Heavy Metals Phytoextraction from Heavily and Moderately Contaminated Soil by Field Crops Grown in Monoculture and Crop Rotation

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Abstract: The uptake of Pb, Cd, Zn and biomass production of the plants *Brassica juncea* v. Opaleska, *Triticale hexaploides* var. Gabo and *Helianthus annuus* v. Maritza were observed in a field (trial) and a pot experiments during four years. The plants were grown in monoculture variants and also in crop rotation. The field experiment (plots about 1 × 1 m) was set up in heavily contaminated Haplic Fluvisol in the Litavka River alluvium. Pb, Cd, and Zn phytoextraction from the identical Haplic Fluvisol and Haplic Cambisol less contaminated mainly by atmospheric deposition was observed in the pot experiment. The application of 0.2 g EDTA (ethylenediaminetetraacetic acid)/kg and 1 g citric acid/kg into the soils of field (250 kg of soil/m² plot) and pot (6 kg of soil/pot) experiments was realized. The comparison was accomplished between natural phytoextraction efficiency of *B. juncea*, *H. annuus*, and *T. hexaploides*. Crop rotation with and without chemical induction was tested. EDTA application had an immediate strong mobilization effect on the elements tested in both experiments and both soils. In the pot experiment, Pb, Cd, and Zn were more mobilized in Cambisol with initial lower mobile contents of elements in comparison with Fluvisol. The highest mobilization by EDTA was achieved for Pb. Strong Pb mobilization in Cambisol after EDTA addition resulted in a high Pb uptake and translocation from the roots of *B. juncea* into the shoots. EDTA application increased Pb phytoextraction by harvested *B. juncea*. Naturally grown *H. annuus* proved the high phytoextraction efficiency for Cd and Zn in the experiment. The assumed effect of the cultivation method, i.e. crop rotation vs. Monoculture, was not statistically proved in our experiments.

Keywords: *Brassica juncea*; Cd; heavy metals contamination; *Helianthus annuus*; mobilization; Pb; phytoextraction; *Triticale hexaploides*; Zn

The use of higher plants to remediate contaminated land is known as phytoremediation, the term known since 15 years ago (VAMERALI et al. 2010). Phytoextraction has been suggested as a viable alternative to the traditional restoration practices for heavy metal-contaminated soils (e.g. soil removal and disposal in landfill areas), in view of the lower costs and lower environmental impact (DE ANDRADE et al. 2009). Phytoextraction is considered as the removal of heavy metals (HMs) from the soil by the harvest of overground biomass where the contaminants are taken up and

transferred by the plant (Wenzel et al. 1999). The uptake and accumulation of HMs were mostly studied on endemic plant species of metallic soils, hyperaccumulators (Prasad & Hagemeyer 1999). Hyperaccumulator plants are able to concentrate high levels of specific metals in the over-ground harvestable biomass. The small shoot and root growth of these plants and the absence of their commercially available seeds have stimulated studies on biomass species, including field crops (Vamerali et al. 2010). Kumar et al. (1995) found out that B. juncea and Brassica nigra have the highest

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ability out of twelve monitored farmed species *Brassica* spp. to receive heavy metals from the substrate and concentrate them in the overground biomass. Sunflower (*H. annuus*) is highly tolerant to heavy metals, showing a large intake of heavy metals into the roots, but poor translocation into the overground biomass (MADEJÓN *et al.* 2002). *Poaceous* species also prove resistance to high contents of heavy metals in soil (ERNST 1996).

The main limiting factors of phytoextraction are contaminant bioavailability in the soil and the biomass yield. Based on the study of element mobility and their transfer into plants (Podlešáková et al. 1999), the elements with high and pH dependent mobility and high transfer into plants can be exempt (Cd and Zn). Lead is an element with a potential mobility (Podlešáková & Němeček 2000) and occurs as a constituent of insoluble compounds (phosphates, carbonates and hydroxides) in soil (LASAT 2002) or bound on soil organic matter (Száková et al. 2003). Different chemical agents (EDTA - ethylenediaminetetraacetic acid, HEDTA – hydroxyethylethylenediaminetriacetic acid, citric acid etc.) mobilize the tightly bound metals from the soil matrix into the soil solution, enhance the plant uptake of metals, and thus increase their phytoextraction from the soil (Kos et al. 2003). Of the five chemical agents tested, EDTA showed the most effective Pb mobilization (Huang et al. 1997), however EDTA is little biodegradable and can enhance leaching of metals to groundwater (GRČMAN et al. 2001). Due to the potential risks, chemical agents with high biodegradability, low phytotoxicity and chelating strength (NTA, citric acid and succinamic acid) were proposed for the chemically assisted phytoextraction of HMs (QUARTACCI et al. 2005).

The calculations of the time required for soils remediation based on laboratory, hydroponics, pot, and trial experiments are based on short-term observation and repetition of the plant grown in monoculture. Monocultures have generally the disadvantage of a significant decline in the biomass yield due to the depletion of nutrients, occurrence of diseases, pests, and weeds, and have a negative impact on the soil fertility (LASAT 2000). The yield reduction then results in a significant reduction in the plant phytoextraction efficiency. Two to three years' monoculture may be acceptable for soil phytoremediation. However, for a longer duration, as considered for most phytoremediation processes, it cannot be expected to clean up the soil only by one plant species used exclusively in monoculture, especially if more harvests per growing season are expected. Banuelos (1998) recommend changing the plant species with risk elements accumulation capacity for technical crops in crop rotation, by which the improvement of economic balance and of the growth and yield of phytoaccumulator can be achieved.

The aim of this paper is to compare the efficiency of two chemical agents, low strength EDTA and citric acid, on the metals uptake by *B. juncea* in the pot and field experiments, and to test the phytoextraction efficiency of *T. hexaploides* and *H. annuus* naturally grown (without chemical treatment) on the tested soil as well. Two differently contaminated soils – Fluvisol (fluvial contamination) and Cambisol (atmospheric contamination) – were used for the detection of Pb, Cd, and Zn mobilization after chemical agent addition, and for the Pb, Cd, and Zn accumulation and the growth ability of the trial plants assessment. The plants were grown in monoculture and in crop rotation.

MATERIAL AND METHODS

The district of Příbram is located in the Central Bohemian Region and belongs to the areas in the Czech Republic most contaminated with heavy metals. The reasons for the contamination are, besides geological conditions, historical HMs mining and metallurgical activities. The vast contamination of the area (approximately 1500 ha including arable land) was caused by atmospheric deposition coming from the lead ores processing. Except this large surface contamination, there is a smaller contamination focus in the alluvium of the Litavka River. The flood water loaded by the wastes from metallurgical sediment storage basins resulted in an extremely high contamination of the alluvium by HMs (BORŮVKA et al. 1996; VÁCHA et al. 2002).

Field experiment

The experimental field was localized for four years near the village of Trhové Dušníky in the Litavka River alluvium on heavily contaminated Haplic Fluvisol. The soil is classified according to the World Reference Base (IUSS 2006). Single experimental plots $(1 \times 1 \text{m})$ were dug up and dolomitic limestone was added to increase the very low pH in the soil depth of 0–20 cm (5 g/kg of soil) in August 2005. The soil

samples were taken up before liming, and 3 weeks and 8 months after liming. All soil samples were air dried to constant weight, sieved through a stainless sieve (diameter 2 mm), and homogenized. Mean total, mobile (extracted with 1 mol/l NH $_4$ NO $_3$) and potentially mobile (0.025 mol/l Na $_2$ EDTA) contents of the risky elements in the experimental soil at the beginning of the experiment are summarised in Table 1.

The plant sowing was done each year in May; the fields were sown with 36 plants per field. Chemical agents were applied into the chosen variants seeded with B. juncea 10 days before the plants harvest for achieving maximal biomass production. 0.2 g Na₂EDTA/kg and 1 g citric acid/kg of soil in different variants were added into the soil in the form of solution. Low strength agents should prevent strong HMs mobilization and subsequent leaching into the groundwater. The harvest was done during the flowering phase, 10 days after the chemical agent addition. The plants were thoroughly washed with drinking water, separated into the shoots and roots, checked for fresh and dry (at 45°C) biomass, and analysed after the harvest. Additional soil samples were taken up by pooled sample method before the chemical agent addition and 4 h and 10 days afterwards. After that, dolomitic limestone in the dose of 2.5 g/kg was applied for the soil stabilization. Single trial variants (Table 2) had two replicates.

Pot experiment

Parallel pot experiment was set up in autumn 2005 and two experimental soils were used: Haplic Fluvisol identical with that in the field experiment, and moderately polluted Haplic Cambisol, contaminated by atmospheric deposition stemming from the lead ores processing; this soil was taken up from the arable land near the local smelter. Both soils were taken up from humic horizon (0-20 cm), sieved through 5 mm mesh, and magnesium limestone in the dose of 5 g/kg was added into each pot with Fluvisol and thoroughly homogenized (6 kg of experimental soil per pot). Cambisol was not limed because of the initial sufficient soil pH. The soil samples of Fluvisol and Cambisol were collected before liming. Further Fluvisol samples were taken up from the pots 3 weeks and 8 months

Table 1. Characteristics of field experiment soil and the limit values for total metals content

| | Pb | Cd | Zn | pH (KCl) | C _{ox} (%) |
|--|-------------------|------------------|-------------------|-------------------|---------------------|
| Haplic Fluvisol | | | | | |
| Total content (mg/kg) | 1099 | 14.2 | 1732 | 5.35 | 3.32 |
| 1M NH ₄ NO ₃ (%) | 0.08 | 5.8 | 3.6 | | |
| 0.025M Na ₂ EDTA (%) | 75.1 | 90.3 | 19.3 | | |
| Limit values (Directive of | the Ministry of t | he Environment o | of the Czech Repu | blic No. 13/1994) | |
| Light-textured soils | 140 | 1 | 200 | | |
| Other soils | 100 | 0.4 | 130 | | |

Table 2. Field experiment variants

| Variant | Remediation process (plot experiment) |
|----------|--|
| Halpic I | Fluvisol |
| 1 | Brassica juncea var. Opaleska grown in monoculture with citric acid application before harvest |
| 2 | Brassica juncea var. Opaleska grown in monoculture with EDTA application before harvest |
| 3 | Brassica juncea var. Opaleska grown in monoculture without application |
| 4 | Crops rotation (Brassica, Helianthus, Triticale, Brassica) with citric acid application before harvest of Brassica |
| 5 | Crops rotation (Brassica, Helianthus, Triticale, Brassica) with EDTA application before harvest of Brassica |
| 6 | Crops rotation (Brassica, Helianthus, Triticale, Brassica) without application |
| 7 | Helianthus annuus var. Maritza grown in monoculture without application |
| 8 | Triticale hexaploides var. Gabo grown in monoculture without application |

after liming. Mean total, mobile (extracted with $1 \text{ mol/l NH}_4\text{NO}_3$), and potentially mobile (0.025 mol/l Na_2EDTA) contents of the risky elements in the experimental soils at the beginning of the experiment are summarised in Table 3.

The plant seeds sowing was performed in the four years in May, the pots were sown with 5 plants per pot. The pots sown with *B. juncea* seeds were treated with 0.2 g Na₂EDTA/kg 10 days before the harvest. The plant and soil samples were taken up and treated in the same way as in the field experiment. Single pot variants (Table 4) obtained three repetitions.

Analyses

The measurements were performed in the central laboratory of the Research Institute for Soil and Water Conservation in Prague. The exchangeable soil pH (0.2M KCl) and the content of organic carbon were measured in the soil samples by the modified Tjurin method. Total soil element concentration was determined after decomposition using a mixture of $HNO_3 + HClO_4 + HF$. The soil samples were extracted with 1 mol/l NH₄NO₃ (mobile) at a ratio of 1:2.5 (w/v) and 0.025 mol/l Na₂EDTA (potentially mobilized risky elements) at a ratio 1:25 (w/v) and the extracts were analysed for the risky element concentrations. The plant samples were washed with distilled water and decomposed using the dry ashing procedure. The elements concentrations in the extracts were determined by flame atomic absorption spectrometry (VAR- IAN FAAS 240 – Cd, Pb and Zn), and flameless one (VARIAN ETA 240Z – Cd and Pb). Certified reference materials RM 7001 Light Sandy Soil and RM 7003 clay-loamy soil were applied for quality verification of the analytical data. For statistical evaluation Statistica 9 programme was used.

RESULTS AND DISCUSSION

Pb, Cd, and Zn mobilization and their contents in the soil

Total content of metals confirmed particularly serious contamination of both soils tested (Tables 1 and 3). The Directive of the Czech Ministry of Environment of the Czech Republic No. 13/1994 of the Coll. was used for the contamination extent assessment. Limit values are listed in Tables 1 and 3. Total content of Pb in Cambisol was comparable with that in Fluvisol whereas the contents of Cd and Zn were significantly lower in Cambisol. Pb was not found in the mobile fraction, but Pb was considerably extracted with Na₂EDTA. On the other hand, Cd was found to be highly represented in the mobile soils fractions and especially in the potentially mobilizable soil fractions. The very low portion of mobile Pb was not further decreased by the liming of Fluvisol. Cd and Zn, as more mobile elements in the soil, decreased their mobile contents in the soil after dolomitic limestone addition; whereas stronger immobilization was found for Zn. EDTA application had an immediate strong mobilization effect on all tested

Table 3. Characteristics of pot experiment soils and the limit values for metals total content

| | Pb | Cd | Zn | pH (KCl) | C _{ox} (%) |
|---------------------------------|-------------------|-------------------|-------------------|--------------------|---------------------|
| Haplic Fluvisol | | | | | |
| Total content (mg/kg) | 1536 | 16.1 | 3136 | 5.90 | 4.16 |
| $1M NH_4NO_3$ (%) | 0.03 | 2.5 | 0.8 | | |
| 0.025M Na ₂ EDTA (%) | 73.8 | 95.6 | 33.9 | | |
| Haplic Cambisol | | | | | |
| Total content (mg/kg) | 1288 | 5.57 | 280 | 6.14 | 2.04 |
| $1M NH_4NO_3$ (%) | 0.10 | 3.2 | 0.4 | | |
| 0.025M Na ₂ EDTA (%) | 89.8 | 92.6 | 16.5 | | |
| Limit values (Directive of | the Ministry of t | the Environment o | of the Czech Repu | ıblic No. 13/1994) | |
| Light-textured soils | 140 | 1 | 200 | | |
| Other soils | 100 | 0.4 | 130 | | |

Table 4. Pot experiment variants

| Variant | Remediation process (pot experiment) |
|----------|---|
| Halpic F | Fluvisol |
| 1 | Brassica juncea var. Opaleska grown in monoculture with EDTA application before harvest |
| 2 | Brassica juncea var. Opaleska grown in monoculture without chelating agent application |
| 3 | Crops rotation (Brassica, Helianthus, Triticale, Brassica) with EDTA application before harvest of Brassica |
| 4 | Crops rotation (Brassica, Helianthus, Triticale, Brassica) without application |
| 5 | Helianthus annuus var. Maritza grown in monoculture without application |
| 6 | Triticale hexaploides var. Gabo grown in monoculture without application |
| Halpic C | Cambisol |
| 7 | Brassica juncea var. Opaleska grown in monoculture with EDTA application before harvest |
| 8 | Brassica juncea var. Opaleska grown in monoculture without chelating agent application |
| 9 | Crops rotation (Brassica, Helianthus, Triticale, Brassica) with EDTA application before harvest of Brassica |
| 10 | Crops rotation (Brassica, Helianthus, Triticale, Brassica) without application |
| 11 | Helianthus annuus var. Maritza grown in monoculture without application |
| 12 | Triticale hexaploides var. Gabo grown in monoculture without application |

elements in both experiments and soils. The highest mobilization by EDTA was achieved with Pb. Pb was more mobilized in the pot Cambisol with the initial lower mobile contents of elements in comparison with pot Fluvisol. Citric acid showed lower ability of metals mobilization, however, its influence had been already statistically proved. The increased Pb, Cd, and Zn mobility caused by chemical agent addition returned after 10 days to the level existing before the application. This could be explained by the long term incidence of liming and thus an increased buffering capacity of the soil regarding the increased CaCO₃ supply. Mobilization effect of EDTA on the observed metals is listed in Table 5. Liming increased pH of the field and pot Fluvisols from 5.35 and 5.9 up to 7.0 and 6.8, respectively, and the chemical agent application did not influence the soil pH.

The trends in the risky elements behaviour after the chemical agent application were confirmed by the results of the experiments in all the years of observation. EDTA and citric acid effects on heavy metals mobilization were proved by Wilcoxon signed rank test. The test confirmed the positive influence of chemical agent EDTA on the metals mobile content in soils as statistically significant on the significance level $\alpha=0.01$ for Pb and Zn ($\alpha=0.05$ for Cd). The influence of citric acid on metals mobile contents in the soils was statistically significant on thr significance level $\alpha=0.01$ for Pb and $\alpha=0.05$ for Cd and Zn. The mobilization

stages are graphically illustrated by the example of lead (Figure 1).

Pb, Cd, and Zn contents and phytoextraction efficiency of plants

The natural ability of *B. juncea* plants to take up heavy metals (especially Pb) was observed (Kumar et al. 1995; Schulman et al. 1999). However, in our experiment The Czech and European regulations (Directive No. 169/2002 Coll. and the Directive 2002/32 EC; chosen for the possibility of comparison of the results) were exceeded in the field experiment with EDTA treatment (Table 6 and 7). The Czech and European limits for Pb were not exceeded even in the plants grown on heavily contaminated soils in the case of naturally grown B. juncea (without EDTA addition). Naturally grown B. juncea blocked Pb mainly in the roots. EDTA addition resulted in an enhanced Pb transfer from the roots into the shoots. The enhanced amount of Pb taken up by B. juncea was proved after EDTA addition into the pot Cambisol and to a smaller extent in the field and pot Fluvisol. The high Pb content in the shoots of B. juncea observed in all experimental years responds to the pronounced mobilization of Pb in the pot Cambisol. Citric acid influenced Pb mobilization in soil and did not influence Pb uptake by the experimental plants. Most of the plants exceeded the

| | 7 | Trial Fluvisol | | | Pot Fluvisol | | | Pot Cambisol | | |
|-------------|--------------|----------------|------|-------|--------------|------|------|--------------|------|--|
| | Pb | Cd | Zn | Pb | Cd | Zn | Pb | Cd | Zn | |
| Before EI | TA applicat | ion | | | | | | | | |
| | 0.001 | 0.76 | 0.07 | 0.004 | 1.24 | 0.17 | 0.01 | 0.98 | 0.06 | |
| 4 h after I | EDTA applica | ation | | | | | | | | |
| 2006 | 11.0 | 38.1 | 5.76 | 1.02 | 13.1 | 1.68 | 14.2 | 37.1 | 4.11 | |
| 2007 | 9.00 | 15.5 | 5.22 | 0.35 | 2.94 | 0.44 | 4.21 | 12.7 | 1.24 | |
| 2008 | 7.69 | 29.0 | 4.78 | 2.07 | 22.9 | 3.08 | 9.76 | 23.7 | 3.96 | |
| 2009 | 3.41 | 1.06 | 0.17 | 0.66 | 0.53 | 0.30 | 0.85 | 0.24 | 0.69 | |

Table 5. Mobile fraction proportions (%) from total risk elements content before application of EDTA and 4 h after application during trial and pot experiment

shoots Pb contents reported by Kabata-Pendias (2001) to be in normal plant 10 mg/kg. Our values of Pb contents in *B. juncea* are comparable with the average 55 mg Pb/kg in the shoots of *B. juncea* planted in soils contaminated by acid sludge (Clemente *et al.* 2005).

The Czech and European regulations (Tables 6 and 7) for Cd, Zn contents in animal feed were more exceeded in the overground biomass than in the roots of *B. juncea* plants grown without EDTA addition. The uptake of Cd, Zn, and Pb into roots significantly prevailed in the case of *T. hexaploides* plants. In spite of that, Cd content in the overground biomass reached the relevant limit values for animal feed in all grown variants. High amounts of Cd and Zn were taken up into the overground biomass by *H. annuus* grown without chemical treatment. The risky elements contents in all naturally grown plants exceeded the relevant limit rates whereas Pb limit

was exceeded just in the roots. The pot experiment results revealed a higher uptake of elements from Cambisol than from Fluvisol.

The biomass yield is also an important factor of phytoextraction efficiency. All plants used in the field experiments proved tolerance and growth ability in soils with a high total content of risky elements. The chemical agents application before the harvest did not reduce the yield of *B. juncea* biomass. A significantly higher yield of the overground biomass and roots was achieved with *H. annuus*, a lower yield was reached with *T. hexaploides*. The plants grown in crop rotation did not show higher biomass yields compared to the same plants grown in monoculture.

The plant potential for phytoextraction use can be also defined according to the risky elements uptake from the soil, determined by the element content taken up by the biomass of plants from the

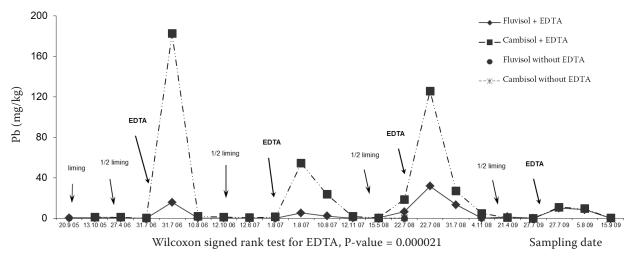


Figure 1. Mobile content of Pb extracted with $1 \text{M NH}_4 \text{NO}_3$ (mg/kg) from tested soils after liming and chelating agents additions through four years of pot experiment

Table 6. Metals average content in shoots and roots of variants grown in all years and limit values exceeding in pot experiment (in mg/kg)

| M | P | b | C | d | Z | Zn | |
|-----------------|--------|-------------------|-------------------|------------------|-------------------|-------|--|
| Variant — | shoots | roots | shoots | roots | shoots | roots | |
| Haplic Fluvisol | - | | | | | | |
| 1 | 45.0 | 30.5 | 4.95 | 5.78 | 361 | 238 | |
| 2 | 6.85 | 27.1 | 6.91 | 5.54 | 387 | 210 | |
| 3 | 32.0 | 70.9 | 4.33 | 7.33 | 364 | 315 | |
| 4 | 29.3 | 58.3 | 6.84 | 7.26 | 411 | 380 | |
| 5 | 6.67 | 51.8 | 5.64 | 7.70 | 403 | 499 | |
| 6 | 3.80 | 105 | 0.99 | 5.30 | 231 | 413 | |
| Haplic Cambisol | | | | | | | |
| 7 | 162 | 156 | 4.20 | 5.58 | 84.6 | 49.1 | |
| 8 | 6.76 | 29.1 | 3.61 | 2.09 | 43.9 | 34.9 | |
| 9 | 54.9 | 108 | 3.27 | 8.76 | 105 | 48.2 | |
| 10 | 5.28 | 70.0 | 2.67 | 3.30 | 38.0 | 37.8 | |
| 11 | 14.6 | 79.3 | 3.62 | 5.60 | 42.4 | 45.1 | |
| 12 | 6.66 | 77.7 | 0.64 | 4.66 | 40.0 | 72.4 | |
| | Direc | tive of the Minis | try of Agricultur | e of the Czech l | Republic, No. 169 | /2002 | |
| T ::4l | 40 | 40 | 1 | 1 | | | |
| Limit values | | Directiv | e 2002/32/EC of | the European P | arliament | | |
| | 40 | 40 | 1 | 1 | | | |

area. The results indicate a significant potential of Cd and Zn phytoextraction in the case of naturally grown H. annus plants. Only B. juncea proved the ability of Pb uptake after EDTA application. The influence of EDTA treatment on Pb transfer from various soil types into the overground biomass of B. juncea plants was statistically tested using two-factor analysis of variance. Both a significant influence of two factors (the EDTA application, soil type) on Pb content in the overground biomass and the factor interaction were proved on the significance level α = 0.01 by the ANOVA.

Remediation factor (Rf) expresses the proportion (%) of metal removed by biomass harvest from the total, eventually mobile amounts in the soil. Rf was determined in 20 cm of soil depth (250 kg soil per plot) where predominant roots distribution is presumed in the field experiment and for the whole volume of the pot (6 kg of soil). Rfs for the environmentally risky mobile metals fraction were determined in relation to 1M NH₄NO₃ extractable metals in the soils. This mobile fraction must be decreased simultaneously to prevent the possible metals movements and leaching into the

groundwater sinks. However, even if preferential metals uptake by plants comes from the available (mobile) fraction, metals are continuously mobilized from the less available fractions in the soil by many factors (Wenzel *et al.* 1999) and it is necessary to observe the mobile fraction in the soil permanently.

Total four-year removal from the pot soils (the sum of shoots and roots) and Rfs based on total metals content in soils are demonstrated in Figure 2. The chemical agents added into the field Fluvisol EDTA increased Pb removal and Rf of B. juncea. Significantly higher Rfs were achieved in the pot experiment, which was caused by the smaller amount of soil and thus a lower Pb supplement in the pot. EDTA addition into the Cambisol had a greater effect on the improvement of Pb removal. Chemical addition did not significantly influence Cd and Zn uptake by B. juncea plants. On comparing the results of Cd and Zn Rfs (numerically expressed as the Rf sum of all experimental years), it may be claimed that H. annuus grown in monoculture without chemical agents application revealed the highest phytoextraction efficiency. The

| Table 7. Metals average content in shoots and roots of variants grown in all years and limit values exceeding in |
|--|
| field experiment (in mg/kg) |

| Vaniant | P | b | C | d | Z | Zn | |
|--------------|--------|-------------------|---------------------|-----------------|--------------------|-------|--|
| Variant - | shoots | roots | shoots | roots | shoots | roots | |
| 1 | 8.96 | 17.7 | 8.52 | 5.60 | 381 | 252 | |
| 2 | 29.7 | 26.4 | 8.27 | 4.31 | 440 | 195 | |
| 3 | 9.16 | 17.2 | 8.02 | 6.83 | 351 | 205 | |
| 4 | 13.2 | 40.2 | 7.57 | 6.65 | 293 | 255 | |
| 5 | 15.2 | 34.6 | 8.51 | 5.88 | 318 | 233 | |
| 6 | 14.1 | 52.4 | 9.06 | 6.88 | 315 | 280 | |
| 7 | 15.7 | 58.2 | 10.2 | 8.51 | 425 | 354 | |
| 8 | 13.2 | 252 | 1.83 | 14.3 | 161 | 551 | |
| | Direc | tive of the Minis | stry of Agriculture | of the Czech Re | epublic, No. 169/2 | 2002 | |
| T · · · · 1 | 40 | 40 | 1 | 1 | | | |
| Limit values | | Directiv | re 2002/32/EC of t | he European Par | liament | | |
| | 40 | 40 | 1 | 1 | | | |

higher phytoextraction efficiency of *H. annuus* in comarison with B. juncea was not fully assumed regarding the results published by Madejón et al. (2002) who mention that H. annuus is tolerant of HMs contamination and proved a high HMs intake into the roots, but a low transfer into the overground biomass was observed. On the other hand, De Andrade et al. (2009) found out that, the chemical agent addition did not induce the heavy metal uptake by *H. annuus*, and that *H. annuus* should be preferred for phytoremediation in the soils of mining and metallurgy areas. SINEGANI & KHALILIKHAN (2010) mention sunflower (Helianthus annuus) as a potential phytoextraction plant accumulating high concentrations of Cd from metal-contaminated soils and give the removal efficiency of Cd from the soil as 0.15% whereas Rf rated by us was 0.42% from pot Cambisol. The effect of the cultivation method (monoculture vs. crop rotation) was not statistically proved in our experiments. However, we cannot exclude the growing importance of this factor due to the plants fragility in monocultures and declining yields of the plant species in monoculture during longer-time experiments.

For the practical use of phytoextraction, the question of the target values should be discussed. These are the values that should be achieved during remediation to minimise the risk of the site in terms of the receptor. The proposal of an amendment to the regulation of the Czech Ministry of

Environment No. 13/1994 Coll. (Sáňka *et al.* 2002) was used for the assessment. The proposal works with three levels of limits – first degree, preventive limits, coming from the upper measure of the background values of the risky elements content in the Czech soils, the values are set for the surface soil horizon.

The indication limit is based on the studies of the risky elements transfer through the soil-plant pathway and indicates the possible level of the element transferring risk from the soil at a level that does not meet the chemical requirements for food or animal feed safety. The values are set for ploughing or humus horizon of the agricultural soils. Rendering limit – the value characterizing the level of the contents of hazardous elements and substances in the soil, in which an imminent risk exists of adverse effects on plants, animals and humans, and possibly other components of the ecosystem.

The values are set for all types of the land use. We counted how much time it would take to clean the contaminated soil used in our experiment to the level of indication limit. The results of the pot experiment are given in Table 8.

The proposal of an amendment to the regulation of the Czech Ministry of Environment No. 13/1994 Coll. (Sáňka *et al.* 2002) also involves the indication limits for the environmentally risky mobile metals fraction extractable with 1M NH₄NO₃. The limit value was only exceeded in the case of Zn, Cd in our experiments. Rfs were determined based on

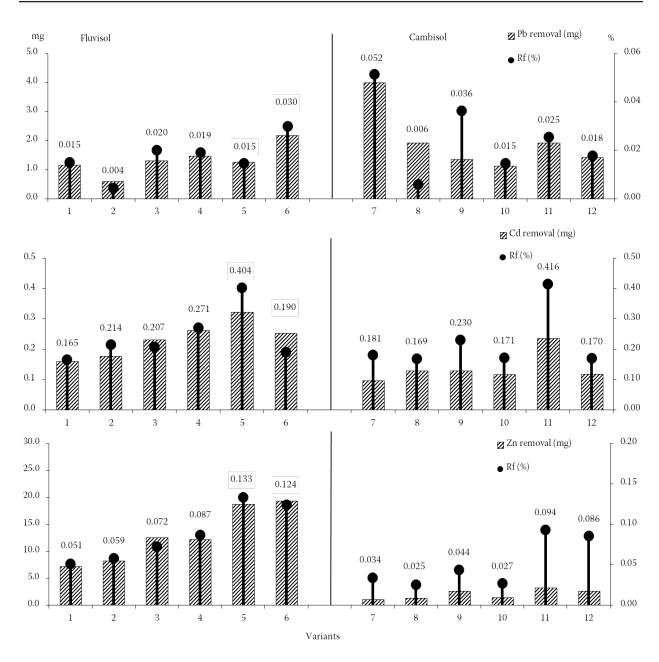


Figure 2. Total four-year removal (the sum of removal by shoots and roots) and Rf based on total metals contents in soils of pot experiment

the mobile metals fraction content and the time needed for the remediation was calculated. The results of the pot experiment are given in Table 9. The results, however, can be misleading, because metals are continuously mobilized from the less available fractions in the soil, the results manifest the favourable phytoextraction efficiency of the trial plantation for the riskiest mobile fraction of heavy metals. The best result was obtained especially with *H. annuus* plants.

The phytoremediation efficiency of the field crops in our experiment was not high and the results confirm that the soil phytoremediation using crop rotation or monoculture is a very long term process. But a great growth potential in the contaminated soils should be positively assessed, as well as the improving of the landscape and reducing of the mobility of the risky elements through water and wind erosion. The phytoremediation potential lies in conjunction with the conventional methods of decontamination in terms of the subsequent cleaning treatment of the site. The methods of phytoremediation are financially accessible and, in general, well accepted by the public.

Table 8. Years needed for remediation to indication limit value (total content) in pot experiment

| | Pb | Cd | 7 | Zn | Pb | Cd | Zn |
|--------------------------|------------|--------------|------|---------|-------|----------------|------|
| Content in soil (mg/kg) | 1536 | 16.1 | 3: | 136 | 1288 | 5.57 | 280 |
| Indicating limit (mg/kg) | 70 | h2 | 1 | 60 | 70 | 2 | 160 |
| Years per remediation to | indication | limit value | | | | | |
| Variant | Н | aplic Fluvis | ol | variant | H | Iaplic Cambiso | ol |
| 1 | 25322 | 2126 | 7399 | 7 | 7335 | 1411 | 4999 |
| 2 | 85291 | 1634 | 6484 | 8 | 63681 | 1512 | 6795 |
| 3 | 18958 | 1692 | 5241 | 9 | 10407 | 1107 | 3887 |
| 4 | 20028 | 1292 | 4364 | 10 | 25911 | 1490 | 6351 |
| 5 | 26208 | 868 | 2847 | 11 | 14861 | 613 | 1832 |
| 6 | 12733 | 1843 | 3058 | 12 | 21245 | 1505 | 2002 |

Table 9. Years needed for remediation to indication limit value (mobile content extractable with NH_4NO_3) in pot experiment

| | Cd | 2 | Zn | Cd |
|-----------------------------|----------------------|----------|---------|-----------------|
| Content in soil (mg/kg) | 0.45 | 3 | 0.18 | |
| Indicating limit (mg/kg) | 0.1 | : | 20 | 0.1 |
| Years per remediation to in | dication limit value | e | | |
| Variant | Haplic 1 | Fluvisol | variant | Haplic Cambisol |
| 1 | 53 | 33 | 7 | 3 |
| 2 | 41 | 29 | 8 | 3 |
| 3 | 42 | 23 | 9 | 2 |
| 4 | 32 | 19 | 10 | 3 |
| 5 | 22 | 13 | 11 | 1 |
| 6 | 46 | 13 | 12 | 3 |

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