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Phytoextraction of Cd and Zn with *Salix viminalis* in field trials

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Abstract. Use of high biomass crops such as the willow *Salix viminalis* to extract metals for soil remediation has been proposed as an alternative to the low biomass-producing hyperaccumulating plants. High yields compensate for the moderate heavy-metal concentrations in the shoots of such species. We report the first long-term trials using *Salix viminalis* to extract heavy metals from two contaminated soils, one calcareous (5 years) and one acidic (2 years). Total metals extracted by the plants were 170 g Cd ha^{-1} and 13.4 kg Zn ha^{-1} from the calcareous soil after 5 years, and 47 g Cd ha^{-1} and 14.5 kg Zn ha^{-1} from the acidic soil after 2 years; in the first year outputs were negligible. After 2 years, *Salix* had performed better on the acidic soil because of larger biomass production and higher metal concentrations in shoots. Addition of elemental sulphur to the soil did not yield any additional benefit in the long term, but application of an Fe chelate improved the biomass production. Cd and Zn concentrations were significantly higher in leaves than stems, highlighting the necessity to collect leaves as well as shoots. On both soils, concentration in shoots decreased with time, indicating a decrease in extraction efficiency.

Keywords: Phytoextraction, cadmium, zinc, *Salix viminalis,* field experiment, high biomass crop

INTRODUCTION

Phytoremediation is the use of plants to decontaminate soil or to render the contaminants harmless. It has been proposed as an environmentally sound option for remediation of soils contaminated by heavy metals (Salt *et al.* 1995). In addition to exploiting the heavy metal uptake of plants (phytoextraction), other benefits of plant cover arise from their water use and root stabilization of the soil which help to prevent export of metals through wind erosion and leaching (phytostabilization). Many phytoextraction studies have been conducted with hyperaccumulating plants, which take up large amounts of heavy metals such as Ni, Zn and Cd and concentrate them in their above-ground plant parts (McGrath *et al.* 1993; Robinson *et al.* 1998). However, in general such species produce a small biomass, and their agronomic requirements are poorly understood, which decreases the efficiency of phytoextraction.

High yielding crops tolerant to metals have been studied in order to develop an alternative phytoremediation strategy to the hyperaccumulator approach. Plants such as *Helianthus annuus*, *Zea mays*, *Nicotiana tabacum*, *Brassica juncea* and *Salix viminalis* produce high annual biomass and have therefore been proposed for phytoextraction on moderately contaminated soils (Huang *et al.* 1997; Kayser *et al.* 2000). Additives such as chelates or acidifying agents, which increase availability of heavy metals to plants, have been used in combination with these species (Blaylock & Huang 2000; Kayser *et al.* 2000). Concentrations of Zn in leaves of different clones of *S. viminalis* grown in soils without additives were 20–330 mg kg^{-1}, and concentrations of Cd were 0.5–7.5 mg kg^{-1} after 20 days growth in a nutrient solution containing 1 mM Cd (Landberg & Greger 1996). High variability of metal uptake between clones of the same species may be an indication that the full potential of *S. viminalis* (and other willows) is still far from being fully exploited. Some of these high biomass plants may also have an additional economic value. For example, the biomass of *S. viminalis* could be used for bio-fuel production and heavy metals recovered after burning. Ledin (1996) observed that in Sweden the economic productivity of short-rotation forestry with willows (*Salix* sp.) was comparable to that of conventional food crops.

Tree species such as *Alnus*, *Betula* and *Salix* have been successfully used for the re-vegetation of heavily contaminated land which had been limed (Dickinson 2000). Punshon *et al.* (1995) and Punshon & Dickinson (1997) suggested that *Salix* might be tolerant enough to decrease the plant-available heavy metal load in contaminated soils, while still maintaining high yields. Felix (1997) tested *S. viminalis* on a Cd-rich calcareous soil in the Swiss Jura (c. 7 mg Cd kg^{-1} soil) and found Cd concentrations in the

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shoots to be 22 mg kg^{-1} dry matter (DM). Kayser *et al.* (2000) set up a long-term field experiment on a nearby calcareous soil with *S. viminalis* and other high biomass and hyperaccumulator species to study the impact of soil amendments such as nitrilotriacetate (NTA) and elemental sulphur on heavy metal uptake. They observed the highest concentrations in the hyperaccumulating species (max. 10 mg Cd kg^{-1}, 2500 mg Zn kg^{-1}) followed by 2-year-old *S. viminalis*. However, total uptake was greatest in *Nicotiana tabacum* (Cd) and *Helianthus annuus* (Zn and Cu) because of their high yields.

In order to follow the long-term extraction of Cd and Zn by *S. viminalis* with and without elemental sulphur addition, we continued the field experiment started by Kayser *et al.* (2000) for a further 3 years (5 years in total). The influence of soil properties on metal uptake and biomass production was assessed and results compared to those obtained with the same clone of *S. viminalis* on a contaminated acidic soil. The aim of these trials was to evaluate the potential of *S. viminalis* to decontaminate soils moderately laden with Cd and Zn. Results are also compared to those obtained with the Cd and Zn hyperaccumulator *Thlaspi caerulescens* grown on the same sites (Hammer & Keller 2003).

MATERIALS AND METHODS

Site descriptions

The two sites are described in Hammer & Keller (2003). The calcareous soil is located at Dornach, northwest Switzerland, and was contaminated with Cu, Zn and Cd emitted by a nearby brass smelter until the mid-1980s. Soil pH ($CaCl_2$) was 7.3, and concentrations of Cd, Zn and Cu extractable with 2 M HNO_3 were on average 2.3, 650 and 550 mg kg^{-1}, respectively, while those extractable with 0.1 M $NaNO_3$ were 2.2, 90 and 700 μg kg^{-1}, respectively, in the 0–0.2 m layer.

The acidic soil is at Caslano, southern Switzerland. Sludges from septic tanks were applied on the site for 20 years, leading to enrichment of the topsoil with organic matter and heavy metals. The pH ($CaCl_2$) was 5.2; average 2 M HNO_3-extractable Cd, Zn and Cu concentrations in the topsoil were 2.8, 1158 and 264 mg kg^{-1}, and the corresponding 0.1 M $NaNO_3$-extractable values were 13, 7400 and 400 μg kg^{-1}, respectively.

Experimental design

Dornach: the experiment was established in 1997 (Kayser *et al.* 2000). Two *Salix viminalis* cuttings were planted in 1.1 × 1.1 m subplots. In 1998, two to four cuttings were added to each subplot. The clone (Swedish clone 78198) was selected on the basis of results of Landberg & Greger (1996), who found it to be both tolerant of and able to accumulate Cd. Prior to each growing season, the subplots were fertilized with 120 kg P ha^{-1}, 200 kg K ha^{-1} supplied as Superphosphat® and Patentkali®, and 40 kg N ha^{-1} supplied as NH_4NO_3 + 7% MgO. To prevent plants from developing chlorosis, Sequestren rapid®, an Fe fertilizer, was applied as 24 kg chelated Fe per hectare (as Fe-EDDHA). In the second year (1998), elemental sulphur (36 mol m^{-2}) was applied to four subplots to increase the soluble heavy metal content (Kayser *et al.* 2000) by inducing acidity.

Caslano: the experiment was established in 2000. Eight plots were subdivided into four 1.0 × 1.0 m subplots. Sixteen of the subplots were planted with four cuttings of *S. viminalis* (same clone as at Dornach). Half of the plots were treated with Sequestren rapid® to protect *Salix* from chlorosis observed in a pot experiment (Hammer & Keller 2002), and to evaluate the impact of the treatment on plant biomass and metal uptake. In 2001 Sequestren rapid® was then applied to all plots. In 2000 no other fertilizer was applied, but in 2001 the same amounts of P, K and N as at Dornach were applied. No sulphur was applied.

Plant analysis

All aerial parts of the plants were harvested by cutting at 0.10 m above ground level and cleaned of soil. For the first two years, plant material from Dornach was processed as described by Kayser *et al.* (2000). For the three following years, plant samples were dried at 60°C for 5 days, leaves and stems were separated and the biomass of the different plant parts was determined for each plot. The same method was used for plant material from Caslano. Dried material was milled and processed as described in Hammer & Keller (2003).

Statistical analysis

Student *t* tests were carried out to determine significant differences ($P < 0.05$). Linear, sigmoidal and power-relationship fits were carried out using Microcal Origin® 6.0.

EXPERIMENTAL RESULTS

Biomass production

Dry matter yield over the 5-year period at Dornach is shown in Figure 1. The yield of both the control and of sulphur (S)-treated plots increased following a sigmoidal time function, but at different rates and with different final biomasses. Fittings using the Boltzmann's formula (equation 1) gave for the control plots: A_1=0.8, A_2=38.07, x_0=1999.8 and dx=1.734 with r^2=0.10; and for the S-treated plots: A_1= –4.71, A_2=35.74, x_0=1999.5 and dx=1.453 with r^2=0.98, with

$$y = (A_1 - A_2)/1 + e^{(x-x_0)dx} + A_2 \qquad (1)$$

Extrapolation of the curve suggests that the control would reach a plateau at about 40 t DM ha^{-1} during the following two years. The set of cuttings planted in 1998 did not grow well due to competition with the 1997 cuttings. The observed plateau might therefore be an upper limit because we used a high plant density, or because the plants became exhausted after repeated cutting. For the S-treated plots there was no clear upper biomass limit (Figure 1). Biomass of these plots estimated from the curve would be between 25 and 35 t DM ha^{-1} during the following two years.

Comparison of growth during the first two years at each site shows that *Salix* grew faster at Caslano than at Dornach (Figures 1 and 2). Although not statistically significant, *Salix* treated with Sequestren in both the first and second year seemed to produce more biomass in both years, even

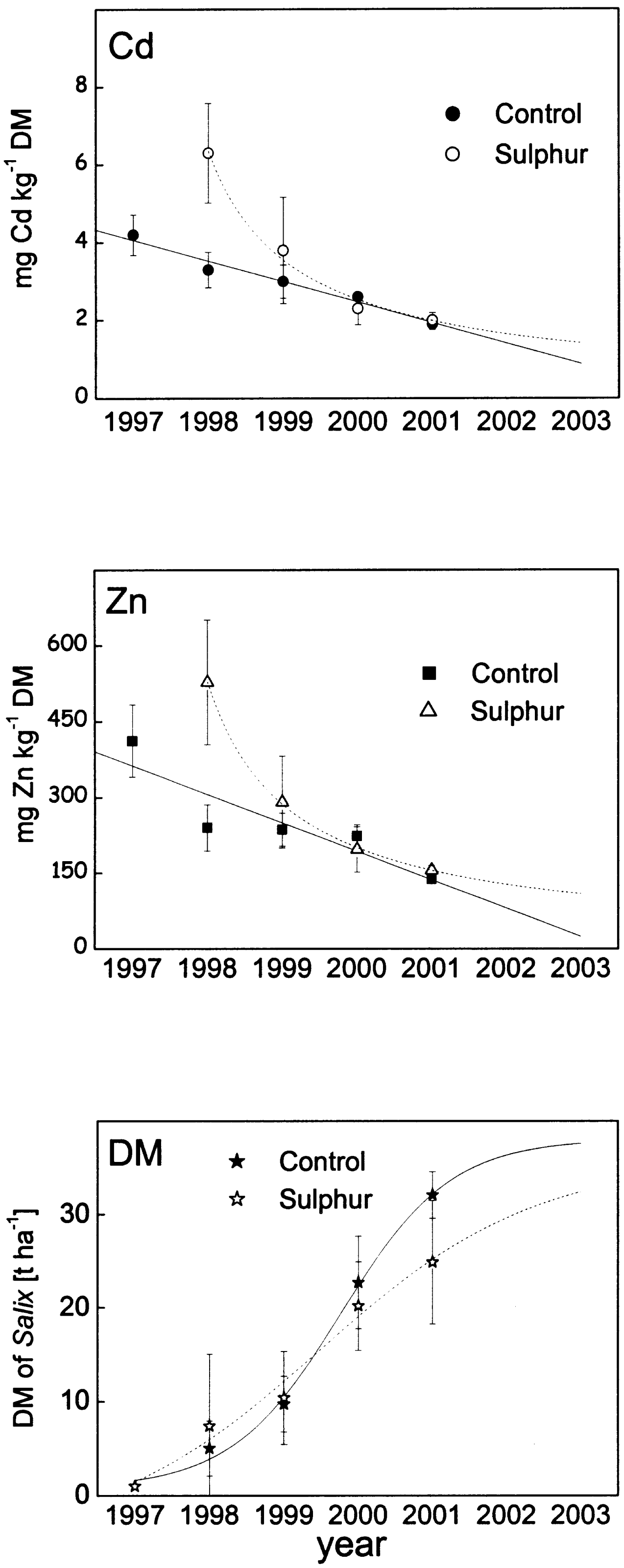

Figure 1. Metal concentrations in shoots and total biomass of *Salix viminalis* grown between 1997 and 2001 at Dornach (calcareous soil) with fitted lines and curves for untreated *Salix* (solid line) and S-treated *Salix* (dashed line).

though Sequestren was applied on all plots in the second year. Indeed, the plants that were not treated in the first year suffered from chlorosis and did not recover.

Heavy metal concentrations in shoots

Total Cd and Zn concentrations in the shoots are given in Figure 1 (Dornach) and Figure 2 (Caslano). At Dornach, in the absence of S, concentrations decreased linearly with time from about 4.2 to 1.9 mg kg^{-1} ($y = 1062 - 0.53x$, r^2=0.97**) for Cd, and 412 to 140 mg kg^{-1} (y = 113193 − 56.5x, r^2 = 0.80*) for Zn. In the first year, S application resulted in a significant (P<0.01) increase in concentration of Cd from 3.3 mg kg^{-1} to 6.3 mg kg^{-1} and Zn from 240 mg kg^{-1} to 530 mg kg^{-1}. Concentrations decreased in subsequent years according to a power relationship: $y = 529x^{-0.88}$ with r^2=0.99** and y = $6.35x^{-0.84}$ with r^2=1.00*** for Cd and Zn, respectively.

The first year Cd concentration in *Salix* grown at Caslano was similar to that measured at Dornach in 1997 (first year), but the Zn concentration was three times higher. By 2001, Cd and Zn concentrations had decreased substantially at Caslano. A similar decline was observed between 1997 and 1998 in the control plants at Dornach. The application of Sequestren in the first year at Caslano had no significant impact on metal concentrations. Cd concentrations were between 1.5 and 3 times higher in leaves than in stems, and Zn concentrations between 4 and 8 times higher (Table 1), irrespective of the soil location. A similar proportional decrease in concentration over time was observed in both plant parts.

Total extraction of Cd and Zn from soil (Figure 3)

The total metal uptake (metal concentration × yield) at Dornach increased between 1997 and 2001 for both the control and S subplots. This uptake followed the same trend as the biomass production, indicating that metal concentrations only slightly influenced the annual metal uptake. Total metal uptake over the five years in control and S-treated plots did not differ significantly. The total uptake of Cd was 170 and 197 g ha^{-1} and Zn uptake was 13.4 kg and 17 kg ha^{-1} in the control and the S treatment, respectively. When data from an anomalous subplot leading to a high standard deviation in 1998 were removed, both treatments led to similar total Cd and Zn uptake. Metal uptake at Caslano (with Sequestren in both years) was twice (47 g Cd ha^{-1}) and 8 times (14.5 kg Zn ha^{-1}) greater than at Dornach (control only) when uptakes after two years were compared.

DISCUSSION

Long-term phytoextraction

Long-term studies with fast growing trees and a direct comparison with a hyperaccumulating plant such as *Thlaspi caerulescens* have not been carried out under field conditions, except in the work of Kayser *et al.* (2000). However, they used data from 1- and 2-year-old *Salix* and were not able to calculate output from mature plants. We observed at Dornach that although the plants were cut every year the biomass increased, whereas metal concentrations decreased linearly during the 5 years of experimentation (Figure 1),

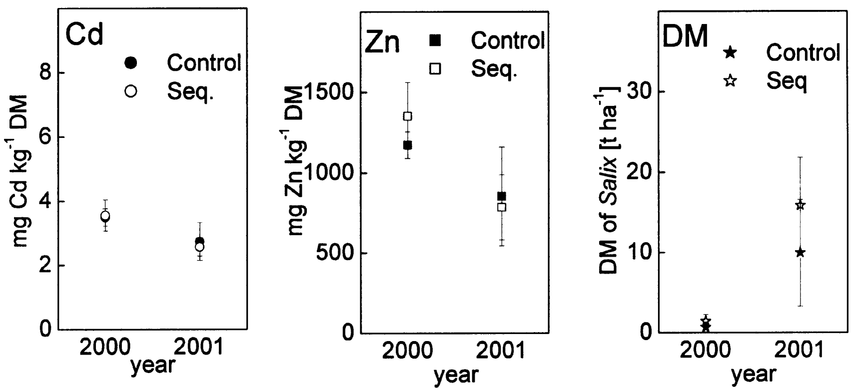

Figure 2. Dry matter (DM) and Cd and Zn concentrations in *Salix viminalis* grown at Caslano in 2000, with and without Sequestren (Seq) and 2001 with Sequestren.

Table 1. Cadmium and Zn concentrations (mg kg^{-1} DM) in leaves and stems of *Salix viminalis* at Dornach and Caslano.

	Cd	1997	1999	2000	2001
Dornach:	Leaves	6.0±1.9	5.1±0.7	6.3±0.9	3.7±0.3
	Stems	3.6±0.3	2.3±0.1	1.9±0.1	1.5±0.1
Caslano:	Leaves	–	–	5.1±0.9	3.6±0.7
	Stems	–	–	2.6±0.4	2.2±0.4
	Zn				
Dornach:	Leaves	1110±110	665±50	860±95	385±5
	Stems	200±20	120±10	110±1	70±5
Caslano:	Leaves	–	–	2695±505	1700±465
	Stems	–	–	570±135	430±85

resulting in an increasing annual metal uptake with time (Figure 3). The results of the first 2 years showed that Cd concentrations in the plants were slightly lower at Caslano, although concentrations of total and $NaNO_3$-extractable Cd in soil were higher than at Dornach (Hammer & Keller 2003), indicating that these factors were not the only ones having effect on metal uptake. Zn concentrations in plants were about 3 times higher at Caslano than at Dornach because total Zn concentration in soil was higher and the pH lower, causing a higher $NaNO_3$-extractable Zn concentration in the acidic soil.

The decrease in metal concentrations in plants from the first year to the second year is likely to continue as was observed in Dornach. In Dornach, we observed that *Salix* roots grew deeper with time (Keller *et al.* 2003). As soil metal concentrations decrease with depth this may lead to less metal uptake and therefore lower metal concentrations in the plants. We can thus hypothesize that the decrease of the metal concentration will stop at Dornach because most of the roots are in the uncontaminated zone, but continue at Caslano until all roots have reached the uncontaminated zone. The use of *Salix* for phytoextraction is therefore likely to be more efficient and appropriate on deeply contaminated soils.

The solubilization of heavy metals by elemental S applied to the Dornach soil in 1998 did not significantly affect cumulative metal extraction over five years. To have a positive long-term effect on plant metal concentration, the S application would have to be repeated several times as proposed by Gupta *et al.* (2000). However, the lower biomass production of S-treated *Salix* throughout the five years' experiment may be an indication that the extra acidity had an adverse effect on the growth of this plant, and repeated S application should be avoided.

Decontamination potential (Table 2)

To evaluate the phytoextraction potential of *Salix viminalis* the number of years necessary to extract Cd and Zn from 0–0.2 m and 0–0.6 m were calculated. Biomass data for the final year of experiments was taken and plant metal concentrations were used from the first harvest (1997 at Dornach and 2000 at Caslano) to calculate decontamination to a depth of 0.2 m, assuming that roots were prevented from going deeper. As *Salix* roots colonized mainly this zone in the first year, it was assumed that the concentration in the plants represented metal extracted from this zone. This also allows comparison between uptake by *Salix* and *T. caerulescens* as the roots of the latter plant species are largely confined to this zone. The calculations for decontamination down to 0.6 m were based on the plant metal concentrations of the last harvest and metal concentrations of the whole soil profile (data not shown). In some cases decontamination of the whole soil profile might be needed and *Salix* is a perennial plant that can rapidly grow its roots below 0.2 m, taking up metals from deeper horizons. Two levels of total heavy metal concentration in soil were chosen as target decontamination values according to the Swiss Ordinance (OIS 1998). The guide value (GV) corresponds to the concentration above which the long-term functionality of the soil is no longer assured (0.8 mg Cd kg^{-1} soil and 150 mg Zn kg^{-1}). The trigger value (TV) is the limit (2 mg Cd kg^{-1}), above which a site-specific risk assessment has to be carried out, which may lead either to a change in the land use or to the necessity to remediate the soil. The GV was taken as target value for Zn decontamination because no TV exists for this element; however, there is no legal obligation to remove Zn to this level.

The calculations for phytoextraction of a 0.6 m deep profile show that neither TV nor GV values for either of the metals would be reached within 10 years, which is assumed

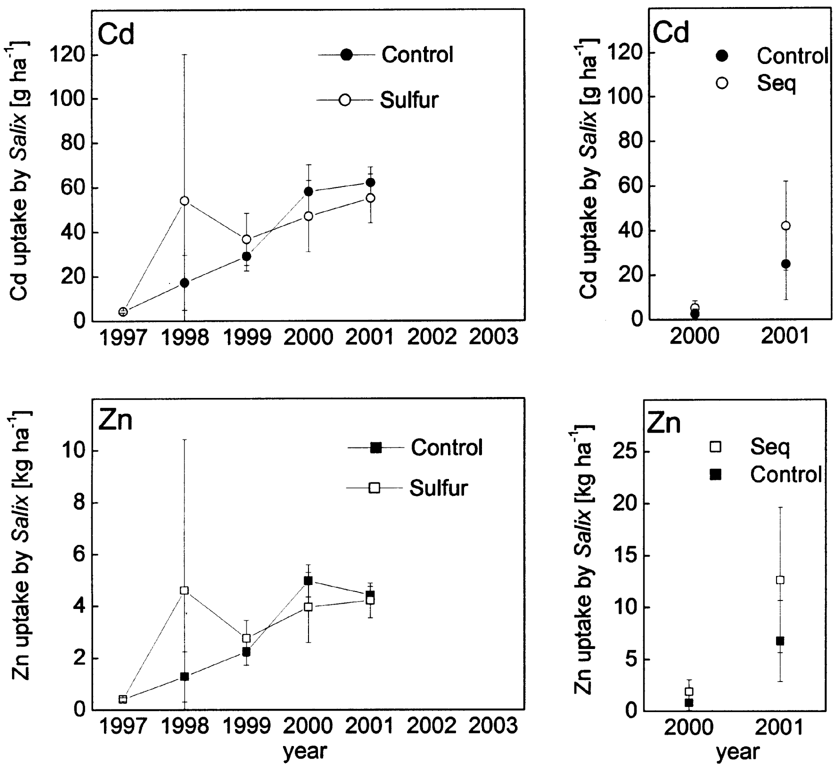

Figure 3. Total metal uptake by *Salix viminalis* grown at Dornach between 1997 and 2001 and Caslano in 2000 and 2001. Seq, with Sequestren.

Table 2. Number of years necessary to phytoextract Cd and Zn from Dornach and Caslano, using the experimental field data in order to reach either the guide values (GV) or the trigger values (TV) of the OIS (1998).[a]

	To depth	Cd (GV)	Zn (GV)	Cd (TV)
Dornach	0.2 m	31	86	6
	0.6 m	142	460	18
Caslano	0.2 m	87	113	35
	0.6 m	175	334	44

[a]Calculations are based on metal concentrations in 1-year-old *Salix viminalis* for 0.2 m, and the last year (2001) concentrations for calculations to 0.6 m with a soil density of 1.4 kg dm^{-3} at Dornach and 1.2 kg dm^{-3} at Caslano.

to be a 'reasonable' time span. In contrast, although technically difficult to achieve, calculations assuming that the roots colonized only the first 0.2 m of the soil indicated that the Cd TV would be reached within 6 years at Dornach and 35 years at Caslano. However, in this case (i.e. shallow contamination) the extraction potential of *Salix* is less than that of *Thlaspi caerulescens* (Hammer & Keller 2003).

Phytostabilization
Salix remains an option for revegetation and soil stabilization purposes, for example, on former mining sites as proposed by Dickinson (2000), owing to its ability to tolerate high heavy-metal concentrations in soil. On our soils, *Salix* performed well if it was given Fe. We have shown that *Salix* could be clear-felled every year prior to leaf fall and still produce an increasing annual biomass. In our study, the limit of annual biomass production seemed to be about 40 t ha^{-1} in Dornach. Literature data suggests, however, that this may be an upper limit (Kopp *et al.* 1997) because we used a high density of plants.

Management of Salix viminalis
The general management of *Salix* used for bio-energy is well described and standardized (for Sweden: Danfors *et al.* 1998) and thus could be adapted for phytoremediation purposes. A lower planting density might be necessary to allow access of machinery used for planting and harvesting. However, in Switzerland and most European countries there is currently no market for coppiced willow bio-fuel.

In general, willows for bio-energy are cut down in winter, when leaves have already fallen. When *Salix* is grown on metal contaminated sites, end-of-summer harvest would be necessary to collect the leaves. It is also necessary to ensure that animals do not consume the foliage.

CONCLUSION

We focused on one clone of one species of *Salix* and compared its phytoextraction potential with the hyperaccumulator *T. caerulescens* (Hammer & Keller 2003). Under the chosen conditions *T. caerulescens* seemed to be better adapted to decontaminate the surface layer of both soils within a reasonable time span. However, owing to the results obtained it is probable that given soils with deeper contamination, *Salix* would perform better and could be used as part of a phytoremediation scheme. Moreover, its tolerance to a range of heavy metals and its fast growth make it a good candidate for stabilization of contaminated soil. The reported results from these field experiments emphasize the need for long-term field trials in order to evaluate the full potential of these plants to extract metals from soils.

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