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Phytoextraction of Cd and Zn with *Thlaspi caerulescens* in field trials

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Abstract. Phytoextraction is the remediation of heavy metal contaminated soils using plants that take up metals. Hyperaccumulating plants such as *Thlaspi caerulescens* are often studied for their possible use for decontamination of Cd and Zn rich soils, but few field trials have been reported, although they are necessary to validate the results of hydroponic and pot studies. This article reports field data for *T. caerulescens* grown on a calcareous and an acidic soil, both contaminated 20 years ago by either atmospheric depositions or septic-tank wastes. Accelerated cropping using transplants grown three times in eight months was compared to *Thlaspi* sown twice during the same period. Both were followed by one crop of sown *Thlaspi*. High Cd and Zn concentrations in the plant shoots compensated for the low biomass production. Annual metal exports with transplanted *Thlaspi* were 130 g Cd ha^{-1} and 3.7 kg Zn ha^{-1} on the calcareous soil and 540 g Cd ha^{-1} and 20 kg Zn ha^{-1} on the acidic soil. We concluded that within the framework of the Swiss legislation, remediation of Cd-contaminated soils could be achieved within less than 10 years with one crop of *Thlaspi* per year, but differences in soil properties could affect the rate of phytoextraction significantly. Total Zn content in both soils was too high to be remediated by *T. caerulescens* in a realistic time span. *Thlaspi* did not decrease the $NaNO_3$-extractable fraction of Cd or Zn in either of the soils.

Keywords: phytoextraction, cadmium, zinc, hyperaccumulation, *Thlaspi caerulescens,* field experiment

INTRODUCTION

Agricultural land in most industrialized countries is contaminated to some extent by atmospheric deposition of heavy metals or through application of metal-rich fertilizers and pesticides. The use of plants to extract heavy metals from soil (phytoextraction) has been proposed by several authors (Brown *et al.* 1994; Kumar *et al.* 1995) for shallow and moderate contamination of soils covering large areas. Many plant species have already been tested for their adequacy to extract metals by either metal hyperaccumulation or by large biomass production (Huang *et al.* 1997; Blaylock & Huang, 2000). McGrath *et al.* (1993) tested the Cd and Zn hyperaccumulator *Thlaspi caerulescens* (referred to hereon as *Thlaspi*) on a sewage-sludge amended soil, and found that it extracted a larger amount of Zn in one harvest than was allowed to be applied as sewage sludge according to EC guidelines. However, uptake potential varies with the populations used (Schwartz 1997; Lombi *et al.* 2000) and soil characteristics. For example, while Brown *et al.* (1995) observed no influence of soil pH on Zn uptake by *Thlaspi*, Robinson *et al.* (1998) found that the metal content in plants collected at a contaminated site was inversely correlated with pH and correlated with the ammonium acetate-extractable fraction. Felix (1997) conducted field trials with different plant species on a calcareous soil in Dornach, Switzerland (close to the field experiment reported in this paper), and found that *Thlaspi* had the highest Zn concentrations (2550 mg kg^{-1}) in shoots but did not reach the hyperaccumulation level. This could either be related to the population used, or to low bioavailability of heavy metals due to high pH, or to the presence of carbonates in the soils, which may fix Cd and other metals in a plant-unavailable form (McBride 1980). In another field experiment conducted at the same site, Kayser *et al.* (2000) measured similar metal concentrations in *Thlaspi* but obtained c. 0.5 t dry matter (DM) ha^{-1} instead of 13.4 t DM ha^{-1} as found by Felix (1997). Low biomass is often observed for hyperaccumulating species. One way to improve phytoextraction efficiency could be to find a way to increase the annual biomass production.

However, assessment of phytoextraction efficiency has to be based on objective goals, including legislative requirements. Unlike most other countries, Switzerland has implemented a three level evaluation of soil contamination with heavy metals (OIS 1998). It consists of guide, trigger and clean-up values for 9, 3 and 4 elements, respectively. In soils exceeding the guide values (GVs) viz. 0.8 mg total Cd kg^{-1}, 40 mg total Cu kg^{-1} and 150 mg total Zn kg^{-1}, the long-term functionality of the soil is no longer assured. The trigger values (TVs) indicate the threshold of a possible

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hazard: 2–10 mg total Cd kg^{-1} depending on the use, 150 mg total Cu kg^{-1}; there is no TV for Zn. A risk assessment has to be carried out when concentrations exceed those values. Clean-up values (CVs) viz. 20–30 mg total Cd kg^{-1}, 1000 mg total Cu kg^{-1} and 2000 mg total Zn kg^{-1} are defined as levels of soil contamination at which stringent measures have to be taken (Hämmann & Gupta 1998; Gupta *et al.* 2000). All three levels include a soluble ($NaNO_3$-extractable) and a pseudo-total (2 M HNO_3-extractable) metal concentration.

The aim of this study was to assess the effect of soil type on phytoextraction efficiency by comparing field experiments performed on a calcareous and an acidic soil. In order to improve the biomass production, different methods of growing *Thlaspi* were tested.

MATERIAL AND METHODS

Site descriptions

The Dornach site, northwest Switzerland, has been described by Kayser *et al.* (2000). The source of the heavy metal contamination was a nearby brass-smelter emitting Cu, Zn and Cd in particulate form until the mid-1980s. The soil has been classified as calcaric regosol (Geiger *et al.* 1993) and characteristics of the topsoil are presented in Table 1 as well as the mean annual temperature and rainfall for the years of the experiment. Further soil characteristics are given in Kayser *et al.* (2000) and Keller *et al.* (2003).

The Caslano site in southern Switzerland was contaminated with sludges from septic tanks spread on the site for 20 years until 1980. This has led to enrichment of the topsoil of the fluvisol with both organic matter and heavy metals (Cd, Cu and Zn) (Table 1). Currently the site is used as a private garden and a meadow for fodder production. The mean annual temperature and rainfall are listed in Table 1.

In the context of Swiss legislation, metal concentrations in both soils exceeded TV for total Cd and Cu, and CV in the case of the acidic soil for soluble Zn, thereby prohibiting the use of this site for fodder production. Other heavy metal concentrations were below TV for both soluble and total concentrations.

Table 1. Climatic conditions and selected topsoil (0–20 cm) properties and heavy metal contents at Dornach and Caslano.

	Dornach[a]	Caslano
pH ($CaCl_2$)	7.3±0.1	5.2±0.3
% $CaCO_3$	14±3	–
% C_{org}	2.5±0.4	6.3±1.9
% Clay	38±1	13±5
% Silt	39±9	19±4
% Sand	23±8	68±8
CEC_{pot}[b] (meq 100 g^{-1})	31±9	28±4
Cd_{tot}[c] (mg kg^{-1})	2.5±0.3	2.8±0.7
Zn_{tot} (mg kg^{-1})	673±115	1158±216
Cu_{tot} (mg kg^{-1})	516±57	264±43
Cd_{sol}[d] (μg kg^{-1})	2.2±0.7	13±11
Zn_{sol} (mg kg^{-1})	0.09±0.02	7.4±5.9
Cu_{sol} (mg kg^{-1})	0.7±0.1	0.4±0.1
Swiss Meteorological Agency, local station	Basel-Binningen	Lugano
Mean annual temp. 2000 (°C)	11.7	13.0
Mean annual temp. 2001 (°C)	11.7	13.6
Annual rainfall 2000 (mm)	787	2100
Annual rainfall 2001 (mm)	984	1500[e]

[a]After Kayser *et al.* (2000); [b]CEC cation exchange capacity; [c]pseudo-total = 2 M HNO_3-extractable; [d]soluble = 0.1 M $NaNO_3$-extractable; [e]data for December not available.

Experimental design

Dornach: prior to the 2000 and 2001 growing seasons all plots (1.25 × 1.25 m) were fertilized with 120 kg P ha^{-1} and 200 kg K ha^{-1} as Superphosphat® and Patentkali®, respectively, and 40 kg N ha^{-1} was supplied as NH_4NO_3 + 7% MgO. In order to prevent plants from developing chlorosis, Sequestren rapid®, an Fe fertilizer, was applied as 24 kg chelated Fe ha^{-1} (Fe-EDDHA) in two applications. In 2000, seedlings of *Thlaspi* (Les Avinières mine, St. Laurent-le-Minier, France) were grown in a controlled chamber for 35 days and then transplanted in the field at a density of 100 plants m^{-2} on four subplots. Three successive crops of *Thlaspi* were grown in the first year. The crops were harvested 69, 78 and 67 days after transplanting, starting on the 20 April 2000. On four other subplots, *Thlaspi* seed was sown twice directly into the prepared soil and harvested after 99 and 115 days in 2000. In 2001, *Thlaspi* seed was sown in April and grown until November. Plants were irrigated with tap water as needed. Four other subplots were kept as control plots, on which only sparse vegetation grew. **Caslano**: the experiment was set up in 2000 and consisted of four plots, each subdivided into four subplots of 1.0 × 1.0 m. As with the Dornach experiment, *Thlaspi* was grown and transplanted into four subplots and sown directly into four others. The transplants were harvested at the same time as at Dornach. The sown *Thlaspi* was harvested after 100 and 110 days, and again in autumn 2001. In the first year no fertilizers were applied, as the original soil was rich in nutrients. In the second year the K, P, and N applications were the same as at Dornach but Sequestren rapid® was added. Additionally on the four control subplots, the pioneer species (*Galinsoga ciliata, Amaranthus retroflexus, Chenopodium albus* and *bonus-henricus*) were harvested twice in 2000, at the same time as the sown *Thlaspi*.

Soil and plant analysis

At both sites, soil samples (0–0.15 m) were taken from all subplots after the field experiments to check for differences in 0.1 M $NaNO_3$-extractable metals (FAC 1989). All aerial parts of the plants were harvested with shears, washed with tap water and rinsed with deionized water. Plant samples were dried at 60°C for four days and ground in a Retsch ZM-1 centrifugal mill. Ground samples (0.5 g) were digested in a mixture of HNO_3 (65%) and $HClO_4$ (70%) (Hammer & Keller 2002).

Cadmium in $NaNO_3$ extracts was measured by Graphite Furnace Atomic Absorption Spectrometry with Zeeman background correction (GF-AAS Perkin Elmer 5100). All other heavy metal concentrations in soil and plant extracts were determined with Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Perkin Elmer Plasma 2000) using certified reference materials.

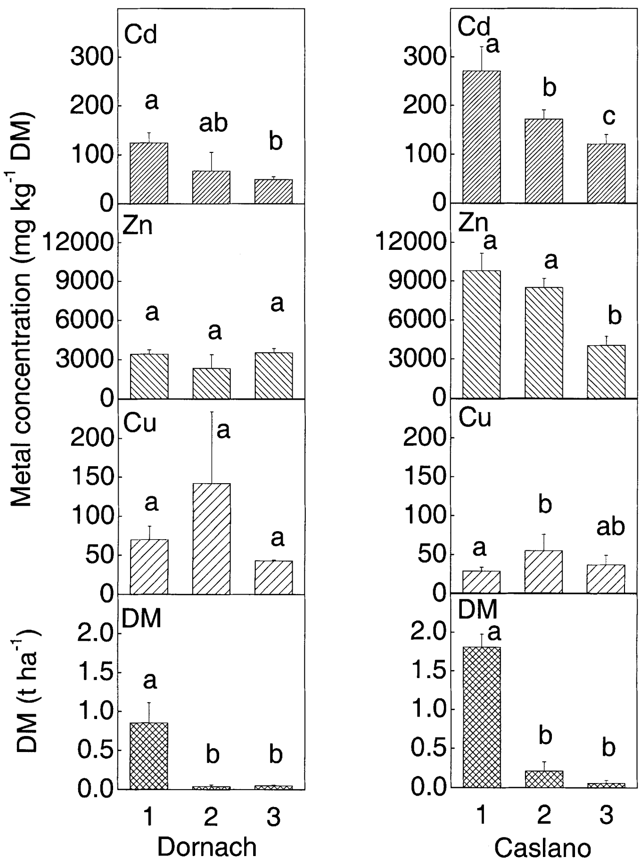

Figure 1. Metal concentrations and dry matter (DM) of *Thlaspi caerulescens* transplanted and harvested three times between spring and autumn 2000 at Dornach and Caslano. The data are means of 4 replicates with standard deviations. Lower-case letters indicate significant differences between harvests ($P<0.05$).

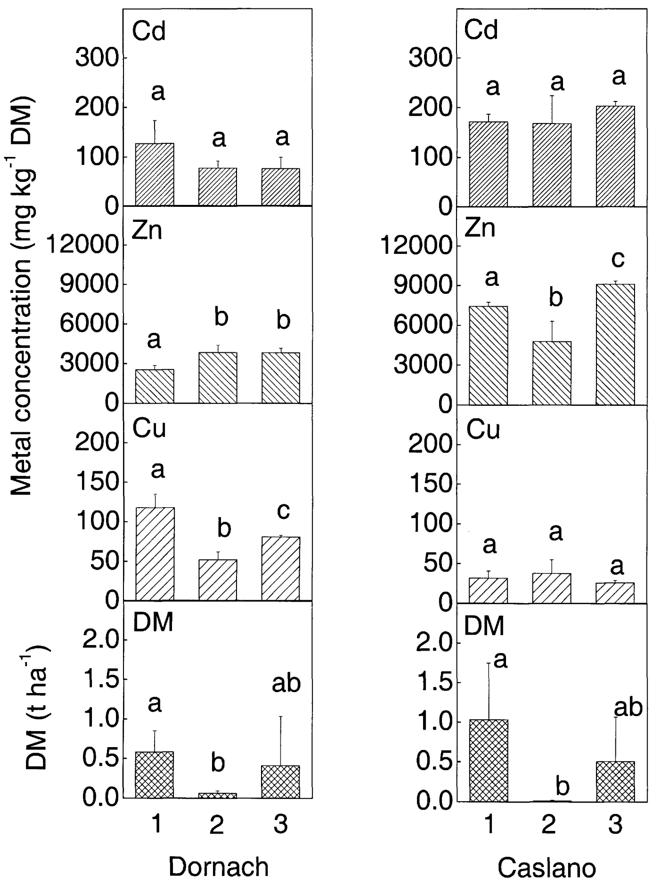

Figure 2. Metal concentrations and dry matter (DM) of *Thlaspi caerulescens* sown and harvested three times between spring 2000 and autumn 2001 at Dornach and Caslano. The data are means of 4 replicates with standard deviations. Lower-case letters indicate significant differences between harvests ($P<0.05$).

Student *t* tests were applied to test metal concentrations and DM weights for significant differences.

EXPERIMENTAL RESULTS

Metal concentrations in plants

Concentrations of Cd, Cu, and Zn in shoots of *Thlaspi* are shown in Figure 1 for transplants and in Figure 2 for sown plants. Cd concentrations in transplanted *Thlaspi* from Dornach were half those from Caslano. They decreased from the first harvest to the third by a factor 2.2 and 2.5 for Caslano and Dornach, respectively. Zinc concentrations in plants of the first harvest were also higher in Caslano (9800 mg kg^{-1}) and decreased in successive harvests as for Cd. However, Zn concentrations in plants growing at Dornach did not vary significantly between harvests, with an average concentration of 3500 mg kg^{-1}. Sown *Thlaspi* showed no significant changes in Cd concentration between the two harvests. In contrast, Zn concentrations in plants increased at Dornach but decreased at Caslano in the second harvest. The third harvest carried out in 2001 showed similar Zn concentrations at Dornach as in 2000, but these were higher than in the two first harvests at Caslano. There was no obvious trend in Cu concentrations.

The effect of management on metal concentrations was significant for both soils (Student *t* test $P<0.05$) in the first harvest for Cd and Zn but in the later two harvests only for Zn at Caslano. However, the mean metal concentrations for the three harvests showed no significant difference between transplanted and sown *Thlaspi* at either site. In contrast to *Thlaspi*, Cd and Zn concentrations in the pioneer plants were low for all species: below 3 mg Cd kg^{-1} and between 220 and 740 mg Zn kg^{-1} (data not shown).

Metal offtake

Metal removal was calculated as the product of metal concentration and biomass of the plants, the results of the croppings performed during the year being added (Table 2). Direct comparison of annual metal outputs between transplanted and sown *Thlaspi* showed that on both soils extraction of Cd and Zn was larger with transplants. This was a direct result of more biomass production and not of differences in metal concentrations. Irrespective of the method of growing, the efficiency of Zn and Cd phytoextraction was higher at Caslano because of larger yields and metal concentrations. In spite of an annual yield of 4.6 t ha^{-1}, the pioneer species extracted 130 times less Cd, and 10 times

Table 2. Dry matter and metal yields of *Thlaspi caerulescens* obtained in 2000 at Dornach and Caslano. Natural regrowth was harvested twice and only at the Caslano site

	Dornach				Caslano			
	Yield DM ($t\ ha^{-1}$)	Cd ($g\ ha^{-1}$)	Cu ($g\ ha^{-1}$)	Zn ($kg\ ha^{-1}$)	Yield DM ($t\ ha^{-1}$)	Cd ($g\ ha^{-1}$)	Cu ($g\ ha^{-1}$)	Zn ($kg\ ha^{-1}$)
Thlaspi transplanted (3 harvests)	0.9±0.3	128±19	76±15	3.7±0.3	2.1±0.2	539±127	65±15	20.0±4.2
Thlaspi sown (2 harvests)	0.6±0.3	85±50	71±37	1.7±0.8	1.0±0.7	184±138	29±13	7.8±5.5
Natural regrowth (2 harvests)	–	–	–	–	4.6±0.4	4±2	59±13	1.9±0.4

Table 3. Metal concentrations extracted by 0.1 M $NaNO_3$ in soil samples taken after the last harvest of *Thlaspi caerulescens* in November 2000.[a]

		Cd ($\mu g\ kg^{-1}$)	Zn ($\mu g\ kg^{-1}$)	Cu ($\mu g\ kg^{-1}$)
Dornach	*Thlaspi* transplanted	1.4 ± 0.3^{A}	48 ± 21^{A}	688 ± 25^{A}
	Thlaspi sown	1.7 ± 1.0^{A}	58 ± 27^{A}	720 ± 139^{A}
	Natural regrowth (sparse)	2.5 ± 1.3^{A}	52 ± 30^{A}	481 ± 44^{B}
Caslano	*Thlaspi* transplanted	7.2 ± 1.2^{A}	8100 ± 400^{A}	530 ± 108^{AB}
	Thlaspi sown	8.9 ± 2.9^{A}	7100 ± 210^{AB}	476 ± 18^{B}
	Natural regrowth (abundant)	4.5 ± 1.7^{B}	4900 ± 1500^{B}	527 ± 35^{A}

[a]Superscript letters indicate significant differences between concentrations in the same soil ($P<0.05$).

Table 4. Number of years necessary to phytoextract Cd and Zn in order to reach either the guide value (GV) or the trigger value (TV) of the OIS (1998).

		Cd (GV)	Zn (GV)	Cd (TV)
Dornach	transplanted	33	279	7
	sown	49	586	10
Caslano	transplanted	9	121	4
	sown	26	310	10

less Zn than the transplanted *Thlaspi*, and 50 times less Cd, and 4 times less Zn than *Thlaspi* that had been sown. At Dornach, the natural regrowth was sparse and not harvested.

Soluble metal concentration in soil after growth of Thlaspi (Table 3)

The $NaNO_3$ extracts of soil samples taken at the end of the experiment at Dornach showed no significant changes in Cd and Zn concentrations, but Cu concentrations were lower on subplots without *Thlaspi* than on subplots with *Thlaspi* (sown and transplanted). At Caslano, significantly lower soluble Cd and Zn concentrations were observed on the subplots without *Thlaspi* compared to the subplots with *Thlaspi*. Soluble Cu concentrations were higher in subplots without *Thlaspi* than in subplots sown with *Thlaspi*. However, the average soluble metal concentrations measured before the experiments (Table 1) were either higher (Cd) or similar (Cu, Zn) to the concentrations measured after plant growth.

DISCUSSION

Impact of the biomass on phytoextraction efficiency

Biomass of *Thlaspi* grown in the field is rarely published and estimates are often based on data extrapolated to a full surface cover by the plant (Robinson *et al.* 1998; Kayser *et al.* 2000). Yields obtained are highly variable; for example, McGrath *et al.* (2000) estimated an annual biomass of between 4 and 6 t DM ha^{-1} and Felix (1997) as high as 13.4 t ha^{-1}, whereas Schwartz (1997) measured a yield of less than 1 t ha^{-1}. In our experiments, we achieved only 1.8 t ha^{-1} for the first harvest at Caslano and 0.85 t ha^{-1} at Dornach, despite full plot covers and large individual transplant biomass (data not shown). Directly sown *Thlaspi* gave lower yields. If the same biomass could be maintained across all three harvests, an annual offtake of 3–6 t DM ha^{-1} would be expected.

Calculations of phytoextraction for total metals

For calculating the time required to decontaminate a soil by phytoextraction, McGrath *et al.* (2000) and Felix (1997) assumed that annual metal extraction would not change over time. Our results show that Cd and Zn concentrations in transplants decreased over three harvests at Caslano. A similar result was obtained for Cd at Dornach. However, for the sown *Thlaspi* no decrease was observed. It appears, therefore, that metal concentrations in plants are somewhat unpredictable but may stay constant throughout the period of phytoextraction in some circumstances. Consequently, as also proposed by McGrath *et al.* (2000), we used a zero-order kinetic equation, which assumes no decrease in plant heavy metal concentrations or yield, to calculate the number of years necessary to decrease the initial total metal concentration to the trigger value (TV) or the guide value (GV) of the Swiss ordinance (OIS 1998). The following additional assumptions were made: the depth for remediation is 0.2 m, soil density is 1.4 kg dm^{-3} at Dornach and 1.2 kg dm^{-3} at Caslano, and one cropping is taken each year. Table 4 summarizes the number of years necessary to decontaminate the soils of Cd and Zn using phytoextraction with either transplanted or sown *Thlaspi*. In the acidic soil

(Caslano) with transplants, it would take 4 and 9 years to reduce the initial Cd concentration (2.8 mg kg^{-1}) to the TV (2.0 mg kg^{-1}) and the GV (0.8 mg kg^{-1}), respectively. Similarly, the use of transplants at Dornach would lead to a decontamination within 7 years to the TV, and 33 years to the GV. As no TV exists for Zn in the Swiss legislation, the GV was taken as the target for phytoextraction. Table 4 shows that for both soils, it would not be feasible to use phytoextraction with *Thlaspi* to reduce Zn concentrations to the GVs. Table 4 also shows that sowing *Thlaspi* would be less efficient than transplanting. With similar calculations, Felix (1997) found it would require 53 years to bring down the Zn concentration at Dornach to the GV. He measured similar Zn concentrations in *Thlaspi* to ours, but he used an annual biomass of 13.4 t ha^{-1}, which appears to be an overestimate.

It may be more appropriate to use first- or even second-order kinetics to describe the decrease of total heavy metal concentrations in soil with time, but the kinetic parameters are not known and may vary from soil to soil. However, simple zero-order kinetics showed that it would take much less time for remediation of the acidic site than for the calcareous site, even though total metal concentrations at the former were higher.

Bioavailability and phytoextraction

The main concern related to Zn at Caslano is its high solubility in this soil. The $NaNO_3$-extractable concentration of about 7.4 mg Zn kg^{-1} was not reduced, and was possibly slightly higher after growing *Thlaspi*, although variability in topsoil metal concentrations made it difficult to identify significant differences. In contrast, Hammer & Keller (2002) found in a pot experiment that *Thlaspi* grown on the Caslano soil decreased the $NaNO_3$-soluble Cd to 25% and Zn to 57% of the initial concentration. In the field trial, soil samples were only collected after the third harvest, which gave a very low biomass. Therefore an explanation for this discrepancy might be that enough time elapsed between the first harvest and the soil sampling for the soluble Zn fraction to be replenished by Zn from less soluble pools. To assess the time required to decrease the soluble Zn concentration below the clean-up value, we would need to quantify the total plant available fraction in the soil and the equilibrium kinetics between this pool and the solid phase. Long-term studies with repeated *Thlaspi* crops would also be needed to obtain a clear picture of the change in bioavailability of these metals under field conditions.

Another explanation for an increase in soluble metals could be that root decay (roots were harvested in the pot experiment but not in the field trial) after each shoot harvest released heavy metals that were in a readily available form, as shown by Perronnet *et al.* (2001).

Transplanting is very labour intensive and it may not be feasible for large areas of contaminated land. However, the similarity in habit of *Thlaspi* to the edible corn salad (*Valerianella locusta*) suggests that similar multiplication and plantation techniques (OCVCM 1997) could be applied to *Thlaspi*, in order to mechanize the remediation procedure. In France, sowing of *V. locusta* is preferred to transplanting, and production is highly mechanized and performed on a large scale. In general, a layer of sand is applied on top of the sown seeds to facilitate harvesting. However, two limiting factors remain: the sowing and harvesting equipment is expensive and the availability of *Thlaspi* seeds for remediation of large areas remains limited.

CONCLUSION

In the framework of the Swiss legislation, phytoextraction with the hyperaccumulator *Thlaspi caerulescens* is a viable remediation technique for a soil that is moderately contaminated with Cd. However, our field experiments demonstrated that phytoextraction efficiency was dependent on both soil properties and the agricultural management. *Thlaspi* transplanted into the acidic soil produced the most efficient Cd phytoextraction. Evaluation of Zn decontamination was more difficult, because of large total Zn concentrations in the soil and a lack of knowledge about the potential of *Thlaspi* to reduce the soluble fraction of Zn in the long term. Similarities between *Thlaspi caerulescens* and the corn salad (*Valerianella locusta*) suggest that mechanization of phytoextraction using *Thlaspi* is feasible, if growing and harvesting techniques are adapted from corn salad production.

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