 and 1500 mg/kg Zn, respectively in the pot experiment at soil B representing annual remediation factor 4.8 and 7.6% for Cd and 2.9 and 1.0% for Zn, respectively (Fischerová et al. 2006). Evidently, the high biomass producing crops were able to reach remediation factors of Cd and Zn comparable to hyperaccumulating plants at moderately contaminated soil where plant growth is not inhibited by high content of elements in soil and the biomass is harvested repeatedly during the vegetation period. Fast growing trees such as willows however demonstrated the highest phytoremediation efficiency for Cd and Zn because of very high biomass production and/or high accumulation of these elements in harvestable parts.

For a practical application of plants in phytoremediation technologies the verification of the results in **field experiment** is necessary. In our experiment the experimental plants demonstrated a different pattern in field conditions compared to the pot trials. The plant yield was not affected by contamination level (Table 6) of the topsoil and the differences in biomass yield among the experimental plants were lower compared to the pot experiments. Evidently, the limited volume of pots and a well developed contact of all roots with the whole amount of the soil support the more significant manifestation of experimental factors. Regardless of the investigated element, the element contents in *C. sativa* and *M. alba* were lower compared to *C. tinctorius* and *M. verticillata*, where a higher level of lead was determined in *C. tinctorius* and of cadmium and zinc in *M. verticillata* (Table 6). Except for zinc the addition of contaminating elements to the soil led to the increase of element contents in plants. However, the differences in element contents in plant biomass at contamination levels 2 and 3 did not correspond to the interval between these levels, suggesting high immobilization rate of element salt solutions added to the soil. Because the differences in biomass yield were relatively low, the total element uptake by harvested plants reflected the ability of individual plant species to accumulate

the investigated elements (Table 7). In most of cases, the total element uptake by plants decreased in order: *C. tinctorius* > *M. verticillata* > *C. sativa* = *M. alba*. Distribution of element uptake by plants between individual vegetation periods (Figure 2) suggested a slightly higher element uptake in the first vegetation period at the soil contamination level 3 but the element mobility was not assessed by determination of mobile portions of elements. Most probably, the mobile forms of elements added to the soil were sorbed and/or bound to individual soil compartments. The differences in element uptake by *C. sativa* and *M. alba* in individual vegetation periods were caused by substantially higher yield of biomass in the second season. Phytoremediation factors calculated as an element removal from 1 $m^2$ of soil to the depth 30 cm did not exceed 0.2% for Pb and Zn and 0.3% for Cd. Evidently, the phytoextraction efficiency differs under field and laboratory conditions by unlimited volume of soil. Also Wieshammer et al. (2005) investigated cadmium uptake by *S. fragilis* and *S. caprea* in pot experiment and found that the values of Cd contents in leaves from pot experiment were higher compared to hydroponic study demonstrating the importance of experimental conditions for an evaluation of element accumulation capacity of plants for possible phytoremediation use. The phytoremediation efficiency in field conditions is likely to be lesser than when determined in laboratory conditions (Nanda Kumar et al. 1995) as confirm our results.

For a possible enhancement of element mobility in soil followed by an increasing element uptake by plants the application of chelating agents such as EDTA (ethylendiaminetetraacetic acid) is frequently discussed (Salt et al. 1998, Bricker et al. 2001, Piechalak et al. 2003) who described a significant increase of Cd and Pb mobility and/or plant uptake. In our pot experiment an inhibition of plant growth was observed at the highest total element level in soil, especially in the case of *C. tinctorius*, the plant showing the best phytoremediation efficiency for Cd at the moderately contaminated soil. Thus, a manipulation with element mobility in soil seems to be reasonable for more tolerant plant species as *T. pratense* in our investigation. However, the transfer of the experiences from the pot experiments to field conditions is the crucial point of sufficient phytoremediation technology. From this point of view the results of precise field experiment showing a lower phytoremediation efficiency but better tolerance of plants to increased element contents in the soil are more promising for further research. The application of chelating agents to increase mobility of elements where the leaching of elements and/or chelating agents from soil column to groundwater must be under control, together with repeated harvest of plant biomass during one vegetation period and optimised crop management can lead to the improvement of element removal from contaminated soil.

Table 7. Total uptake of elements by aboveground biomass of individual plant species according to the level of total element contents in soils – biennial field experiment; $n = 4$, the data marked by the same letter did not significantly differ at $\alpha = 0.05$ within individual columns of data and individual element levels in soil

| Soil | Species | Cd | Pb | Zn |
|---|---|---|---|---|
| | | | mg/$m^2$ | |
| Level 1 | *Cannabis sativa* | 0.248 ± 0.064$^{a}$ | 5.47 ± 1.37$^{a}$ | 44.9 ± 15.8$^{a, b}$ |
| | *Malva verticillata* | 0.932 ± 0.139$^{b}$ | 8.23 ± 1.41$^{a}$ | 33.6 ± 6.0$^{a}$ |
| | *Melilotus alba* | 0.258 ± 0.015$^{a}$ | 9.05 ± 1.08$^{a}$ | 30.0 ± 4.7$^{a}$ |
| | *Carthamus tinctorius* | 1.22 ± 0.39$^{b}$ | 20.4 ± 6.0$^{b}$ | 55.8 ± 13.2$^{b}$ |
| Level 2 | *Cannabis sativa* | 0.641 ± 0.123$^{a}$ | 17.0 ± 3.8$^{a, b}$ | 59.4 ± 10.9$^{c}$ |
| | *Malva verticillata* | 2.07 ± 0.50$^{b}$ | 23.2 ± 6.6$^{b, c}$ | 41.9 ± 6.1$^{b}$ |
| | *Melilotus alba* | 0.555 ± 0.103$^{a}$ | 10.3 ± 3.6$^{a}$ | 25.2 ± 7.4$^{a}$ |
| | *Carthamus tinctorius* | 2.27 ± 0.64$^{b}$ | 28.5 ± 6.9$^{c}$ | 53.7 ± 12.4$^{b, c}$ |
| Level 3 | *Cannabis sativa* | 1.08 ± 0.15$^{a}$ | 17.2 ± 3.1$^{a}$ | 47.1 ± 6.1$^{a}$ |
| | *Malva verticillata* | 3.74 ± 0.10$^{b}$ | 25.2 ± 7.8$^{a}$ | 48.4 ± 8.0$^{a}$ |
| | *Melilotus alba* | 0.845 ± 0.296$^{a}$ | 18.6 ± 7.1$^{a}$ | 36.8 ± 12.5$^{a}$ |
| | *Carthamus tinctorius* | 6.78 ± 0.98$^{c}$ | 120 ± 17$^{b}$ | 104 ± 19$^{b}$ |

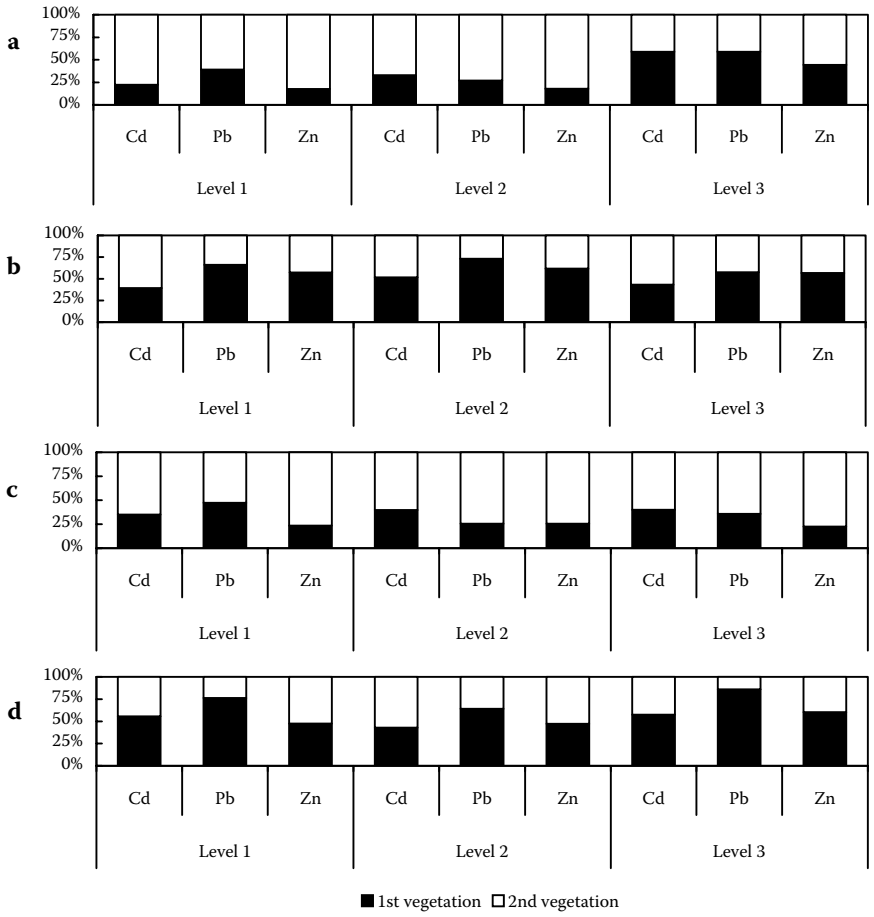


Figure 2. The distribution of uptake of investigated elements among individual harvests of aboveground biomass of *C. sativa* (a), *M. verticillata* (b), *M. alba* (c), and *C. tinctorius* (d) during two subsequent vegetation periods – field experiment

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