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Root development and heavy metal phytoextraction efficiency: comparison of different plant species in the field

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Abstract

Heavy metal phytoextraction is a soil remediation technique which implies the optimal use of plants to remove contamination from soil. Plants must thus be tolerant to heavy metals, adapted to soil and climate characteristics and able to take up large amounts of heavy metals. Their roots must also fit the spatial distribution of pollution. Their different root systems allow plants to adapt to their environment and be more or less efficient in element uptake. To assess the impact of the root system on phytoextraction efficiency in the field, we have studied the uptake and root systems (root length and root size) of various high biomass plants (*Brassica juncea, Nicotiana tabacum, Zea mays* and *Salix viminalis*) and one hyperaccumulator (*Thlaspi caerulescens*) grown in a Zn, Cu and Cd contaminated soil and compared them with total heavy metal distribution in the soil. Changes from year to year have been studied for an annual (*Zea mays*) and a perennial plant (*Salix viminalis*) to assess the impact of the climate on root systems and the evolution of efficiency with time and growth. In spite of a small biomass, *T. caerulescens* was the most efficient plant for Cd and Zn removal because of very high concentrations in the shoots. The second most efficient were plants combining high metal concentrations and high biomass (willows for Cd and Zn and tobacco for Cu and Cd). A large cumulative root density/aboveground biomass ratio (L_A/B), together with a relative larger proportion of fine roots compared to other plants seemed to be additional favourable characteristics for increased heavy metal uptake by *T. caerulescens*. In general, for all plants correlations were found between L_A/B and heavy metal concentrations in shoots ($r = 0.758^{***}$, $r = 0.594^{***}$, $r = 0.798^{***}$ ($P < 0.001$) for Cd, Cu and Zn concentrations resp.). Differences between years were significant because of variations in climatic conditions for annual plants or because of growth for perennial plants. The plants exhibited also different root distributions along the soil profile: *T. caerulescens* had a shallow root system and was thus best suited for shallow contamination (0.2 m) whereas maize and willows were the most efficient in colonising the soil at depth and thus more applicable for deep contamination (0.7 m). In the field situation, no plant was able to fit the contamination properly due to heterogeneity in soil contamination. This points out to the importance and the difficulty of choosing plant species according to depth and heterogeneity of localisation of the pollution.

Introduction

Plants that are used to extract heavy metals from contaminated soils have to be the most suitable for the given contamination. This includes tolerance to specific heavy metals, adaptation to soil and climate characteristics, heavy metal uptake capability and spatial fitting of roots to pollution distribution. At present, there are basically two phytoextraction strategies available: one is the use of hyperaccumulators like *Thlaspi caerulescens* or *Alyssum bertolonii*, as proposed by McGrath (1998) or Robinson et al.

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(1998): these plants take up specifically one or two metals and produce often a low biomass compensated by very high metal concentrations in the shoots (Baker and Brooks, 1989; Reeves and Baker, 2000). The other option is the use of high biomass plants that are usually not metal-specific and contain low to average heavy metal concentrations which are compensated by their high biomass. The knowledge gathered over years on their agronomic requirement is an additional advantage. In that case, however, additives such as chelating agents (EDTA, NTA) are very often needed to enhance heavy metal uptake (Blaylock and Huang, 2000) but they may cause leaching off-site and thus threaten the surrounding environment and/or the groundwater (Vangronsveld and Cunningham, 1998).

For all the plants, root system morphology is a compromise between costs of resource capture, transport and prospecting efficiency. The resulting pattern allows some plants to be more efficient than others in nutrient uptake in the case of infertile soils or other stress conditions (Fitter and Stickland, 1991). On the other hand, the root system possesses a certain phenotypic plasticity (de Kroon and Hutchings, 1995): variability of the root system development enables plants to cope with a wide range of soil factors and the heterogeneity and patchiness of the soil. The opportunistic strategy for major nutrients was already discovered in the last century. Eissenstat and Caldwell (1988) and Campbell and Grime (1989) found preferential root proliferation in favourable (fertilised) micro sites, but root reactions also depended on element mobility in the soil (Fitter, 1987). On this point dicotyledones (including hyperaccumulators) exhibit a higher plasticity than grasses (Eissenstat, 1992; Taub and Goldberg, 1996). Concerning heavy metals, a major difference between *T. caerulescens* (hyperaccumulator) and non-accumulating plants is that *T. caerulescens* exhibits also a preferential root development where heavy metals are present (Schwartz et al., 1999; Whiting et al., 2000), thus enhancing their ability to both accumulate specific heavy metals and remediate efficiently heterogeneously contaminated soils. As for major elements one can also expect different root reactions depending on metal distribution and mobility. But unlike high biomass crops, most of hyperaccumulators (*Thlaspi, Alyssum*) seem to have a restricted root system (Ernst, 1996) although this is not a general rule. In non-accumulating and tolerant plants, trace element resistance may be due to physiological adaptation mechanisms which allow some plants to grow roots in contaminated soils better than others (Palazzo and Lee, 1997). Avoidance of heavy metal contaminated zones has also proven to be an efficient survival strategy, especially for trees (Dickinson et al., 1991). For example, it has been shown that beech roots grew differently while passing through different Pb-contaminated layers, thus allowing the plants to avoid the most toxic parts of the environment (Breckle and Kahle, 1992). The same behaviour was observed with 2,4,6-Trinitrotoluene (TNT) and parrot feather (*Myriophyllum spicatum*) in a flooded soil: while the plant was very efficient in breaking down this compound, after a certain time new roots avoided the hot spots of TNT in the soil (Schnoor et al., 1995).

From what has been said above, it can be understood that plants may react differently to the presence of heavy metals or stress conditions. The ability of the root system to pump heavy metals and to fit and colonise the contaminated zone are thus key factors for phytoextraction efficiency, along with translocation to the shoots and accumulation. Their tolerance and specific behaviour at the root level must be taken into account while choosing plants for phytoextraction.

Root systems of different plants can only be compared if the soil and climate characteristics are similar. For a given site, one can then expect differences between plants due to their genetic background and also to their sensitivity to stress factors such as heavy metals. Also, the ability to accumulate heavy metals in the shoots might be related to the ratio shoot biomass/root biomass or length.

In order to investigate the impact of the root system on phytoextraction efficiency, we have studied in the field the uptake and the root systems (root length and root size) of various crop plants (*Brassica juncea, Nicotiana tabacum, Zea mays* and *Salix viminalis*) and one hyperaccumulator (*T. caerulescens*) and compared them with heavy metal concentrations along the soil profile. Changes from year to year have been studied for an annual (*Z. mays*) and a perennial plant (*S. viminalis*) in order to assess the impact of the climate on root systems and the evolution of efficiency with time and growth.

Materials and methods

Site description

The site and the experimental design have been already described in details in Kayser et al. (2000). Briefly, the site is located in the village of Dornach

near Basel, NW of Switzerland. It has been contaminated by atmospheric emissions of Cd, Cu and Zn from a nearby brass smelter over approx. 4.5 km^2 (Hesske et al., 1998). The field experiment is located 300 m east of the smelter.

The mean annual temperature (1960–1990) is 9.5 °C and mean annual rainfall is 790 mm (Basel-Binningen, meteorological station, SMA, 1992). Evapotranspiration represents 65% of the total rainfall. The experiment was performed in 1997, 1998 and 1999. The year 1997 was fairly similar to the average although warmer (10.7 °C) and sunnier with less rainfall (750 mm). The year 1998 was as warm as 1997 but with a close-to-average rainfall amount (803 mm). The year 1999 was steadily warmer (10.9 °C) and with more rainfall (1151 mm).

The soil has been classified as a calcaric regosol disturbed by human activities and has been already described by Kayser et al. (2000). The pH_{CaCl_2} is around 7.3 at the surface and increases with depth till 7.6 at 0.6 m. Calcium carbonate content varies between 100 and 400 g kg^{-1}; organic carbon content is 35 g kg^{-1} in the first 0.2 m and decreases slowly. Clay content is the highest at the surface with 380 g kg^{-1} in the first 0.1 m then remains around 320 g kg^{-1}. Silt and sand are around 330 g kg^{-1} each. Two-molar HNO_3-extractable heavy metal concentrations are on average 2.5 mg Cd kg^{-1}, 650 mg Zn kg^{-1} and 550 mg Cu kg^{-1} in the first 0.2 m. 0.1 *M* $NaNO_3$-extractable concentrations are low with 2.3, 100 and 750 μg Cd, Zn and Cu kg^{-1}, resp. More details on this point can be found in the results section.

Experimental design

The field experiment comprises 28 plots (divided into four subplots labelled A, B, C and D) of 2.5×2.5 m each. This allowed four replicates of seven plant species (*Brassica juncea*, *Nicotiana tabacum*, *Zea mays*, *Salix viminalis*, *Helianthus annuus*, *T. caerulescens* and *Alyssum murale*) in the first year (Kayser et al., 2000). Not all plants could be sampled the first year and only Indian mustard (*B. juncea* cv. 426308) and tobacco (*N. tabacum* cv. Badischer Geudertheimer) were sampled that first year. The second year the same species were planted and willows were left to re-grow from the first year stumps. Three out of four subplots for all plants were treated with additives (1 and 2 mmol nitrilotriacetate (NTA) kg^{-1} soil and 36 mol S_8 m^{-2} soil) in order to enhance phytoextraction (Kayser et al., 2000) and one subplot of each plot was left as control. Maize (*Z. mays* cv. LG11) and 2-year-old willows (*S. viminalis* clone No. 78198) were sampled the second year in the control subplots. The third year all plots except willow and hyperaccumulator plots were planted with maize. The control subplots planted with maize, willows (3-year-old) and *T. caerulescens* (Les Avinières population, St-Laurent-Le-Minier, France) subplots were sampled (all control subplots without treatment).

Indian mustard and maize were sown respectively at 0.3 m and 0.65 m row distance. Tobacco and *T. caerulescens* were planted as pre-grown seedlings at a rate of 27 890 plants (row space=0.6 m, between plant space=0.5 m) and 1 000 000 (row space=0.1 m) seedlings per ha, respectively. Willows were planted as woodcuttings the first year at a density of eight cuttings per plot that is 12 800 cuttings per ha. Without removing the plants, other cuttings were added after 1 year. However, competition with the older plants resulted in a very limited growth of these new plants. All plants were sown or planted beginning of May except *T. caerulescens*, which was planted end of July. The *T. caerulescens* plots were covered to prevent direct sun and watered manually according to need.

The following fertilisation plan was applied each year on all plots: 120 kg ha^{-1} P (Superphosphat®) 200 kg K ha^{-1} (Patentkali®) and 40 kg N ha^{-1} (NH_4NO_3+7% MgO) for *T. caerulescens*, Indian mustard, willows, 120 kg N ha^{-1} for maize, and 200 kg N ha^{-1} for tobacco. This fertilisation was meant to cover all plant needs. Additionally, Fe fertilizer (Sequestren rapid®) was applied to *T. caerulescens* and willows to prevent chlorosis at a rate equivalent to 24 kg Fe ha^{-1}. Weeds were removed either by harrowing or manually. Overall biomass and concentration results of the first 2 years are reported in Kayser et al. (2000).

Plant harvest and analysis

Indian mustard, tobacco, maize and 2-year-old willows were sampled and treated as described by Kayser et al. (2000): aerial parts of the plants were bulked and harvested the first week of September. Fresh weight was measured after cleansing of the plants. Each sample was coarsely ground. A sub-sample was taken for dry matter determination after drying at 105 °C and another sub-sample was dried to 65 °C, finely ground and 0.25 g were microwave-digested in a mixture of 5 mL HNO_3 (65%), 2 ml H_2O_2 (30%) and 2 mL purified water (Milli-Q reagent grade water). The third year, 3-year-old willows and maize were harvested the

third week of September due to a delayed growth season. Shoots were dried to 65 °C until constant weight was reached. Willow leaves were separated from stems and weighed separately. Fallen leaves were collected and added to the leaves biomass. *Thlaspi caerulescens* was harvested mid of October, that is $2\frac{1}{2}$ month after planting and processed as described above for willows. Finely ground samples (0.5 g) were digested in 8 mL HNO_3 (65%) and evaporated. The residue was re-dissolved in 1 mL $HClO_4$ (70%) and heated 1 h to 235 °C before dilution to 20 mL with purified water. All samples were run together with certified reference material.

All glassware and PE-flasks used for digestion and stock solutions were soaked in 10% HNO_3 (v/v) overnight and rinsed three times with purified water.

Soil and root sampling and preparation

Immediately after plant harvest (second harvest only for Indian mustard), soil cores were taken for soil analysis and root measurement. A Humax auger of ∅ 0.05 m (Max Hug, Luzern, Switzerland) was used to collect four undisturbed core soil samples per sampling profile down to 0.75 m depth. The first year the four auger profiles were taken at a distance of 0.75 m from the plot edges with two profiles taken between two rows and two within rows. The second and third years, profiles were taken at a distance of 0.25 m from the edges of the subplots. For willows they were sampled so that two profiles were taken next to plant stems and two were sampled at equal distance between two plants. For *T. caerulescens*, the choice was made at random since the plant density was high. In some cases, due to the presence of stones, the depth of 0.75 m could not be reached (e.g. Indian mustard). Each profile was divided into 0.10-m-thick layers and the four samples from the same depth were mixed in order to obtain one sample per depth and per plot. For each mixed sample and before root washing, one sub-sample was taken directly by separating the roots from the soil by hand in order to perform soil analysis.

Furthermore, 2×1 m^2 outside the field experiment were infiltrated with the mobile dye tracer Brilliant Blue FCF (C.I. Food Blue 2, C.I. 42090; Flury and Flühler, 1995) as described by Flury et al. (1994) and profiles were dug in order to evaluate infiltration paths and detect possible compacted layers. These profiles were also analysed every 0.1 m for soil characteristics.

After the soil sample was taken for analysis, roots were washed with a hydro-pneumatic washer (Smucker et al., 1982) in order to collect all roots with a diameter >75 μm. The largest organic particles other than roots were removed using tweezers. Roots were kept frozen until measurement. Additionally, roots were collected manually from the topsoil (0–0.2 m) samples for heavy metal analysis. They were cleansed from soil and washed for 5 min with 0.02 *M* EDTA (ethylenediaminetetra-acetic acid) to remove metals adsorbed onto the roots. The roots were then rinsed three times with deionised water before being dried at 80 °C. They were prepared like the aerial parts for digestion and analysis.

Soil analysis

Soil samples were dried at 40 °C, and 2 mm-sieved samples were analysed for 2 *M* HNO_3- and 0.1 *M* $NaNO_3$-extractable Cd, Cu and Zn concentrations (FAC, 1989) and pH (0.01 *M* $CaCl_2$). Other characteristics were determined on the two soil profiles outside the experiment following the FAL (1998) procedures.

Soil and plant samples were always run together with reference materials. Zinc, Cd and Cu were measured by flame atomic absorption spectrometry (Flame-AAS, VarianSpectrAA 400) or inductively coupled plasma atomic emission spectroscopy (ICP-AES, Perkin Elmer Plasma 2000). Cadmium was analysed by graphite furnace atomic absorption spectrometry (VarianSpectrAA 300 or Perkin Elmer HGA 600 Zeeman-corrected graphite furnace) in soil $NaNO_3$ extracts and digested plant samples.

Root measurement

Root length is a parameter that expresses the potential for absorption of nutrients or water from soil (Atkinson, 2000). It has also been found to show more statistical differences between experimental treatments than total root weight in the case of wheat (Box and Ramseur, 1993) and it is more important for solute uptake than root weight (Nye and Tinker, 1977). It was measured either manually (roots with diameter >4 mm) or using a desktop scanning device and is expressed as root length density (L_V) for the different soil layers or as cumulative root density (L_A) calculated for the uppermost 0.2 or 0.75 m of soil depth. Root diameter was measured because it responds to soil physical conditions (Atkinson, 2000) and diameter distribution was determined for each depth. Surface area of cylindrical roots was also calculated. Two different systems were used for the measurements of the root samples of

Table 1. Average HNO_3- and $NaNO_3$-extractable concentrations of heavy metals for the soil profiles sampled in the Dornach field experiment

Depth in m	pH_{CaCl_2}	HNO3-extractable[a]			$NaNO_3$-extractable[b]		
		Cd mg kg^{-1}	Cu mg kg^{-1}	Zn mg kg^{-1}	Cd μg kg^{-1}	Cu μg kg^{-1}	Zn μg kg^{-1}
0–0.1	7.2±0.1	2.0±0.4	529±60	654±80	2.1±0.5	598±154	77±30
0.1–0.2	7.3±0.1	2.0±0.5	520±64	636±82	1.9±0.7	722±112	81±40
0.2–0.3	7.3±0.1	2.0±0.5	460±128	601±147	2.3±0.7	705±214	123±65
0.3–0.4	7.3±0.1	1.8±0.6	370±163	521±220	2.0±1.3	539±257	83±72
0.4–0.5	7.4±0.1	1.6±0.7	255±167	371±228	1.1±0.1	297±205	22±8
0.5–0.6	7.5±0.1	1.3±0.7	184±201	270±272	0.8±0.4	83±51	30±18
0.6–0.7	7.4±0.2	0.8±0.5	73±107	116±150	1.5±0.8	60±49	20±0
0.7–0.75	7.5±0.1	0.5±0.5	18±17	36±18	4.2	8	bdl[c]

[a] 20 plots.
[b] 3 plots.
[c] bdl=below detection limit.

the three experimental years. However, differences between the two methods were found to be negligible. Extraneous objects in the root samples with a length-to-diameter ratio of less than three were excluded from the root data.

Before scanning roots were thawed and stained with Pararosaniline chloride (No. P1528, Sigma). Thereafter, roots were spread in a glass tray in 2–3 mm of water. If necessary, root samples were divided into sub-samples for more accurate measurement. The first year, roots images were analysed with the system described by Chassot et al. (2000). The system RHIZO (Régent Instruments, Quebec, Canada, 1995) was employed for the second and third years samples. The software WinRHIZO (version Pro 3.10b) was used to analyse images acquired using a desktop scanner STD1600 (Epson) provided by Régent Instruments. It is equipped with a positioning system (for trays receiving the roots) and two light sources preventing shadows and allowing for distinction between overlapping roots and forks. No root staining was used for the second method. Limitations and accuracy of the technique have been tested by Bauhus and Messier (1999). All measurements were carried out at a resolution of 400 dpi.

Statistical data analysis

Pearson correlation coefficients were calculated between the different plant parameters. Mean values for the root parameters were compared using paired Student *t* test. All tests were performed with SYSTAT® 8.0 from SPSS Inc (1998).

Results

Compaction of the soil profiles

Pore distribution and compaction within the soil profile were assessed by the brilliant blue infiltration test. These factors are known to have an impact on root characteristics (Bennie, 1996). Both profiles had a well-distributed and fine porosity, but a compacted layer with reduced infiltration and few preferential pathways was found in one of the profile between 0.2 and 0.4 m. This pattern is expected to occur in some of the profiles dug within the field experiment perimeter and thus may locally influence root penetration and density. In some plots, this compacted layer was indeed visually observed or detected when taking soil cores, although no quantitative measurements were done.

Distribution of the heavy metal contamination in the soil profiles

The heterogeneity of the topsoil was less pronounced than in the subsoil as shown in Table 1 by the average HNO_3-extractable concentrations obtained for all the 20 plots sampled (eight plots were sampled twice but only 1 year was taken into account). Standard deviations increased between 0.2 and 0.7 m, which expressed an increasing heterogeneity of contamination in depth: the contaminated layer varied between 0.2 and 0.7 m of thickness. For example in some profiles Zn concentration was already less than 50 mg kg^{-1} at 0.4 m (with a sharp decrease below 0.2 m) whereas in other profiles it was still above 250 mg

kg^{-1} at 0.75 m. Fifty mg kg^{-1} corresponded to the Zn background concentration at the site (although the Swiss guide value for total Zn in soil is 150 mg kg^{-1} (OIS, 1998)) and for the profiles analysed, the decrease was always sharp between a value close to 250 mg $kg^{-1}$and the background value. As Cd, Zn and Cu contaminations were present simultaneously as shown in Table 1, phytoextraction of the three metals would be desirable in order to comply with the Swiss legislation based on three levels of concentration (guide, trigger and clean-up values) for both total and soluble metals (OIS; 1998).

Plant concentrations and removal of heavy metals

Plant concentrations and uptake are shown in Table 2. Copper concentrations and outputs (concentrations× biomass) were small for all plants. *Thlaspi caerulescens* gave the largest Cd and Zn outputs due to high shoot concentrations, whereas 3-year-old willows came second mostly because of much larger biomass production. Cadmium output by tobacco was similar to the one calculated for willows (harvest 1999). Maize gave low Cd outputs although it had the highest biomass. Indian mustard gave the lowest results because of a small biomass and low concentrations. More detailed results on 1st and 2nd year-maize, I. mustard and tobacco can be found in Kayser et al. (2000). For all plants analysed, root concentrations were always low and below shoot concentrations, with a shoot/root ratio above 1. Transfer coefficients (TC=concentration in the aboveground biomass/concentration in the 0–0.1 m soil layer, Sauerbeck (1989)) were below 1 for all plants for Cu and Zn except *T. caerulescens* (TC_{Zn}=8). For Cd, TC was between 1 and 2 for all plants except I. mustard and maize (TC_{Cd} less than 0.5) and *T. caerulescens* (TC_{Cd}=91). *Thlaspi caerulescens* reached the hyperaccumulation level for Cd (more than 0.01% Cd in DM) but not for Zn (less than 1% Zn in DM) as defined by Reeves and Baker (2000).

Root length distribution

Table 3 gives the average cumulative root density (L_A) along the soil profile for all species as well as some other plant root characteristics. There were large differences between plant species: calculated for a soil depth of 0.75 m, the largest L_A was found for willows and maize and the lowest for *T. caerulescens*. Willows, maize and tobacco colonised best the first 0.2 m with their largest root length in this layer. However, there were large variations within plant species as emphasised by standard deviations calculated for the four plots. There were also large variations from year to year for maize (planted twice): climatic conditions as well as harvest time might have influenced the results. Differences observed for willows included both the L_A and the root density along the soil profile with more roots at depth the second year of measurement. Apart from this perennial plant (willows), there seemed to be steadiness in maximum root depth and root distribution patterns: *T. caerulescens* exhibited a shallow root system with length per soil volume decreasing rapidly with depth. This was to be expected for a plant growing naturally in harsh environments, and which was only $2\frac{1}{2}$-month-old. All the other plants had grown roots down to 0.75 m. However, root density decreased rapidly for I. mustard and tobacco (10 and 22 times less roots at the maximal rooting depth than in the first 0.1 m resp.) whereas it remained more stable for willows and maize (both years) with resp. three and five times less roots at depth.

Root area and root diameter

Differences between plants species were significant. In addition, the year seemed to have a large impact on this factor: for example, the root area of maize was divided by two between 1998 and 1999. Although no impact of age could be seen on L_A for willows root area increased with age. Also, L_A was rather small for *T. caerulescens*, but its root area was on average larger than that of I. mustard and tobacco. Most of the roots were found in the smallest diameter classes (0–100 and 100–200 μm) for all the species (Figure 1 shows root diameter distribution over depth for some of the plots). In general for all plots there was a sharp decrease in root length between 200 and 500 μm and then again after 800 μm. In contrast to the other species, *T. caerulescens* and willows collected in 1998 had a large proportion of roots from the layer 0–0.1 m in the 0–100 μm class. For maize and willows from 1998 the root diameter class with the largest root length was shifted towards larger diameters with increasing soil depth.

Root characteristics and plant concentrations and uptake of heavy metals

Due to its special characteristics (high accumulation levels of Cd and Zn and low biomass) in compar-

Table 2. Heavy metal concentrations in shoots and roots of I. mustard, tobacco, willows, maize and *T. caerulescens* in the Dornach field experiment and mass balance calculations

Harvest year	Plant species	Age year		Concentrations in shoots			Concentrations in roots			Yield t ha^{-1}	Outputs		
				Cd mg kg^{-1}	Cu mg kg^{-1}	Zn mg kg^{-1}	Cd mg kg^{-1}	Cu mg kg^{-1}	Zn mg kg^{-1}		Cd g ha^{-1}	Cu g ha^{-1}	Zn g ha^{-1}
97	Indian mustard[a]		m	1.0	20	124	0.3	32	45	7.3	6.95	146	894
			sd	0.1	2	13	0.0	3	3	0.7	0.80	15	104
97	Tobacco[a]		m	3.5	38	146	0.6	26	36	12.6	41.7	474	1834
			sd	0.8	6	21	0.2	2	2	2.1	16.9	96	384
98	Willows[a]	1+2	m	3.3	12	240	nd[b]	nd	nd	0.8	3	8	200
			sd	0.5	5	46	–	–	–	0.5	2	6	152
98	Maize[a]		m	0.6	10	129	0.7	38	41	15.6	9	163	1998
			sd	0.2	1	30	0.3	11	10	3.8	3	52	576
99	Willows	2+3	m	3.4	14	294	nd	nd	nd	13.2	44	187	3851
			sd	0.4	1	38	–	–	–	2.3	7	28	598
99	Thlaspi		m	184	53	5265	168	71	693	0.9	179	50	5052
			sd	38	3	612	32	5	159	0.2	79	9	1680
99	Maize		m	0.3	8	83	0.1	36	94	14.2	4.7	108	1221
			sd	0.1	1	21	0.2	3	18	2.9	0.4	14	515

[a] After Kayser et al. (2000) for shoots concentrations and yield.
[b] nd=not determined.

Table 3. Root lengths and areas measured for I. mustard, tobacco, willows, maize and *T. caerulescens* along the whole soil profiles and comparison with shoot biomass. Letters refer to the significance of the differences: for a given parameter, similar letters mean no difference between the means and different letters mean significant differences between means with $2p < 0.05$

Plants	I. Mustard	Tobacco	Willows	Maize	Willows	Maize	*Thlaspi*
Sampling year	1997	1997	1998	1998	1999	1999	1999
Root length density (L_V)				in m dm^{-3}			
Depth in m							
0–0.1	14.8±2.5	26.8±5.1	23.6±7.5	33.0±5.0	19.1±7.7	14.6±14.0	16.5±7.8
0.1–0.2	12.2±4.9	9.7±3.1	20.4±10.7	33.0±12.9	15.9±1.4	9.4±4.4	9.8±2.7
0.2–0.3	5.2±1.0	7.1±3.7	14.9±5.4	14.7±1.3	9.0±0.9	4.8±4.8	2.6±1.4
0.3–0.4	7.8±5.2	3.4±1.2	11.1±3.7	16.9±5.6	3.0±1.0	3.9±3.2	0.4±0.3
0.4–0.5	7.0±0.4	3.3±0.7	6.9±1.6	15.0±9.3	4.5±1.3	2.4±1.4	0.07±0.08
0.5–0.6	1.6	1.5±0.5	7.1±7.2	11.3±1.5	4.0±1.2	2.6±1.9	–
0.6–0.7			10.2±9.2	9.9±4.9	3.3±1.8	3.7±1.6	–
0.7–0.75			8.1±7.4	5.3±3.0	6.0±4.8	3.4±0.2	–
				in km m^{-2}			
Cumulative root density L_A till 0.75 m depth	4.9±1.4 a	5.4±1.5 ad	9.8±4.9 bdc	13.6±4.2 b	6.2±1.8 c	4.3±3.1 ac	2.9±1.2 a
Cumulative root density L_A till 0.2 m depth	2.7±0.7 ae	3.7±0.8 bd	4.4±1.8 abc	6.6±1.8 c	3.5±0.9 bd	2.4±1.8 abd	2.6±1.1 de
				in m^2 m^{-2}			
Total root area till 0.75 m depth	1.3±0.1 a	1.8±0.4 a	5.0±1.2 b	8.7±1.7 c	6.5±1.4 bc	4.7±3.5 abc	2.2±0.9 a
				kg m^{-2}			
Shoot biomass	0.7±0.1 a	1.3±0.2 b	0.6±0.4 a	1.6±0.4 b	1.3±0.2 b	1.4±0.3 b	0.1±0.0 c
L_A till 0.75 m/Shoot biomass	7	4	16	9	5	1	31
L_A till 0.20 m/Shoot biomass	4	3	7	4	3	2	28

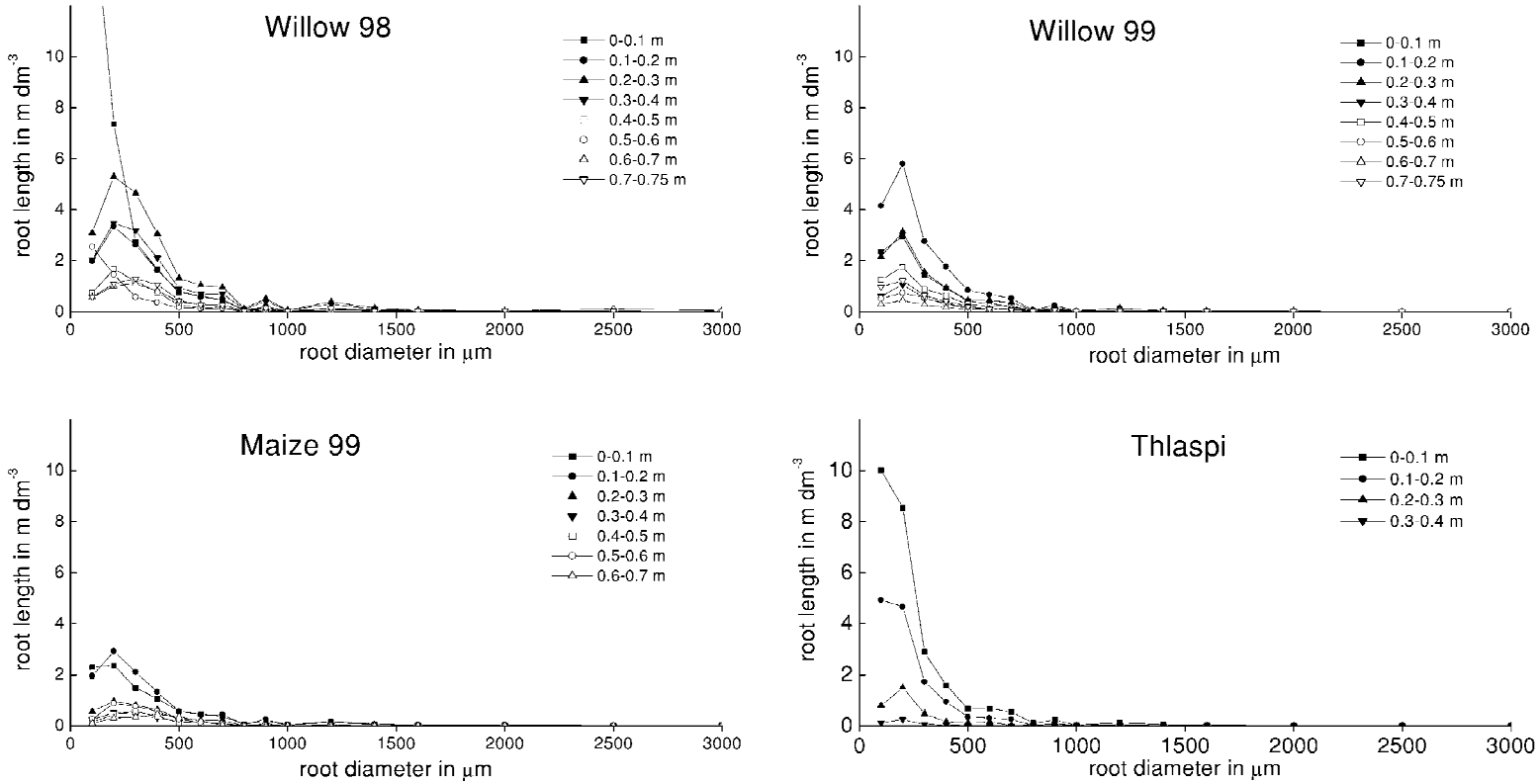

Figure 1. Root diameter distribution over depth for some representative plots. L_V=total root length per layer in m dm^{-3}.

Table 4. Correlations between root and shoot parameters calculated for all the plants except *T. caerulescens* in the Dornach field experiment

n=24	Area	L_A [a]	Biomass	Ratio L_A/B [b]	Conc. in shoots			Output	
					Cd	Cu	Zn	Cd	Cu
L_A [a]	0.857***								
Shoot biomass	0.572**	0.236							
Ratio L_A/B [b]	0.595**	0.750***	0.207						
Cd in shoots	−0.383	−0.076	−0.166	0.042					
Cu in shoots	−0.457*	−0.307	−0.158	0.273	0.484*				
Zn in shoots	−0.167	0.197	−0.101	0.216	0.770***	−0.037			
Cd output	−0.508*	−0.196	0.298	0.333	0.651***	0.559**	0.427*		
Cu output	−0.333	−0.212	0.308	0.361	0.347	0.859***	−0.132	0.738***	
Zn output	−0.212	0.068	0.559**	0.169	0.254	0.069	0.423*	0.772***	0.393[c]

[a] L_A=cumulative root density till 0.75 m.
[b] Ratio L_A/B=cumulative root density till 0.75 m/total shoot biomass.
[c] r=0.776*** without tobacco.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

ison with the crop plants, *T. caerulescens* had a large impact on linear correlation coefficients calculated between heavy metal concentrations, outputs by shoots, biomass and root characteristics. There was no correlation between heavy metal concentrations or outputs and biomass (except for Zn output) when only non-hyperaccumulating plants were taken into account (Table 4). However, these parameters were negatively correlated when taking *T. caerulescens* into account ($r < -0.632^{***}$ for Cd, Cu and Zn concentrations and biomass ($p < 0.001$)). On the contrary, correlation were found between Zn, Cu and Cd outputs in crop plants (showing no heavy metal specificity) whereas including *T. caerulescens* would give a correlation between Cd and Zn outputs only ($r = 0.686^{***}$). This was to be expected, as *T. caerulescens* is a Cd and Zn hyperaccumulator. In fact, only correlations between Cd and Zn concentrations in plants were significant with ($r = 0.994^{***}$) or without ($r = 0.770^{***}$) *T. caerulescens*, which means that these two elements were taken up together by all the plants tested.

The root area is by definition a function of root length and root diameter and as such is very well correlated with L_A. It was also well correlated with the shoot biomass (when *T. caerulescens* was not included) but not with the heavy metal outputs. The highest positive correlations were found between the 'cumulative root density/total shoot biomass' ratio (L_A/B) and heavy metal concentrations in shoots when *T. caerulescens* was included ($r = 0.758^{***}$, $r = 0.594^{***}$, $r = 0.798^{***}$ ($p < 0.001$) for Cd, Cu and Zn concentrations resp.).

Discussion

Before a decision is made on the remediation technique to be applied on a heavy metal contaminated soil, different parameters have to be taken into account such as the initial level of contamination and the target value (total and/or available) to be reached after remediation, the type and the use of the soil to remediate, the area and depth of soil concerned as well as the time and price allowed for remediation. Once phytoextraction has been found to be suitable, plants have to be selected and a timetable with plant rotation, agronomic practices and management has to be set up. The most suitable plants must show tolerance to the site conditions together with a high biomass and high heavy metal concentrations in the shoots. The latter are also related to the specific physiology of the plant, which may have a great influence on observed differences in total metal output. However, this point will not be discussed here as our aim was to focus on the impact of the root system morphology on phytoextraction efficiency. Indeed, plants have also to be able to reach the metal to be removed, which means that their root system must develop within the contaminated zone. In our experiment the depth of contamination varied between 0.2 and 0.7 m and was suspected to be critical for assessing the efficiency of phytoextraction. Although heterogeneity is considered to be a typical pattern for soil contamination, such variations in contamination depth questions the use of a single species to extract heavy metals located at variable depths.

Table 5. Matching of roots and Zn soil contamination expressed as the ratio between the maximal depth with a root length density >5m dm^{-3} and the soil contamination depth; (a) contamination as measured in the soil profile of each plot, (b) hypothetical shallow contamination (0.2 m), (c) hypothetical deep contamination (0.7 m). (b) and (c) depths are extreme values found in the field experiment in Dornach. Depth of contamination is calculated after substraction of 150 mg kg^{-1} Zn (Swiss guide value; OIS, 1998). '>' means that the deepest root sampling gave a root length density above 5m dm^{-3}

	Contamination		
	(a) true	(b) shallow	(c) deep
Indian mustard	0.9±0.3	1.9±0.8	>0.5
Tobacco	0.5±0.2	1.3±0.3	0.4±0.1
Willows 98	1.3±0.5	>2.9	>0.8
Maize 98	1.4±0.6	>3.3	>0.9
Willows 99	1.5±0.7	>3.2	0.9±0.2
Maize 99	0.4±0.2	1.4±0.5	0.4±0.1
Thlaspi	0.4±0.1	1.0±0.0	0.3±0.0

Although most species go on average as deep as 0.7 m (Polomski and Kuhn, 1998), the root density decreases rapidly with depth. In some cases, contamination as deep as 0.7 m or more will need species with deep root system or soil will have to be prepared and the plantation procedure will have to be adapted so that roots can go as deep as possible (Schnoor, 1997). The only way to assess the potential efficiency of plants to extract metals from a certain layer in the soil is thus to study the root distribution along the soil profile and the soil horizons.

Total outputs calculated for the different plants tested were rather low except the 3-year-old willows and *T. caerulescens* (and to a lesser extent tobacco and Cd). However, with a calculated TC larger than 1, *T. caerulescens* was the only plant able to concentrate significantly soil Cd and Zn in its biomass as expected from a Cd and Zn hyperaccumulator (Reeves and Baker, 2000). The other plants seemed to be more efficient for Cd than Zn. All calculated TC (Cd, Cu and Zn) were within the range given by Sauerbeck (1989), except TC_{Cd} for *T. caerulescens*.

Cumulative root density (L_A) is a convenient parameter to assess the efficiency of the root system. It shows large variations between plants: this is a common feature due to plant intrinsic differences (Box, 1996) but in our case it is also probably due to the high heterogeneity of soil characteristics as already described above. It varied also according to the year (climatic conditions) and the sampling time: indeed, maize root length usually reaches its maximum around flowering stage and then decreases slowly till harvest (e.g. Foch, 1962; Upchurch and Ritchie, 1984). Temperature has also an effect on root longevity (Fitter, 1996). There were 3-weeks delay between the first and the second year maize harvest due to climatic conditions, thus harvest did not take place exactly at the same physiological stage of the plant. Willows presented large differences between the first and the second year of measurement. As plants were just cut and not removed after the second year, they were one year older and grew roots in deeper layers. It can be hypothesised that the reduction in L_A the second year was compensated by root growth below the depth of measurement: indeed, willows were the only plants exhibiting an increasing root length below 0.7 m. Unfortunately, no measurements could be performed below 0.8 m because of the stones found at depth (alluvial bed).

Maize root system is known to increase down to variable depths (up to 0.9 m) (Kirkham et al., 1998). Willows are known to grow shallow adventitious roots (Fitter, 1996) as well as deep roots down to 2 m (Polomski and Kuhn, 1998) or more. These features allowed a more homogeneous colonisation of the soil profile by these two plants than by the other plants. It appeared also that the root extension in depth was responsible for their larger L_A. In some profiles (mostly for I. mustard and maize) a decrease in L_V was observed around 0.3–0.4 m and might be associated with the compacted layer identified by the infiltration test. Indeed, mechanical impedance can be responsible for reduced root length with roots localised in cracks or large pores (Bennie, 1996). Although Kirkegaard et al. (1992) have shown that a reduced root growth in a localised compacted layer is usually compensated by higher growth in other layers and thus has globally no effect on nutrient uptake, it may have an impact on phytoextraction efficiency as the compacted layer is then less prospected whereas the need for heavy metal removal is the same as for the other layers.

The ratio calculated between L_A and the aboveground biomass gives a good idea of the efficiency of the plant to prospect and concentrate elements into the shoots: with a ratio of 31 *T. caerulescens* exhibited the largest root system per kg of shoot dry matter. This might favour soil prospecting and opportunity to take up nutrients as well as heavy metals. Willows presented also a rather large ratio, especially when calculated for the whole soil profile, indicating that their high bio-

mass was supported by large root propagation. When taking into account the first 0.2 m only, all plants gave the same ratio except *T. caerulescens* having a higher ratio, which again speaks for a better efficiency of this plant in the case of shallow contamination.

Root area is a factor that might have an effect on uptake efficiency. It is related to the root size and plants with large root area have also more roots with small diameters. Assuming that fine slowly growing roots are partly responsible for a better control of ion movement into the shoots (Eshel and Waisel, 1996), then *T. caerulescens* would be potentially more efficient at removing heavy metals such as Zn and Cd that are already preferentially taken up by this plant (Schwartz et al., 1999).

The root parameters were combined with the general plant characteristics in order to assess the impact of the root system described by the root length density, root area and the ratio L_A/B biomass on the plant efficiency to remove heavy metal from soil. This general comparison did not yield any trend except the positive correlation between root area and shoot biomass (crop plants only) indicating a positive effect of the area of the root system on plant growth and probably an indirect effect on metal outputs although no significant correlation could be found between root area and outputs. When *T. caerulescens* was included in the calculations, L_A/B gave positive correlations with metal concentrations and outputs (except Cu) indicating that a large root system in comparison to plant biomass might help to concentrate heavy metals in the shoot. In general, including *T. caerulescens* in the calculations led to a bimodal population with the crop (or 'accumulating') plants on one hand and the hyperaccumulator plant on the other hand. An explanation for the poor correlations observed between root parameters and heavy metal uptake, might be that mycorrhizal associations were not taken into account (all the plants studied may have been associated with micorrhiza except the *Brassicacea*). However, although the positive role of micorrhiza in plant nutrition has been clearly demonstrated (Marschner, 1997), the role of mycorrhiza on heavy metal uptake is still in discussion (Hagemeyer and Breckle, 1996).

The direct way to assess the spatial adequacy of the root system on heavy metal removal is to compare root profiles with heavy metal distribution at depth. Figure 2 gives a picture of the fitting between root length distribution and total Zn contamination. All data from all layers and plants were used. Only zinc data are shown because of the high correlation found between Cd, Cu and Zn in the soil (as described above), but conclusions apply to Cu and Cd as well. From the graphs it is quite obvious that for none of the plant species root distribution matched soil contamination perfectly. Willows and maize presented an increase in root length with increased contamination because most of the roots and the highest contamination are both located in the upper part of the soil profile. This trend was limited for maize from 1999 and the I. mustard but more accentuated for willows and maize collected in 1998. If it is assumed that metal uptake is a partially passive process driven by evapotranspiration, then a high root density measured in layers with low concentrations will lead to diluted heavy metal fluxes to the shoots and thus to reduced concentrations and outputs compared to the same plant with roots located in the contaminated zone only. An indirect confirmation is given by the higher heavy metal concentrations measured in 2-year-old willows (with most probably shallow roots) compared to 3-year-old ones (deeper roots) collected the same year (data not shown). For tobacco and *T. caerulescens* the high root density matched high Zn concentrations because their root systems were restricted to the top layers where the highest contamination took place. From this it comes that some species are more adequate for shallow contamination remediation and others for deep contamination. Table 5 gives the ratio between the maximum depth with a L_V >5m dm^{-3} and the depth of contamination according to three scenarios (5m dm^{-3} was chosen as limit because below this value root density decreased sharply): (a) the true field conditions (high variability in the contaminated layer thickness), (b) an hypothetical even shallow contamination (0.2 m) for the whole field experiment, and (c) an hypothetical even deep contamination (0.7 m), (b) and (c) being the minimal and maximal contamination depths found in the field. Similar results were obtained with a L_V limit set to 1m dm^{-3}. For shallow contamination *T. caerulescens* appeared to be best suited with a ratio equal to 1, which means that all *T. caerulescens* roots were well distributed within the contaminated layer and in this layer only. A ratio larger than three indicated that willows and maize from 1998 had more than half of their roots out of the contaminated layer, that is in the underlying uncontaminated layer. In the case of a deep contamination, deep rooting plants (maize, willows) were able to reach contamination at depth (ratios close to 1). *Thlaspi caerulescens*, I. Mustard and tobacco were able to extract metals only from the upper part of the contaminated layer as shown by a ratio below 1. In

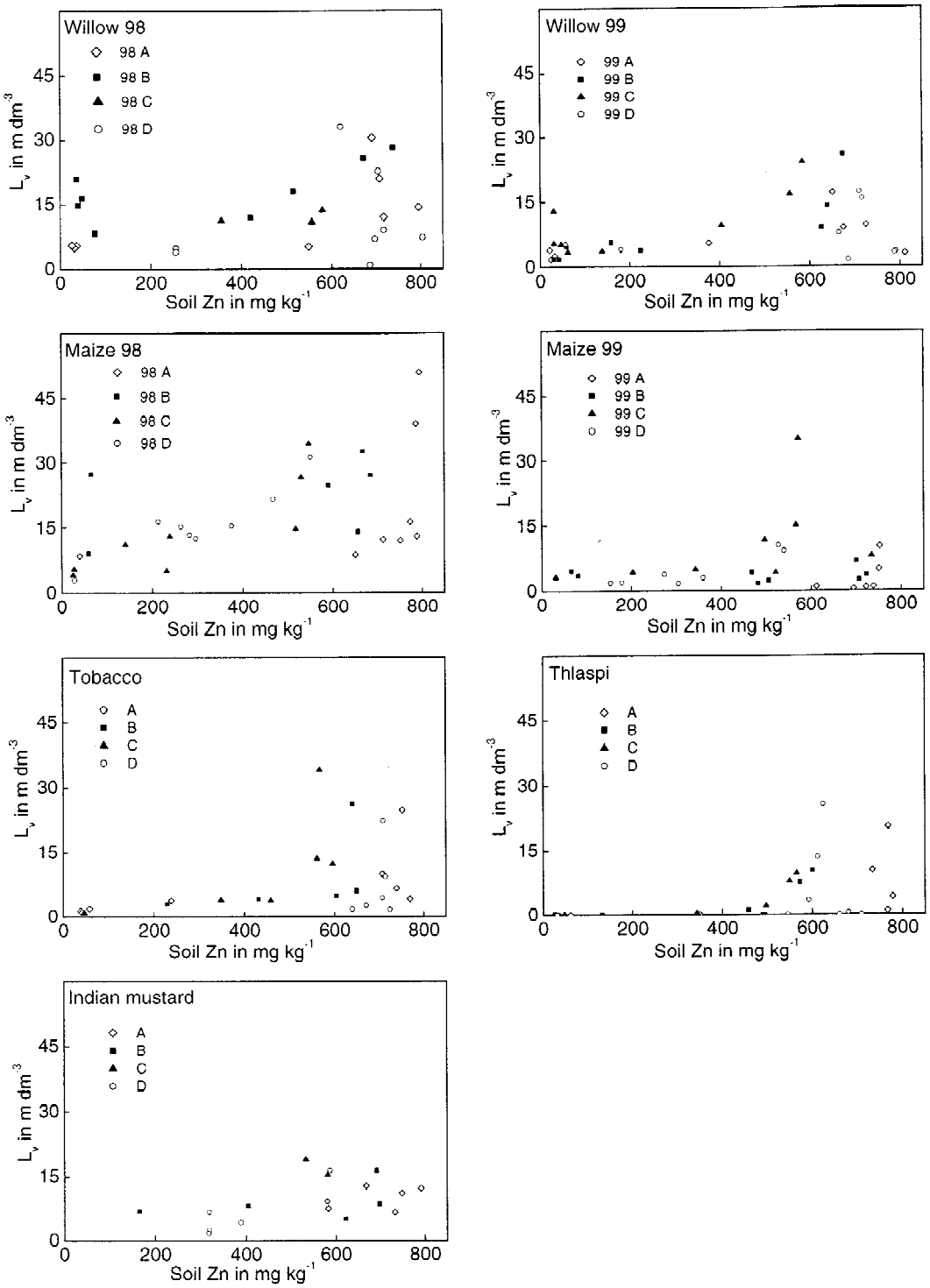

Figure 2. Relations between root length density (L_V) and total Zn concentrations calculated for each soil layer and per plant and year. For each plant, individual data from the four plots (replicates A, B, C and D) were used.

the true field situation as measured by soil analyses, no plant gave a ratio close to 1 except the I. mustard. This points to the importance and difficulty of choosing plant species according to soil contamination depth.

From the results, there was no evidence that some plants would avoid growing roots in the contaminated layer as it has been shown by Breckle and Kahle (1992) for trees growing on Pb contaminated soil. To optimise phytoextraction of Cd and Zn it is thus necessary to match root system development with the extent of soil contamination. In the case of deep contamination, if the root system is restricted to the upper contaminated part, agronomic strategies are to be chosen either to remove the decontaminated layer or to mix it regularly with the underlying layer. As already mentioned, because of their historical background, contaminated soils are often heterogeneous and deep mixing of the remediated layer with the contaminated one located below might be neither technically possible nor environmentally or politically acceptable. Additionally, this might not be realistic in the case of agricultural soils. An alternative could be the use of mixed crops or rotation of different accumulating plants to compensate the locally limited efficiency of one single species.

Summary and conclusion

So far, very few studies have dealt with the direct relationship between root system, heavy metal uptake and extraction efficiency by plants in the field. Three aspects were found to be important in assessing phytoextraction efficiency: heavy metal concentrations in shoots, root density and spatial distribution of roots. In Dornach, *T. caerulescens* was the most efficient plant although its biomass was small, followed by plants combining high metal concentrations and high biomass (willows for Cd and Zn and tobacco for Cu and Cd). The ratio between cumulative root density and aboveground biomass, together with a relative larger proportion of fine roots compared to other plants seemed to be additional favourable characteristics for increased element uptake, including heavy metals. For these two characteristics *T. caerulescens* was distinctively different from the other species studied. Spatial distribution of roots deserves attention as it might obliterate the theoretical benefit of choosing a specific plant for its high uptake. Although root systems are fairly plastic and are sensitive to soil characteristics and climatic conditions (i.e. watering), under the same soil and climatic conditions different species will exhibit different root distribution along the soil profile and thus might be differently efficient at extracting heavy metals. A sound agronomic management is thus required to optimise the process of phytoextraction.

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