30 Efficiency and Limitations of Phytoextraction by High Biomass Plants: the Example of Willows

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30.1 INTRODUCTION

Phytoextraction with crops and bioenergy plants or trees has been proposed as a suitable alternative to the destructive techniques used thus far to clean up soils contaminated with heavy metals. This is one of the two possible approaches that are followed in phytoextraction; the other one is the use of hyperaccumulating plants. The crop and energy plants have opposite characteristics to hyperaccumulators: they are well known plants with large yields and fast growth, but they are usually not metal specific and they accumulate below the hyperaccumulation thresholds. Plants or seeds are readily available and some have already been tested in the field [1–3]. On the other hand, very few species (if any) of hyperaccumulators are presently available for field application under temperate climate (lack of nurseries or seed producers), so the use of hyperaccumulators might need to be dismissed for remediation of large areas.

In general, the use of plants to remove metals from soils is environmental friendly and its cost is low compared to engineering-based techniques such as soil capping, soil washing, vitrification, land-filling, etc. Additionally, it is an *in situ* and solar-generated technique that could help to rehabilitate large areas of agricultural soils contaminated mostly in the upper layer, by maintaining or even restoring soil fertility. It also produces less waste because the biomass may be recycled for energy and metals. Unlike phytostabilization — its counterpart among the phytoremediation techniques — phytoextraction potentially resolves most of the legislative requirements that ask for metal removal down to a given threshold. Another interest of bioenergy plants or trees — including willows — is that they may also be used in phytostabilization [4].

The general drawbacks of phytoextraction are well known [5] and its optimization still requires a lot of research: phytoextraction is a slow process in comparison to the previously mentioned techniques (10 years is the goal upon which most people agree to recognize phytoextraction as economically acceptable). Its application is limited because of the lack of established methods and successful completed remediation case studies, the lack of recognized economic performance, and the risk of food chain contamination. Additionally, it may not be able to remove 100% of the contaminants and its efficiency has been proved for some contaminants only. Alternatively, the plants might not be able to withstand highly toxic concentrations of the contaminants.

This chapter does not aim to review all the drawbacks in detail; a preliminary comparison with the other available techniques should determine whether these negative aspects are limiting or not for choosing phytoextraction for a given site. In addition, for the reasons mentioned earlier, phytoextraction with hyperaccumulators will not be discussed here.

Once it has been decided to use phytoextraction with crop or bioenergy plants as a cleanup technique, many pitfalls may appear that will limit its efficiency — that is, its ability to reach the goal set in a reasonable time and with limited negative side effects. They may be due to the specificities of the site, soil, plants, and contamination characteristics, as well as to external constraints like legislation. Highlighting these factors will help to define the boundary conditions for the use of phytoextraction and thus the ability to offer an efficient technique to landowners or local authorities as main remediation tool or as part of a "remediation package."

This chapter focuses on phytoextraction with the high biomass willow *Salix viminalis*. It draws examples from field experiments with *S. viminalis* and pot experiments, with additional references to other soils and plants. The field experiments of reference will be presented first. Then, the site characteristics (including the local climatic and edaphic conditions as well as the nature and extent of the contamination), plant characteristics, and aspects related to the legislative background that may be limiting for phytoextraction efficiency will be presented successively. It should be remembered that all these aspects may interact and thus are not truly independent parameters.

30.2 CHARACTERISTICS OF THE EXPERIMENTS

30.2.1 DESCRIPTION OF EXPERIMENTAL SITES

The three sites chosen to illustrate these different aspects and their impacts on the final efficiency of phytoextraction are presented in Table 30.1. Two are agricultural soils and the third is a landfill that has been closed and revegetated with trees. The first two have been tested for phytoextraction [6,7] and the third one was studied in view of a general site rehabilitation [8]. They are all moderately contaminated with metals, mostly Cu, Zn, Cd and Pb with some additional metals in lower concentrations, but no significant organic contamination.

- The Dornach site in northwest Switzerland has been described by Kayser et al. [2]. 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 [9]. The site is currently included in a crop rotation.
- 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 this acidic fluvisol with organic matter and heavy metals (Cd, Cu, and Zn). Currently,

TABLE 30.1
Site Descriptions

	Dornach	**Caslano**	**Les Abattes**
Location	Jura edge	Southern Alps	Jura chain
Altitude in meters	300	280	1000
Mean temperature in °C	10	11.7	5.3
Mean rainfall in millimeters	800	1860	1470
Number of samples for analysis 0–0.2 m	20	4	49
pH	7.2	4.9	7.4 (0.1)[a]
% Clay	30[b]	12	43 (13)[a]
% Organic carbon	2.5[b]	5.2	5.6 (2.1)[a]
Total Zn in mg $kg^{-1(c)}$	645 (81)	1158 (216)	245 (179)
Total Cu in mg $kg^{-1(c)}$	525 (62)	264 (43)	188 (158)
Total Cd in mg $kg^{-1(c)}$	2.0 (0.4)	2.8 (0.7)	1.2 (0.7)
Soluble Zn in mg $kg^{-1(c)}$	0.08 (0.03)	7.4 (5.9)	bdl
Soluble Cu in mg $kg^{-1(c)}$	0.7 (0.2)	0.4 (0.1)	bdl
Soluble Cd in mg $kg^{-1(c)}$	2.0 (0.6)	13 (11)	bdl

[a] 11 samples.
[b] 3 samples.
[c] FAC, *Methoden für die Bodenuntersuchungen,* Eidgenössische Forschungsanstalt für Agrikulturchemie und Umwelthygiene, Bern-Liebefeld, Switzerland, 1989.
[d] bdl = below detection limit.

Note: Soil parameters are average values with standard deviations given in parentheses.

Sources: After Kayser, A. et al., *Environ. Sci. Technol.,* 34, 1778, 2000 (Dornach and Caslano); Hammer, D. et al., *Soil Use Manage.,* 19, 187, 2003 (Dornach and Caslano); Keller, C. et al., *Plant Soil,* 249, 67, 2003 (Dornach and Caslano); and Keller, C. et al., unpublished data, 1997–2004 (Les Abattes).

the site is used as a private garden and a meadow for fodder production. At both sites, the contaminated layer is between 0.20 and 0.60 m thick.

- The third experimental site was designed for forest rehabilitation and is located on the former landfill "Les Abattes" (Le Locle, Switzerland), in the Swiss Jura mountain chain. The 2-ha landfill was used for inert and uncontaminated waste material deposits [10], but the landfill was capped in 1990 with a final top layer (0.05 to 0.60 m thick) made up by a mixture in varying proportions of contaminated compost originating from contaminated sewage sludge and uncontaminated local calcareous soil and gravel (calcaric anthroposol). Thus, heavy metal concentrations in the final top layer were spatially highly variable as a consequence of the heterogeneous mixing of the components [8].

In addition, for illustration of some specific points, results from other soils are presented. The important characteristics of these soils are given together with the results.

30.2.2 PLANTS FOR PHYTOEXTRACTION

Phytoextraction experiments have been performed on the two agricultural sites with crop plants (tobacco, maize, Indian mustard, sunflower, bioenergy plants [*Salix viminalis*], and hyperaccumulators [*Thlaspi caerulescens*, *Alyssum montanum*, and *Iberis intermedia*] on miniplots. Only results obtained with the crop plants and *S. viminalis* (Swedish clone n°78980) are presented here. Both experiments have been conducted according to agronomic standards; detailed descriptions of the experiments can be found elsewhere [2,7]. On the landfill, various tree species have been planted in groups of 10 plants of the same species, including pioneer (and bioenergy) trees like *S. viminalis* [8]. In addition to this grid, to study the behavior of *S. viminalis* more precisely, two clones (a local one and the Swedish clone) of *S. viminalis* were planted as cuttings in 1997 or as 1-year-old plants (1 m high) in 1998 in 30 groups of 12 plants with a random distribution of the 30 groups on a 30-m side grid. The whole field was maintained as a typical forest plot (no weed removal but grass around the young plants was cut once per year).

Table 30.2 gives an overview of the main characteristics of the experiments as well as some results obtained with *S. viminalis* that will be presented later. In the case of Les Abattes, only the results of the 1-year-old plants are given. Additionally, the two clones of *S. viminalis* were tested in hydroponics as well as in pots (with various soils) in order to measure their extraction potential in controlled conditions. They were compared to other willow species, *Salix aurita* and *S. irrorata*, found to be tolerant to Cd and Zn, and *S. aurita*, which was also able to accumulate Cd and Zn in its shoots (tests performed in hydroponics) [11].

TABLE 30.2
Overview of *Salix viminalis* (Clone 78980) Results

	Dornach	**Caslano**	**Les Abattes**
Type of experiment	Phytoextraction	Phytoextraction	Forest rehabilitation
Plant density in plants ha^{-1}	12,800 (+20,000)	40,000	10,000
Type of planting	Cuttings	Cuttings	1-year-old plant
Yield after 2 years in t DM ha^{-1}	6.5 (3.8)	15.8 (6.0)	0.9 (0.7)[a]
Yield after 5 years in t DM ha^{-1}	32 (2.5)	24 (11)[b]	—
Yield after 2 years in t DM ha^{-1} normalized to 10,000 plants ha^{-1}	5.08	3.95	0.9
Concentrations after 2 years in mg kg^{-1} DM			
Zn in whole plants	240 (46)[c]	785 (202)	252 (77)
Zn in leaves	665 (50)	1700 (465)	475 (181)
Zn in stems	120 (10)	430 (85)	183 (42)
Cu in whole plants	10 (1)	13 (1)	5.2 (0.7)
Cu in leaves	13 (1)	13 (1)	5.6 (0.7)
Cu in stems	9 (1)	13 (1)	5.1 (0.8)
Cd in whole plants	3.3 (0.5)	2.6 (0.5)	1.3 (0.5)
Cd in leaves	5.1 (0.7)	3.6 (0.5)	1.5 (0.5)
Cd in stems	2.3 (0.1)	1.2 (0.5)	1.2 (0.5)

[a] Plants not cut the first year.

[b] Yield after 4 years in t DM ha^{-1}.

[c] Analyses performed on 3-year-old plants for Zn, Cu, and Cd.

Note: Average yield and concentrations of 4 (Dornach and Caslano) or 30 plots (Les Abattes) are given with standard deviations in brackets.

Sources: After Kayser, A. et al., *Environ. Sci. Technol.,* 34, 1778, 2000 (Dornach and Caslano); Hammer, D. et al., *Soil Use Manage.,* 19, 187, 2003 (Dornach and Caslano); Keller, C. et al., *Plant Soil,* 249, 67, 2003 (Dornach and Caslano); and Keller, C. et al., unpublished data, 1997–2004 (Les Abattes).

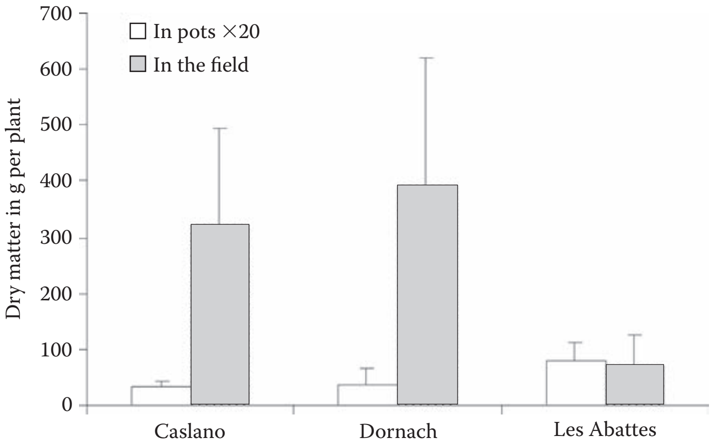

FIGURE 30.1 Comparison of the biomass produced by plants of *Salix viminalis* (clone 78980) grown in three different soils in pots (3-month-old plants) and in the field (second growing season). Ratios between dry biomass produced in pot and in the field are 118, 190, and 18 for Caslano, Dornach, and Les Abattes, respectively. (Modified from Keller, CNATO Series, 2004, in press.)

30.3 FACTORS LIMITING THE EFFICIENCY OF PHYTOEXTRACTION

30.3.1 Climate and Soil Characteristics

Site characteristics include the climatic conditions; the nature of the soil that is contaminated; volume of soil concerned (surface and depth); the nature and the extent of the contamination; the metals to be removed, their concentrations, their availability to plants, and their toxicity; the presence of other inorganic or organic contaminants; and the degree of heterogeneity of the contamination.

30.3.1.1 Climate

Climatic conditions (mostly temperature and rain) play a role directly in biomass production and indirectly in metal concentrations (that are influenced by plant health). The results obtained for *S. viminalis* and presented in Figure 30.1 are typical of the impact of the climatic conditions on the growth of plants. In a pot experiment, no significant difference was measured in the biomass production of *S. viminalis* (clone 78980) grown 3 months in Les Abattes, Dornach, and Caslano soils [12,13]; however, in the field, the yield per plant in Les Abattes was lower due to a lower average temperature and high rainfall (Table 30.1 and Table 30.2). Also, Van Splunger et al. [14] have found that drought increases root length and rooting depth, thus affecting root distribution of Salicaceae and the ability to forage and take up nutrients from soil.

30.3.1.2 Soil Characteristics

Soil characteristics (pH, percent of C, percent of clay, nutrient availability, water table, and compaction) are responsible for the general soil fertility and have a large impact on plant biomass and composition [15] (Figure 30.1, results in pot, and Figure 30.2). In Figure 30.2, the biomass production of four *Salix* clones grown in pots under controlled conditions varied with the type of soil and these variations were not correlated (see, for example, the differences between *S. aurita* and *S. irrorata* for Dornach and soil 1). The type of soil also influences the root depth prospecting as shown in Table 30.3, where root depth may vary from 40 to 500 cm for the same species growing in different uncontaminated soils. For willows, it has been shown that root development varies

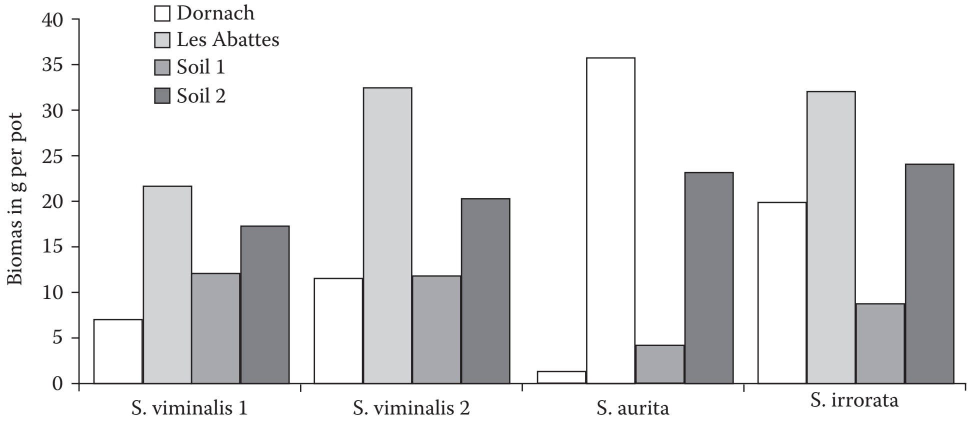

FIGURE 30.2 Biomass production of *S. viminalis* (1 = Swedish clone 78980; 2 = clone Les Abattes), *S. aurita*, and *S. irrorata* grown 8 months in pots in the Dornach and Les Abattes soils and two other agricultural soils —soil 1 and soil 2 — presented for their Zn content in Figure 30.6. (After Keller, C., Raduljica, O., and Hammer, D., unpublished data, 1997–2004.)

TABLE 30.3
Examples of Average Root Depth for Several Tree Species and According to Soil Type and Age of Trees

Soil type	Acer pseudoplatanus		*Populus* spp	Populus tremula	Betula pendula	
	Root depth (cm)	Age (year)	Root depth (cm)	Root depth (cm)	Root depth (cm)	Age (years)
With skeleton, carbonates, average depth	—		—	—	40–60	40–50
Deep, without skeleton, silty clay	50 500	2 20	120–140	90–150	100–150	40–50
Nonhydromorphic sand, sandy soils	110–140	60–70	100–260	30	150–450	10–20
Different soils, slightly hydromorphic	130–140	60–70	110	—	—	
Hydromorphic soils	40–50	50–60	—	—	90–130	90
Presence of groundwater table	40	50-60	80	—	—	

Source: Modified from Polomski, J. and Kuhn, N., *Wurzelsysteme*, Paul Haupt, Bern, Switzerland, 1998.

according to soil texture that drives plant-available soil water and the maximum capillary rise. For example, sand retains too little water and silt is usually too compact and oxygen deficient to allow root growth at depth, whereas sandy loam or clay loam allows a deep root system [16].

Like the three sites studied located within the boundaries of towns, many contaminated soils in urban areas are fertile, especially kitchen gardens. Unfortunately, in addition to the contamination, many other contaminated sites are degraded in a broader sense [17]. They are poor in nutrients and have low water retention and more generally heterogeneous biological, chemical, and physical properties unfavorable for plant growth that may induce deficiencies. For example, Bending and Moffat [18] found that the establishment of trees on mine spoils was impaired by acidity, salinity, N deficiency, compaction, and poor water-holding capacity. Agronomic management, irrigation, fertilization, and various additives may need to be used intensively to obtain optimal results; this adds to the costs of phytoextraction. For example, *S. viminalis* has a high nitrogen requirement that must be satisfied to obtain efficient metal removal [19,20]. Iron deficiency may also occur and requires the use of an Fe fertilizer to reduce chlorosis, as observed on *S. viminalis* at Dornach and Caslano [6].

Although the origin of the contamination influences the speciation of the metals present in the soil, soil characteristics have an impact on metal speciation in the soil matrix and soil solution. This is best exemplified in unpolluted environments [21,22], but also occurs in polluted soils [13]. This in turn has an impact on the availability of metals and, ultimately, on metal uptake. For example, at Les Abattes, the large C content combined with a high pH and clay content resulted in undetectable soluble Cd, Zn, and Cu as compared to the Dornach and Caslano soils (Table 30.1).

Kayser [2] has studied the relation between pH and $NaNO_3$-extractable Zn and Cd (defined according to Swiss law [23] as the soluble metal fraction in two soils [one is the Dornach soil and the other is named Rafz] amended with increasing amounts of elemental sulphur in order to decrease soil pH and, consequently, metal bioavailability to plants) [2,24,25]. The increase in $NaNO_3$-extractable Cd and Zn was more pronounced in the Rafz soil (initial pH $CaCl_2$ = 6.8, HNO_3-extractable Zn = 813 mg kg^{-1}, and Cd = 0.9 mg kg^{-1}) than in the Dornach soil (pH 7.4; see Table 30.1) with 26- and 13-fold increases in Rafz for Cd and Zn, respectively, and in Dornach of 15- and 11-fold with the highest S8 addition (400 mmol sulphur kg^{-1} soil). This illustrates that the changes in soil pH (artificially induced or through natural acidification) may affect metal availability in different soils differently.

Additionally, the effect on Cd and Zn concentrations in *S. viminalis* was 4.6-fold for Zn and 1.6-fold for Cd compared to the control plants grown on the non-amended Rafz soil and 2.4- and 1.5-fold for Zn and Cd, respectively, in *S. viminalis* grown on the Dornach soil [25]. This highlights the fact that no strict relationship between plant uptake and metal bioavailability is assessed by chemical extractants. The question of bioavailability in relation to plant uptake is discussed in the next section.

30.3.1.3 Nature and Extent of the Contamination

30.3.1.3.1 Toxicity of the Contaminants

An overall evaluation of the particular contamination issue and site must be made before starting a phytoextraction program. Indeed, sites to be remediated are very seldom contaminated with single metals. Multicontamination with organic and inorganic pollutants (and sometimes with physical constraints, such as compacted soil) is common and may induce toxicity to plants that are not tolerant to all the contaminants present. Plants are potentially sensitive to a broad range of elements if their concentrations in the soil are above a given threshold. However, the most toxic metals for higher plants and certain microorganisms are Hg, Cu, Ni, Pb, Co, Cd, and, possibly, Ag, Be, and Sn [26].

The effect of organic pollutants (toxicity) on plants and their fate in plants is poorly known. Attempts have been made to use plants to reduce their concentrations in soils. However, their uptake by roots, translocation to shoots, volatilization, and/or degradation seem to be highly plant, soil, and component dependent [27–29]. It is also clear that the presence of herbicide residues may reduce or even prevent plant establishment.

In general, critical limits in plant can be quickly reached when a large amount of a metal contaminant is in a form available in the soil for plant uptake, especially when it is combined with a large soil–plant transfer coefficient [30,31] or when the threshold is low. For example, copper is a widespread contaminant and no example of successful extraction of Cu by *Salix* species from contaminated soil has been reported thus far [32]. The remediation efforts are mostly directed towards Cu immobilization through the use of additives [33]. However, its toxicity has been found to reduce phytoextraction efficiency because, for most of the plants, the toxic level is already reached when the Cu concentration in the plant is above 15 to 20 mg kg^{-1} DM [31] or when the concentration in the soil solution reaches 0.02 to 0.06 mg L^{-1} [34]. As a result of Cu toxicity, the biomass is reduced and the metal uptake is impaired [35–37].

In contrast, Punshon et al. [38] have shown that *Salix* species (*S. caprea*, *S. cinerea*, and their hybrids with *S. viminalis*) originating from contaminated sites exhibit Cu tolerance, but that there is a large variability between willows at the species and the population levels. Thus, soils with undesirable contaminants might not be easily decontaminated and a soil pretreatment might be necessary to remove or inhibit the toxic compounds. Alternatively, specific willow clones may need to be selected for these sites. Additionally, metals may have antagonistic or synergic effects on their toxicity and uptake as demonstrated for Cd by Costa and Morel [39].

30.3.1.3.2 *Heterogeneity of the Contamination*

Contamination is highly heterogeneous at polluted sites; this includes spatial variation in composition and concentration [4,17] as well as variation with depth [40]. An example of surface and at depth heterogeneity is displayed in Figure 30.3 for HNO_3-extractable Cd and Cu concentrations at Dornach. The standard deviations (SD) increased with depth, illustrating that the thickness of the contaminated layer varied between 0.2 and 0.7 m within a 400-m^2 area. For Cd, the SD was smaller and similar along the soil profile because, although Cd was brought to the soil by atmospheric deposition along with Cu and Zn, it also had a geogenic origin [41,42]. The high heterogeneity in the thickness of the contaminated layer prevents an optimal root colonization of the contaminated layer (see Table 30.4, third column) because phytoextraction can only be achieved when roots are within the contaminated layer. The question of root colonization will be developed later.

30.3.1.3.3 *Limited Availability of Metals*

From a different perspective, low availability of a metal to be remediated may reduce the potential for its phytoextraction. Although the metal available fraction in soil may be determined by different methods, it is indeed usually better correlated to plant uptake than total metal content in soil. In Figure 30.4, the low Cd uptake by *S. viminalis* is explained by the low $NaNO_3$-extractable (s) Cd content in the soil compared to hydroponics that show the uptake potential by this plant. Similar

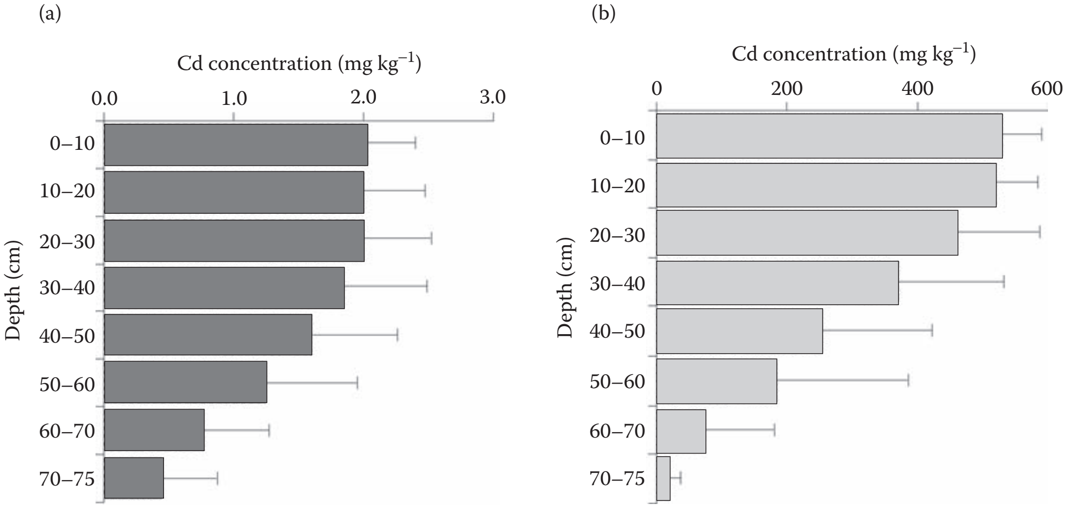

FIGURE 30.3 Profiles of HNO_3-extractable Cd and Cu at Dornach. These are means of 15 profiles collected within a 20 × 20 m area with the Humax technique that allows for sampling of undisturbed soil cores. The increasing SD with depth illustrates the variability in the contamination depth.

TABLE 30.4
Cumulative Root Density

	LA (km m^{-2})[a]	**LA/shoot biomass[b]**	**Depth of the contamination**		
			True	**Shallow**	**Deep**
Indian mustard	2.7 (0.7)	4	0.9 (0.3)	1.9 (0.8)	>0.5
Tobacco	3.7 (0.8)	3	0.5 (0.2)	1.3 (0.3)	0.4 (0.1)
Maize	6.6 (1.8)	4	1.4 (0.6)	>3.3	>0.9
Willows[c]	3.5 (1.8)	3	1.5 (0.7)	>3.2	0.9 (0.2)
Thlaspi (Ganges)	2.6 (1.1)	28	0.4 (0.1)	1.0 (0.0)	0.3 (0.0)

[a] LA = cumulative root density between 0 and 0.2 m (depth) in km m^{-2}.

[b] LA 0 to 0.2 m/shoot biomass.

[c] 2- and 3-year-old plants.

Notes: Calculated for five crops grown at Dornach and matching of roots and Zn soil contamination expressed as the ratio between the maximal depth with a root length density > 5000 mm dm^3 and the soil contamination depth, according to three scenarios: (1) contamination as measured in the soil profile of each plot; (2) hypothetical shallow contamination (0.2 m); and (3) hypothetical deep contamination (0.7 m). (shallow) and (deep) depths are extreme values found in the field experiment. Depth of contamination is calculated after removing 150 mg kg^{-1} Zn (Swiss guide value) from the total soil Zn concentration. ">" means that the deepest root sampling gave a root length density above 5000 mm dm^3. Standard deviations are in brackets.

Sources: Swiss guide value: OIS (ordinance relating to impacts on the soil), Swiss Federal Legislation, SR 814.12, 1998; modified from Keller, C. et al., *Plant Soil,* 249, 67, 2003.

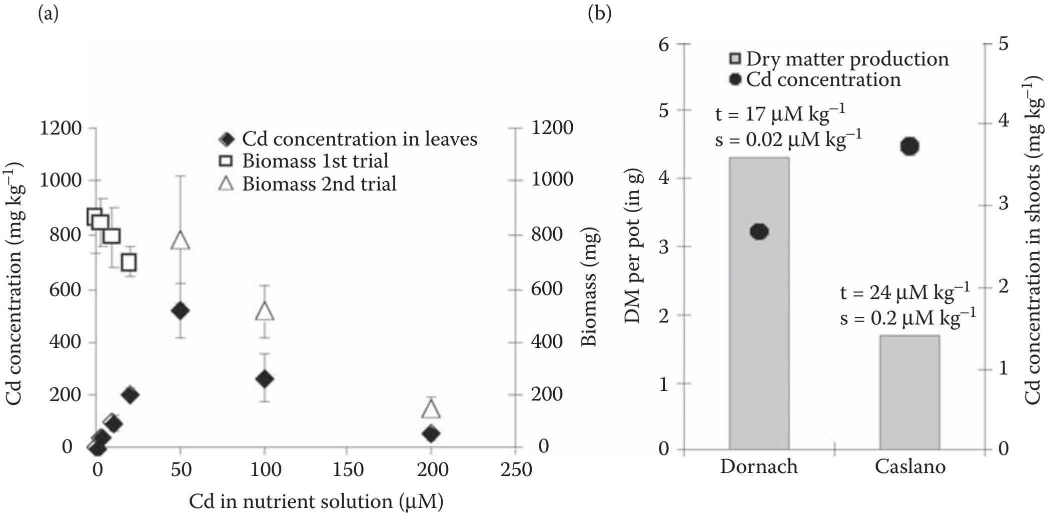

FIGURE 30.4 Cadmium concentration and biomass production: comparison between a) *Salix viminalis* (clone 78980) grown in hydroponics with increasing Cd concentrations and b) the same clone grown in pots in the Dornach and Caslano soils. s = $NaNO_3$-extractable Cd (soluble) and t = HNO_3-extractable (pseudototal). (After Hammer, D. and Keller, C., *J. Environ. Qual.,* 31, 1561, 2002 and Cosio, C. et al., unpublished data, 1998–2002.)

Cd concentrations in solution in both cases give similar Cd concentrations in the shoots of *S. viminalis* (clone 78980).

A low metal uptake due to low metal availability increases the number of years necessary for metal removal. It may lead to the use of additives to increase bioavailability [43–47]. EDTA, NTA, sulphur, and other chelating agents have been tested on willows [2,48], as well as on other crop plants, but results have not been conclusive thus far. EDTA is often preferred because it enables large metal mobilization, especially for Pb phytoextraction [47,49]. However, the main drawbacks are its persistence, the risk of groundwater contamination through uncontrolled leaching [50,51], and its high cost. Its use should therefore be limited to a situation in which leaching can be controlled or prevented and thus it may not be applicable at any site.

In contrast, Fisher et al. [52] tested different depths of incorporation of lime and organic matter on metal uptake and biomass production of Geyer willow (*Salix geyeriana*). They found that these additives improved the growth performance without decreasing Cd uptake, but increased the overall Cd extraction. However, it decreased Zn uptake and may not be suitable at Zn-contaminated sites. This approach needs to be investigated and tested carefully prior to application, but may provide an alternative to the addition of mobilizing agents.

30.4 FACTORS LIMITING THE EFFICIENCY OF PHYTOEXTRACTION SPECIFICITY OF WILLOWS

Ideally, the plant chosen for phytoextraction should be

- Contaminant tolerant
- Adapted to the site conditions
- Able to produce a large biomass quickly with high metal concentrations in the shoots
- Easy to manage, dispose of, and/or recycle
- Able to be grown continually or repeatedly

No plants that possess all these characteristics are available; however, crop plants and bioenergy plants usually produce a large biomass in a short time. Crop plants are usually annual but tree species — including willows — are perennial and thus do not require annual planting. In addition, their agronomic requirements are well known and their harvest is mechanized. They accumulate metals in their tissues moderately, but could be used for multiple contamination because they may be able to take up more than one metal. In the end, the choice will depend on the final total extraction obtained multiplying the yield with the metal concentration in the shoots. This will require preliminary studies to estimate these two values.

A large genetic variability exists in willows and active breeding programs for the bioenergy production have already produced a broad range of clones, especially of *S. viminalis* [53]. They have been selected for rather rich and fertilized agricultural soils and may thus not adapt easily to contaminated sites (see earlier comments). However, clones also exist that are adapted to extreme environments and grow spontaneously at contaminated sites [54–56]. They may have developed and enhanced metal tolerance to the metal present in their original soil [38,57] and may also be able to stand a limited nutrient availability. Vandecasteele et al. [56] proposed to use *Salix* species as bioindicators because the foliar content in Zn and Cd of *Salix* spp. grown on contaminated dredged sediments reflected the soil metal content, indicating that metal accumulation is probably a common trait to many *Salix* species. However, they also stressed the risk of metal transfer to the food chain. This is a recognized risk of phytoextraction, but is probably less problematic with willows than with hyperaccumulators.

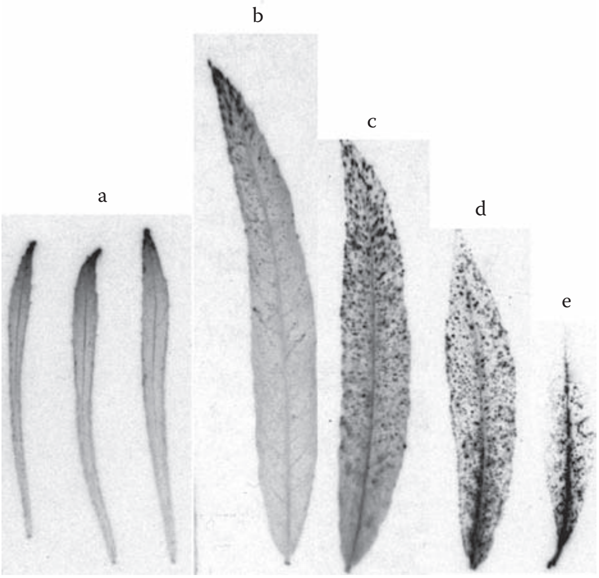

FIGURE 30.5 Cadmium visualization in leaves of *S. viminalis* (clone 78980): autoradiographs of *S. viminalis* leaves grown in hydroponics and after 30 days exposure to 10 m*M* Cd spiked with 2.2 10^{-3} m*M* of 109 Cd; (a) leaves n°1–3; (b) leaf n°11; (c) leaf n°16; (d) leaf n°21; and (e) leaf n°27. Leaves were counted from the top to the base of the stem; leaf n°1 is the first identifiable leaf. The analyzed plant had a total of 29 identifiable leaves. (After Cosio, C., Ph.D. thesis n°2937, Swiss Federal Institute of Technology, Lausanne, Switzerland, 2004.)

Thus, because of the wide range of wild species that can be collected at contaminated sites, as well as the existing nurseries for biomass production, a large choice of species and clones can be tested according to site specifications and metal tolerance.

30.4.1 CONCENTRATIONS IN PLANT PARTS

As already mentioned, high biomass plants may be more or less tolerant to a range of contaminants, depending on the contaminant, the species, and even the cultivars or clones. *Salix* spp. presents a large plasticity in Cd tolerance and accumulation [58]. For this reason, Cd extraction seems to be the most likely application of phytoextraction using willows. However, some critical limits may also be quickly reached for other elements, such as boron, which has a narrow deficiency/toxicity range varying widely between plant species [26].

In addition to having various accumulation capabilities, plants do not react in the same way to increasing concentrations in the soil [59]. In general, their efficiency will decrease with increasing soil concentrations [60].

Another point is the distribution of metals inside the plants: metal concentration in the plant varies with the organ and the age of this organ [31,61–63]. Figure 30.5 shows Cd distribution within leaves of *S. viminalis* grown in hydroponics: Cd is located at the edges and tips of the younger leaves but is mostly at the base of the older leaves [64]. In addition, concentration varies with the species [65], the metal [66,67], and the soil characteristics. In general, Zn, Cd, and Ni tend to accumulate in shoots, whereas Cu and Pb remain in roots. This has consequences for the time of the harvest and the parts to be harvested.

As illustration, in Figure 30.6, Zn concentrations in shoots of *S. viminalis* and *S. aurita* grown in two different soils are shown: in soil 1 they were larger in leaves than in stems or roots. In addition, the ratio of concentration between leaves and roots was smaller for *S. aurita* than for the two *S. viminalis* clones. Conversely, in soil 2, Zn concentrations were larger in roots. This means

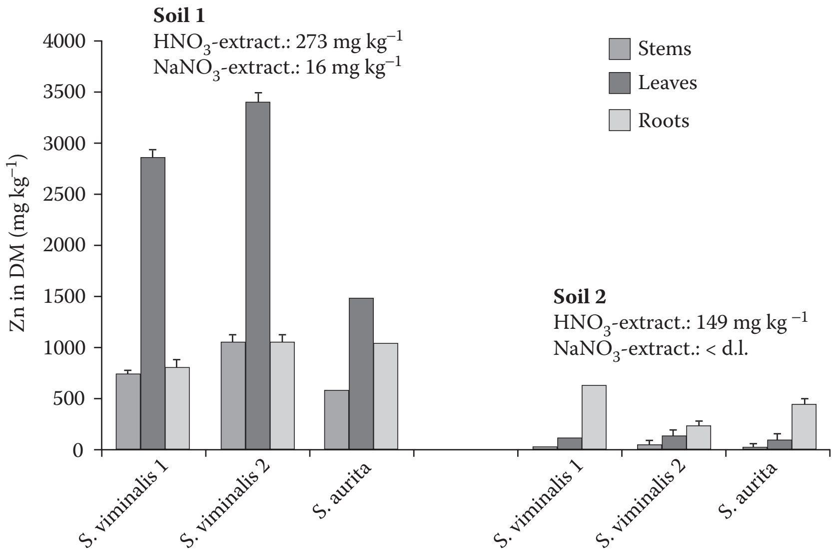

FIGURE 30.6 Zn concentrations in different parts of two *Salix viminalis* clones (Swedish clone 78980 and local clone from Les Abattes) and *Salix aurita* grown during 3 months in pots in two different soils. (After Keller, C. et al., in *ICOBTE: 5th Int. Conf. Biogeochem. Trace Elements*, Vienna, Austria, 1999, 518.) According to the Swiss legislation (OIS: Ordinance relating to impacts on the soil), Swiss Federal Legislation, SR 814.12, 1998), soil 1 is above the guide value for the HNO_3-extractable Zn and above the cleanup value for the $NaNO_3$-extractable Zn. In soil 2, HNO_3- and $NaNO_3$-extractable Zn concentrations are below the guide values.

that extrapolation of given results to different situations (different metals, soils, and clones) is difficult and that leaves and stems must be collected if *Salix* is used for phytoextraction because concentrations are the highest in leaves. In general, pot experiments may thus be necessary prior to applying full-scale phytoextraction.

30.4.2 ROOT PROSPECTING

As demonstrated earlier, the extent of the root systems varies with the site conditions and the age of the plants (Table 30.3). However, for a given soil, plants develop very different root systems [20] as well as different root:shoot ratios. This is true for willows known to present clonal variation in rooting strategy [68]. Such characteristics have been measured at Dornach for various species and the results are presented in Table 30.4. Maize had the largest root length density and thus was able to prospect the whole soil profile efficiently; *T. caerulescens* had the largest root:shoot ratio (together with a relative larger proportion of fine roots), expressing an ability for increased element uptake [40]. Willow gave intermediate results.

Root colonization of the soil is mostly driven by plant type and soil characteristics and may not match the metal distribution properly. Figure 30.7 displays several of the possible situations that lead to optimal or suboptimal metal extraction. Because of the localization of the contaminated layer and the size of their root system, as observed for various species, plants may [69,71]:

- Not colonize the whole layer (case III)
- Not be able to reach the contaminated layer if it is at depth (IV)
- Grow deeper than the contaminated layer (V)
- Avoid the contaminated spots (VI)

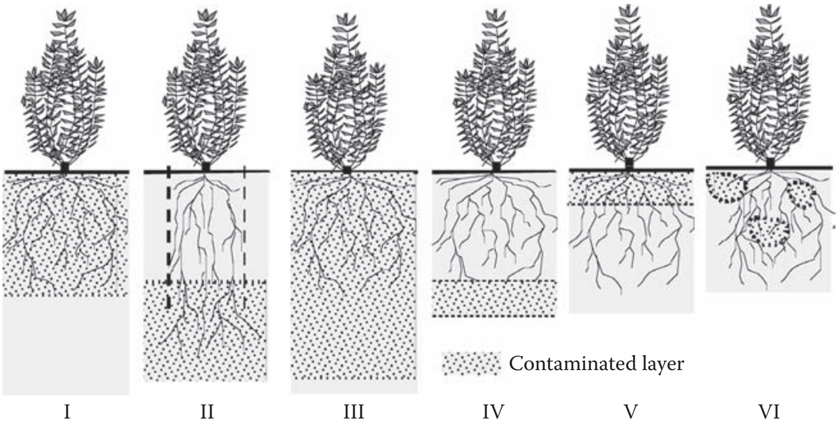

FIGURE 30.7 Various possible combinations of root distribution and contaminated layers. *Salix viminalis* is taken as an example. I: optimal situation where the root system develops only within the contaminated layer; II: situation where the roots are forced to reach the contaminated layer; III to VI: situations where the root system distribution does not match the contamination.

On the other hand, root systems may be manipulated to a certain extent to force the root to reach the contaminated layer [72].

Practically, this may result in a decrease in extraction efficiency as shown in Table 30.4: for each miniplot, soil cores were taken down to 0.75 m and metal concentrations of the soil were measured every 0.10 m, as well as total root length for the same layer. A coefficient taking into account the true depth of contamination and the maximal root depth (with root length density > 5000 mm dm^3) was calculated. The same calculation was made for a hypothetical steady shallow (0.20 m, case III of Figure 30.7) or deep (0.70 m, case V of Figure 30.7) contamination. In general, maize and willows were more suitable when the contaminated layer was thick. However, in the specific case of Dornach, none of the species was optimal, so a mixed culture or a rotation with plants with various root colonization would be best. These results can probably be extended to other contaminated sites and emphasize the necessity of an appropriate and site-specific plant management.

Additionally, total root length in the contaminated layer (and thus the extent of the root prospecting) and the root length/shoot biomass ratio may have an impact on metal concentrations in the biomass. For example, Van Noordwijk et al. [73] found a positive correlation between Cd uptake by maize and root length.

30.4.3 PLANT MANAGEMENT

As discussed with Figure 30.6, the field management applied for the bioenergy production may not be efficient for phytoextraction with willows: for biomass production, *Salix* spp. are left 20 to 30 years on site and cut in winter every 3 to 4 years [16]. From Figure 30.6 and data obtained at Dornach and Caslano, it was found that Cd and Zn concentrations were the highest in leaves. At Dornach, leaves accounted for approximately 20 and 15% of the biomass produced after 3 and 4 years, respectively, but concentrations were between twice (for Cd) and five times (Zn) larger than in stems [6]. Thus, leaves must be harvested in order to reach the maximal extraction efficiency during phytoextraction and to prevent metal accumulation in the litter and the topsoil up to toxic levels. Because leaves must be harvested, the chipping process (chopping stems into small pieces) used in biomass production is not suitable; thus, other harvesting machines like adapted stick harvesters may need to be used. This problem may increase the price of the harvesting process and

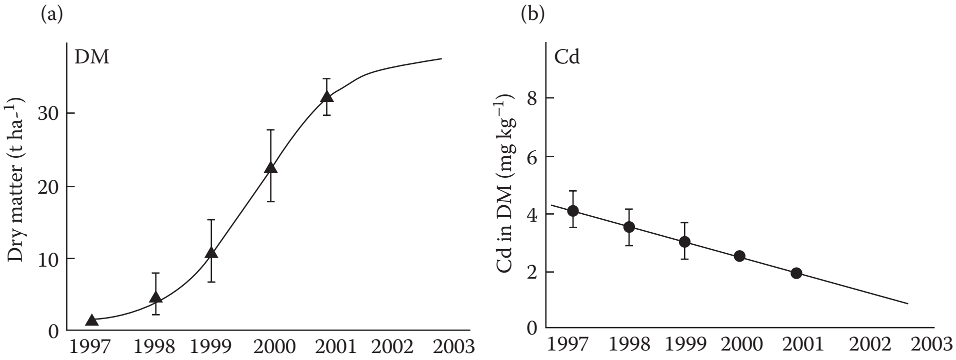

FIGURE 30.8 Biomass production and Cd concentrations measured in *S. viminalis* grown at Dornach between 1997 and 2001. (Modified from Hammer, D. et al., *Soil Use Manage.,* 19, 187, 2003.)

thus phytoextraction drastically. In addition, many studies are necessary to assess the decrease in plant vigor that may arise from a systematic leaf harvest.

In addition, Cd and Zn concentrations measured in leaves and stems of *S. viminalis* decreased with time (Figure 30.8) — most probably because roots had progressively extended downwards outside the contaminated layer [40], thus reducing the extraction efficiency with time. Similar results were obtained at Caslano after 4 years (Keller et al., unpublished data) and are attributed to the same reason. Therefore, it may be necessary to remove the plants every 2 or 3 years to keep an optimal extraction.

On the other hand, whereas Cd and Zn concentrations in shoots were lower at Les Abattes than those recorded at Dornach and Caslano, they increased with time (from 62 in 1998 to 175 in 1999) and 252 (2000) Zn mg kg^{-1} DM). This reflects the progressive establishment of the plants on the site and also the fact that the roots probably remained within the contaminated layer because the underlying layer was composed of marl and lime and compacted, and thus did not facilitate root colonization (Figure 30.9). Again, these contrasting results emphasize the need for a careful study of the site to be remediated and for monitoring plant concentrations to assess metal extraction in the long term.

The choice of the planting technique may have also a long-term effect on phytoextraction efficiency. At Les Abattes, the field management was conducted as a forest management with limited weed control, and cuttings and 1-year-old plants (1 m high) were planted. At Dornach and Caslano, only cuttings were used and weeds were systematically removed. In the first case, cuttings did not survive, but in the second, a good survival percentage was obtained and maintained. Weed competition and subsequent neglect may thus influence plant survival and the result of phytoextraction [74].

Another point to take into account before starting phytoextraction is an assessment of the plant disposal that may differ according to the site location, the volume of "waste" produced, the time of the year and duration of the biomass production, the accessibility of disposal facilities, and the legislation (these parameters may have an impact on the plant species to be chosen).

Finally, the choice of the species (one or several) needs a thorough study of the plant's potential and its suitability for the site. Willows have a high potential for use in phytoextraction, but other alternatives must not be discarded before thorough evaluation. As it has been shown, the results are not easily predictable and pre-experiments in pots or even in miniplots will be necessary before applying phytoextraction to the site.

30.5 LEGISLATION AND TIME REQUIRED

Models have been proposed to evaluate the time needed to decontaminate a given soil with a given plant [2,3,75]. This time varies with the target value and the depth of contamination, as well as

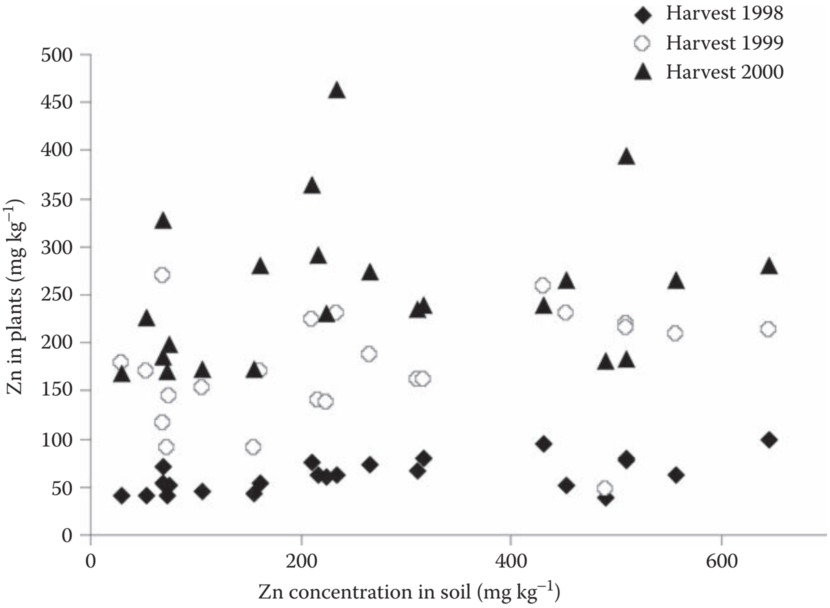

FIGURE 30.9 Zn concentrations in shoots of *S. viminalis* clone 78980 grown in the field at Les Abattes (planted as 1-year-old plants) and sampled after 6, 18, and 30 months after planting.

with the type of function (linear or exponential) used to describe the decrease of metal concentrations in soil. Indeed, concentrations in the plants and yields may not be constant with time, and efficiency may thus decrease because the available pools decrease and also because the roots are going deeper or because perennial plants need a certain time to establish themselves. Unfortunately, not enough long-term data sets are available to validate either of these models.

The target values are also highly variable and depend mostly on legislation and the perception of a clean (or uncontaminated) soil. Some countries have already developed legislation that gives a framework for soil remediation; others have not [76]. If the target value in a given country is very low, it will take more time to clean up a soil below this value than it will in a country where the target value is high. In the second case, phytoextraction may be an option, but it might take too long in the first one. Using phytoextraction as a stripping technique to remove the bioavailable pool only has also been proposed. The extent of the replenishment of the metal available pool is difficult to assess in the first place, and it appears also that, in some countries like Switzerland [23], total and soluble contents must be reduced below a given threshold; the BSM approach may not be suitable for this reason. Finally, as mentioned earlier, the decrease of metal concentration in soil may not be linear and it remains to be tested whether the target values can effectively be reached.

In addition to the total or soluble metal content, target values could theoretically be based on the sum of metals, functional criteria and toxicological aspects, exposure pathways, or the idea of restoring soil multifunctionality. This will lead to a large range of concentrations that may have an impact on the technical and economical feasibility of phytoextraction whatever the option chosen is.

Thus far, calculations made from preliminary experiments or, at most, from a few years of field experiments, have led to various results (results obtained with addition of large amounts of chelating agents like EDTA are not taken into account), predicting remediation within few years, decades, or centuries [1–3,6,75]. Obviously, all the factors presented previously added to obtain such a large range of results. However, Cd seems to be the element that is the most readily taken up by willows and for which the best results in the shortest time would be expected. This emphasizes again the necessity to choose the sites to be remediated by phytoextraction, as well as the planning of the procedure to be applied, carefully.

30.6 CONCLUSION

Phytoextraction has a great potential for cleaning soils contaminated with heavy metals, especially in cases in which conventional technologies are not efficient, not possible, or too expensive. From the early results obtained in the lab and the field, it is clear that phytoextraction could be applied as the main remediation tool or, more likely, as a "polishing" technique combined with other conventional and "bio" techniques. Some evidence also indicates that, under temperate climate, some sites are unlikely to be cleaned by high biomass plants or hyperaccumulators; elements such as Pb, Cr, and Cu are not easily translocated to the plant shoots, whereas Cd is more suitable for extraction with willows [32].

There is obviously no single phytoextraction technique and each site will need a tailor-made scheme of the willows that may be part of it. The main difficulty in doing so is the optimization of the efficiency. Indeed, it is difficult to spot the limiting factors and even more to try to find a hierarchy among them because of their large number and their interdependency. To help practitioners choose or discard phytoextraction technology, decision trees have been proposed (see, for example, the US-EPA phytoremediation decision trees at: www.clu-in.org/techdrct/techpubs.asp [77]) that include some of these factors. However, once phytoextraction has been selected as the appropriate technique, much effort must be devoted to reach a full control of the technique and to obtain a maximal efficiency — in particular, in the long term. Research has a key position on that aspect and is responsible for the successful development of phytoextraction.

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