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Phytoextraction capacity of trees growing on a metal contaminated soil

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Abstract

Phytoremediation is an innovative biological technique to reclaim land contaminated by heavy metals or organic pollutants. In the present work, we studied the ability of five woody species to extract heavy metal (copper, zinc or cadmium) from a polluted soil to their above-ground tissues. Metal content in leaves and twigs was determined. *Salix* and *Betula* transferred zinc and cadmium to leaves and twigs, but *Alnus*, *Fraxinus* and *Sorbus* excluded them from their above-ground tissues. None of the species considered transferred copper to the shoots.

Introduction

A vegetative cover on a heavy metal polluted soil may help to avoid dispersion of contaminants through wind erosion and by reducing the volume of water percolating through the soil. This may keep contaminants away from underlying ground water by stabilising them in the soil profile (Vangronsveld et al., 1995). Some plants phytostabilise heavy metals in the rhizosphere through root exudates immobilisation (Blaylock and Huang, 2000) whilst other species incorporate them into root tissues (Khan, 2001). Some plant species also transfer metals to their above-ground tissues, potentially allowing the soil to be decontaminated by harvesting the above-ground parts of the plants (Brun, 1998). In extreme cases, such as mine spoils, revegetation of contaminated soils may raise problems, due to the phytotoxicity of heavy metals, requiring the use of metal-tolerant plants to provide a vegetation cover (Johnson et al., 1997; Smith and Bradshaw, 1979).

The soil of a former landfill in Switzerland is polluted by heavy metals, particularly copper and zinc, due to the application of sewage sludge compost following closure. A phytoremediation project was initiated to investigate the possibilities of extraction and/or stabilisation of metals in the soil. The choice of the species responded to different criteria: they had to be local climate adapted species. Among these, two (*Salix viminalis* and *Betula pendula*) were chosen for known Zn and Cd phytoextraction ability, whereas the other three (*Alnus incana*, *Fraxinus excelsior* and *Sorbus mougeotii*) were chosen to test it. A pot experiment performed with Salix viminalis allowed investigation of Cd and Zn partitioning between shoots and roots under controlled conditions.

Materials and methods

Site description

The experimental site is located on the former landfill 'Les Abattes' close to the town of Le Locle (Neuchâtel, Switzerland), in the Swiss Jura mountain chain (approximately 1000 m above sea level). The natural soil of the region is calcareous, typical of the Jura chain. Winters in the region have long-lasting snow cover and summers are cool and wet

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(1470 mm average annual precipitation, 5.3 °C annual temperature).

The 2-ha landfill was used for inert waste material (lime and marl from nearby building areas). Heavy metal concentrations in these materials were very low, as shown in Table 1 (Dubois, 1991), but the landfill was capped with a final top layer (0.05–0.60 m depth) made up by a mixture of contaminated compost originating from sewage sludge contaminated with copper, zinc and cadmium and uncontaminated earth and gravel. The respective proportions of contaminated sewage sludge and uncontaminated earth and gravel varied randomly, as did the total thickness of the final top layer. Thus, heavy metal concentrations in the final top layer in the field were spatially highly variable as a consequence of the heterogeneous mixing of the components. The concentrations of the metals in the final top layer are given in Table 1. Other average characteristics of this final top layer were: organic carbon 56 ± 1 mg kg^{-1}, pH$_{\mathrm{CaCl2}}$ 7.4 ± 0.1, clay 430 ± 130 mg kg^{-1}, sand 300 ± 120 mg kg^{-1}. The site was submitted to temporary deforestation permit and, according to local laws, it had to be replanted with trees after closure of the landfill.

Two experimental areas of 300 m^2 each were established to reduce heterogeneity of the soil cover. One was covered with the same contaminated compost that was used to cover the landfill. The other was covered with non-contaminated arable soil as a control. Metal concentrations in the soil of these two areas are given in Table 1. Soil thickness on these experimental areas was in the range of 1.0–1.2 m.

Between 1997 and 1999, 180 groups of five species of local trees (each group contained by 10 trees of the same species) were planted. The five species planted were Grey Alder (*Alnus incana*), Silver Birch (*Betula pendula*), Ash (*Fraxinus excelsior*), Basket Willow (*Salix viminalis*) and Mougeot Rowan (*Sorbus mougeotii*). The plants were issued from clones or from germinated seeds and were prepared at the Swiss Federal Research Institute WSL. Growth was measured once a year at the end of the vegetative period (data not shown).

Sampling and preparation

Seven soil samples were collected from the two areas at 0–0.25 m depth, 3 years after planting. Samples were collected using a Humax auger of ∅ 5 cm (Max Hug, Luzern, Switzerland). Only four of them were taken on the contaminated area and three on the control because both areas had a limited extension and were assumed to be homogenous over their whole surface and down to a depth of 1.20 m. The samples were kept at +4 °C before drying. Soil samples were oven dried at +40 °C for 2 days, then crushed and sieved to 2 mm through a nylon sieve. Total heavy metal concentrations were determined after digestion with boiling 2 m HNO_3 (FAC, 1989) as required by the Swiss legislation (OIS, 1998).

Metal concentrations in plants were determined 3 years after planting. Plant material was sampled at approximately the same position (0.10–0.30 m below the top of the trees) from the trees growing on the two experimental areas. After rinsing with tap water, they were dried for 4 days at 80 °C. Leaves and twigs were separately ground in a Rentsch titanium mill, then 0.5 g samples were mineralised in 50 mL tubes with 8 mL HNO_3 65% s.p. (Merck) and heated till dry. $HClO_4$ was added (1 mL) and samples were further heated at 235 °C for 1 h. The clear solution was then made up to 20 mL with purified water (Milli-Q reagent grade water system by Millipore Corporation, Bedford, MA). All glassware or PE-flasks used for digestion and stock solutions was soaked in 10% HNO_3 overnight and rinsed three times with Milli-Q reagent grade water.

Pot experiment

The pot experiment was performed with the contaminated compost only and two *Salix* clones: (i) the local clone (Le Locle clone) was compared to (ii) a Swedish clone (called no 78980 clone). The first one is well adapted to the local climatic conditions because it was collected in the surroundings of the landfill. It is the same clone as the one planted on the landfill. The latter is known to accumulate high concentrations of Cd and Zn (Landberg and Greger, 1996) and was collected initially in Sweden and further propagated in Switzerland. The compost was the same material originally mixed with the arable soil to make up the final top layer of the landfill and the experimental contaminated area.

Plastic pots were prepared in 3 replicates with 2000 g (fresh weight) of compost passed through a 1 cm sieve. Twenty-cm-long willow cuttings were planted into pots (one per pot), set in a greenhouse (light 16 h, temperature day/night: 20–28/16 °C) and watered with partially deionised water. Two of the replicates were harvested after 90 days and the last one was harvested after 250 days of growth. Twigs, leaves

Table 1. Concentrations of copper, zinc and cadmium (2 m HNO_3-extraction) and pH in the non-contaminated arable soil (control), in the sewage sludge contaminated compost (contaminated area), in the contaminated compost used in the pot experiment, in the final top layer capping the landfill and in the inert waste material, compared to the Swiss guideline values of OIS (Swiss Confederation, 1998)

	Cu [mg kg^{-1}]	Zn [mg kg^{-1}]	Cd [mg kg^{-1}]	pH
Control	21 ± 6.61	65 ± 3.71	1 ± 0.14	7.5
Contaminated area	557 ± 51	620 ± 41	1.8 ± 0.20	7.4 ± 0.01
Pot experiment compost [a)]	1140 ± 25	848 ± 15	1.7 ± 0.05	7.9
Final top layer	302.5 ± 281.5	461 ± 401	1.71 ± 1.38	7.25 ± 0.25
Inert waste material	1.15 ± 0.45	6.1	0.08 ± 0.04	8.65 ± 0.25
Guideline values of OIS	40	150	0.80	–

[a)] according to DanielHammer, personal communication.

and roots were analysed separately for Cd, Cu and Zn. All plant parts were then processed as described for the field samples.

Heavy metal determination

Copper, zinc, and cadmium were measured by Inductively Coupled Plasma Atomic Emission Spectrometry (ICP-AES, Perkin Elmer Plasma 2000) for soil and plant samples. Bioconcentration factors were calculated by dividing the total heavy metal concentration (FAC, 1989) in the soil by the heavy metal concentration in the above-ground plant tissue.

Results

Heavy metal content in the soils of the two areas

The metal concentrations in the polluted final top layer that was spread onto the landfill were above the OIS guidelines values (Swiss Confederation, 1998) for all three metals analysed (Table 1). The metal concentrations on the contaminated area were slightly higher than the concentrations in the final top layer (Table 1). The non-contaminated arable soil (control) had metal concentrations under or at the limit of the guideline values (Table 1). The metal concentrations in the pot experiment compost were above the guidelines values (OIS, 1998) for all three metals analysed (Table 1). The relatively high Cd concentration found in control soil (above the guide value set to 0.8 mg kg^{-1}) is probably due to a particularity of the Jura Mountains: a geogenic origin of cadmium has been proposed by Atteia et al. (1994), Baize and Sterckeman (2001) and Benitez and Dubois (1999).

Heavy metal content in the trees

Copper

Concentrations of copper in plant tissues were similar for samples collected from the trees growing on the contaminated and non-contaminated control soil (Figure 1). Copper concentration in the above-ground vegetative tissues was quite low for all five species considered both on contaminated area and on control. The concentrations found in leaves were comparable to those measured in the twigs. Bioconcentration factors to the above-ground plant tissues were all very low (Table 2).

Zinc

Both willow and birch growing on the contaminated area as well as on the control had higher Zn concentrations in their leaves and twigs compared to the other species analysed. Concentrations were higher in the trees of these species growing on the contaminated area than in those growing on the unpolluted control (Figure 1).

Alnus, *Fraxinus* and *Sorbus* growing on the polluted soil showed a low zinc concentration in their above-ground tissues (leaves and twigs). There were no differences in zinc concentration in their tissues, either growing on sewage sludge contaminated area or non-polluted control soil.

Bioconcentration factors from soil to the above-ground parts of the plants for zinc were quite low (Table 2) and only willow and birch showed a slightly increased bioconcentration factor for zinc, compared

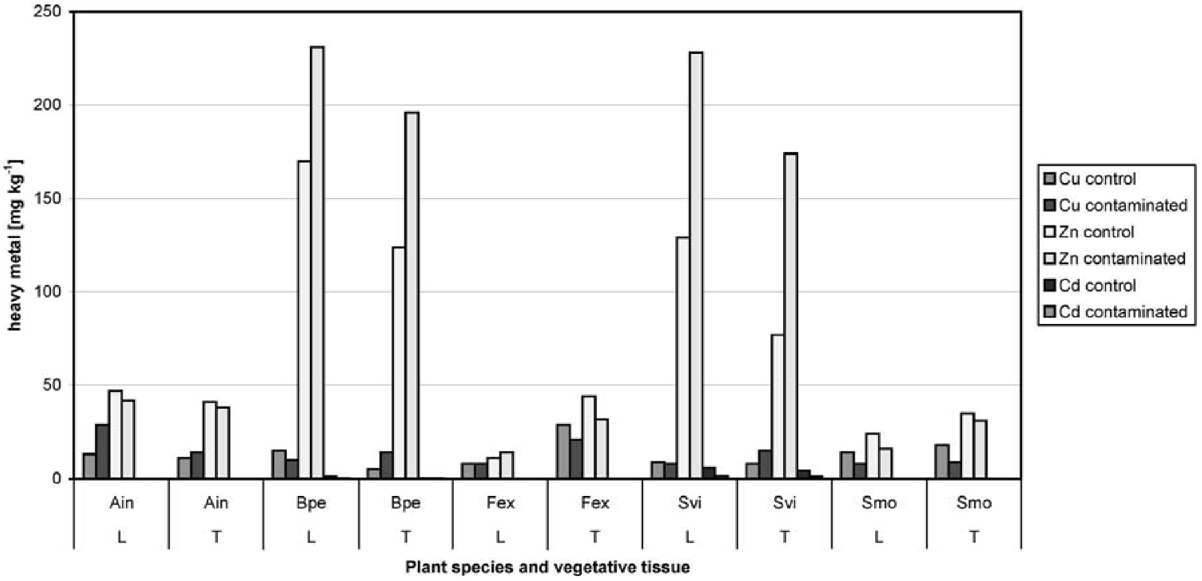

Figure 1. Concentrations of copper, zinc and cadmium in leaves and twigs (mg kg^{-1} of dry matter) of 5 species planted on the sewage sludge contaminated compost area (contaminated) and non-contaminated arable soil area (control). Uptake and transfer to the above-ground tissues succeeded in *Betula* and *Salix*. (Ain, *Alnus incana*, Bpe, *Betula pendula*, Fex *Fraxinus excelsior*, Svi, *Salix viminalis*, Smo, *Sorbus mougeotii*, L, leaves, T, twigs).

Table 2. Bioconcentration factors to the above-ground plant tissues for copper, zinc and cadmium for the trees growing on the contaminated area

	Bioconcentration factor for Cu		Bioconcentration factor for Zn		Bioconcentration factor for Cd	
Plant species	Leaves	Twigs	Leaves	Twigs	Leaves	Twigs
Alnus incana	0.05	0.03	0.07	0.06	–	–
Betula pendula	0.02	0.03	0.37	0.32	0.06	0.11
Fraxinus excelsior	0.01	0.04	0.02	0.05	–	–
Salix viminalis	0.01	0.03	0.37	0.28	0.83	0.72
Sorbus mougeotii	0.01	0.02	0.03	0.05	–	–

to the other species. Nevertheless, the calculated bioconcentration factor was lower than 1 for all samples analysed.

Cadmium

Very low or undetectable cadmium concentrations were measured in the vegetative tissues analysed, except for *Salix* and *Betula* (Figure 1). In both cases, concentrations were higher in leaves than in twigs.

In addition to heavy metal analysis in plant tissues, plant growth was checked yearly. However, it was not affected by the presence of metal-contaminated compost (data not shown).

The pot experiment

Both *Salix* clones gave similar results after 3 and 8 months although the Swedish one had slightly higher Cd uptake (Figure 2). Growth was even and replicates produced a similar amount of biomass. After 8 months, Zn concentrations were higher than after 3 months (on average 3 times more, with the largest increase observed in leaves (Figure 2). No significant increase was measured for Cd. Except for Zn at 3 months, Zn and Cd concentrations in roots were not greater than in shoots and did not increase with time for both clones. Copper concentrations in roots were 10 times (Swedish clone) and 20 times (local clone) larger than concentrations in shoots after 3 months. The ratio increased to more than 30 times after 8 months. Cadmium and copper concentrations in leaves and in twigs of the local clone were similar to those found in *Salix viminalis* grown on the contaminated area in the field, but Zn concentrations were 2 to 4 times higher. Bioconcentration factors calculated for the local clone were 0.95 for Zn, and 1.42 for Cd in leaves, both higher than in the field. However, for the woody parts, these coefficients were 0.29 for Zn and 1.12 for Cd and, thus, close to those calculated for the plants in the field. For copper, bioconcentration factors after 3 months and 8 months were very low and similar to those obtained in the field: 0.01 in leaves and even less in twigs.

Discussion

All species established successfully on the heavy metal-polluted soil, whether or not metals were taken up into their above-ground tissues. The concentration of copper that was found in the above-ground plant tissues was similar in samples collected from the contaminated area or non-contaminated control soil (Figure 1) and were also similar to the concentrations naturally found in plants (Kabata Pendias and Pendias, 1992). Low Cu concentration in the above-ground tissues may suggest that copper is not taken up by the plants. In fact, the soil characteristics are highly favourable to copper binding to the soil. Copper is known to strongly bind to many soil components (Turner and Dickinson, 1993) and to be hard to mobilise from the soil, as already discussed by Sauerbeck (1989). On the other hand, previous studies measured quite high bioconcentration factors from soil to plant for copper (Kloke et al., 1984). Alternatively, the low bioconcentration factor for copper to the above-ground tissues (Table 2) suggests that copper may not be transferred from the roots into the above-ground parts of the trees, as observed in the pot experiment where the ratios between roots and shoots are, respectively, 22 and 13 for the local and the Swedish clone. Such a low copper transfer to the above-ground plant tissues may be explained by a storage mechanism of copper in the root tissues (Alloway, 1999; Khan, 2001; Marschner, 1995) or by the low mobility of Cu in plants due to binding to the xylem (Lepp, 1991; Nissen and Lepp, 1997). High copper concentrations have previously been found in *Betula* roots (Kozlov et al., 1995; Maurice and Lagerkvist, 2000), as well as in *Salix* roots (Punshon and Dickinson, 1997). Arduini et al. (1996) found a good uptake of copper by roots but low above-ground copper in *Fraxinus*.

High zinc concentrations in *Betula* and *Salix* growing in non-polluted conditions, agree with previous established values (Kabata Pendias and Pendias, 1992; Mertens et al., 2001; Nissen and Lepp, 1997). These authors showed a higher zinc concentration in leaves than in twigs and the highest Zn-transfer factor to the above-ground parts among the species analyzed. This may be a mechanism to eliminate the metal from the organism, via autumnal leaf fall (Dahmani-Muller et al., 2000). Nevertheless, *Betula* and *Salix* cannot be called accumulators in our case study, since the metal concentration in their tissues does not exceed that in the contaminated soil. In general, *Betula* is known to have naturally high zinc concentrations in leaves (Adriano, 1986). This could explain the high concentration that was found in the plants growing on the control.

The results of the field experiments are confirmed by the pot experiment under controlled conditions. However, higher bioconcentration factors show that *Salix viminalis* was potentially more efficient under controlled conditions. The local clone was also as efficient as the selected Swedish one in accumulating Zn, but it is probably better adapted to the local conditions. In the pot experiment, metal concentrations in the vegetative tissues were always higher after 8 months than 3 months. Additionally, in general, higher concentrations are expected in plants grown in pot experiments as compared to those grown in the field. This can be explained by the restricted volume of soil prospected by the roots and thus their better efficiency.

No toxicity symptoms were detected on plants grown in the pot experiment. *Salix* has been shown to accumulate zinc (Kayser et al., 2000) and cadmium (Felix, 1997; Landberg and Greger, 1996) and has been used in phytoextraction experiments (Hammer et al., 2003; Landberg and Greger, 1996; Nissen and Lepp, 1997). However, in our case, zinc did not reach sufficient concentrations for the plant to be considered an accumulator. The major interest of this plant is its large potential biomass production (Felix, 1997; Hammer et al., 2003; Kayser et al., 2000). *Betula* is not considered as an accumulator but as a metal tolerant plant (Brown and Wilkins, 1985; Kopponen et al., 2001; Kozlov et al., 1995). Among the species analysed, *Betula* and *Salix* may be useful for phytoextraction as they transfer reasonably high concentrations of metal to their above-ground parts.

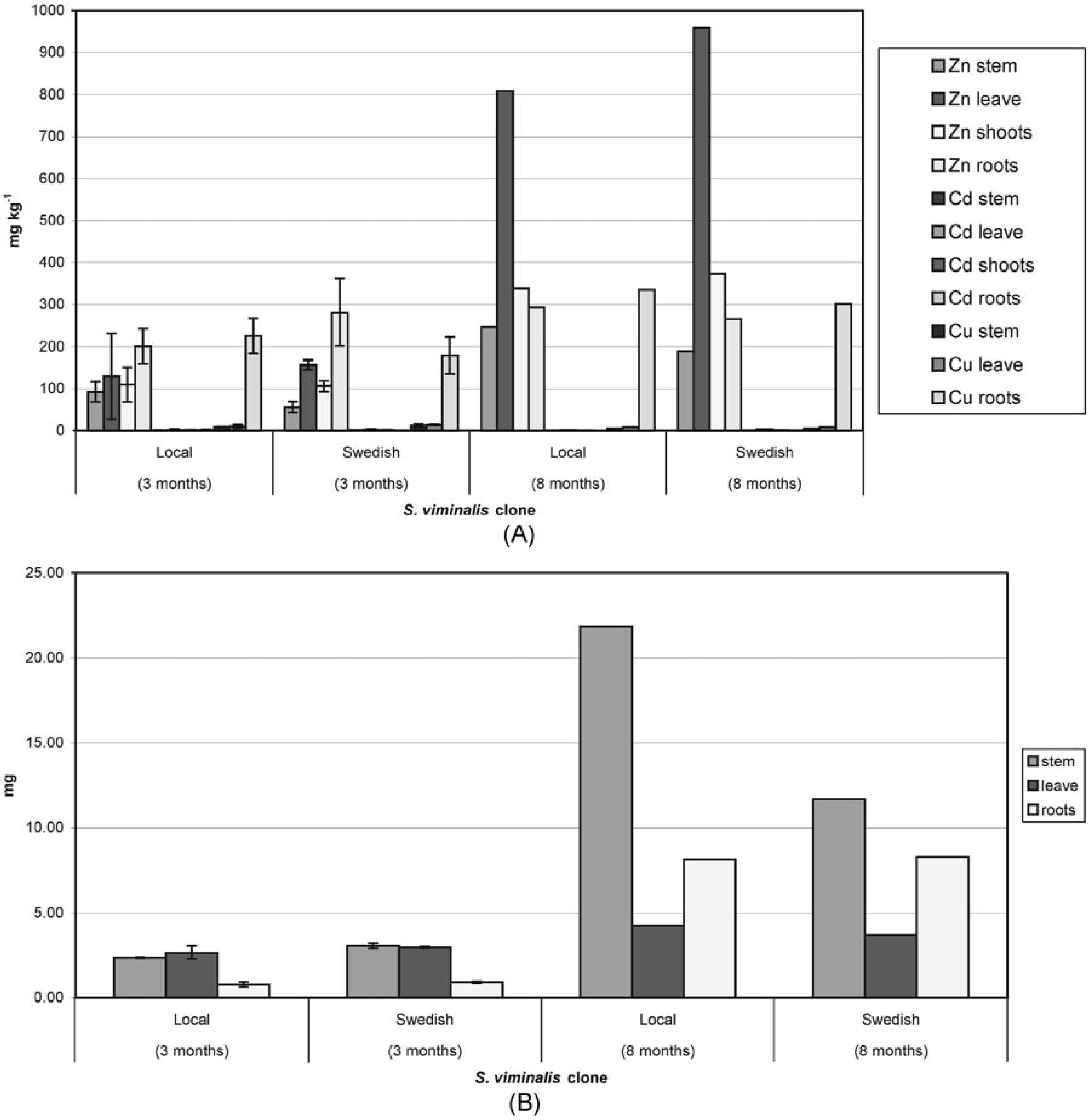

Figure 2. (A) Zinc, cadmium and copper concentrations (in mg kg^{-1} dry matter) and (B) biomass production (in mg per pot) in 2 *Salix* clones (local and Swedish) in a pot experiment after 3 and 8 months of growth. (Shoots = mean between leaves and wood, taking into account the biomass.)

Cadmium concentrations in the vegetative above-ground tissues analysed were very low or undetectable, except for *Salix*. The highest bioconcentration factors to the above-ground tissues for Cd were also detected in *Salix*. Similar results were obtained in the pot experiment after 8 months. Some clones of *Salix* are known to accumulate cadmium (Landberg and Greger, 1996). According to these authors, the Swedish clone tested was a Cd accumulator. However, it did not accumulate more Cd than the local one. For both clones, the bioconcentration factors were above 1 in pots and close to 1 in the field (but up to 3.3 in the control). This reflects a special ability of these plants to accumulate Cd. *Betula* is also able to extract cadmium, although at lower rate and with a lower bioconcentration factor to the above-ground tissues than *Salix*.

Alnus, *Fraxinus* and *Sorbus* growing in the polluted soil showed a low metal concentration in their above-ground tissues (leaves and twigs). They did not show any difference in metal concentration in their above-ground tissues either growing on sewage sludge contaminated area or non-polluted control soil. *Alnus*, *Fraxinus* and *Sorbus* can be considered as heavy metal excluders. These three plant species may have a mechanism to avoid metal uptake by stabilising it in the rhizosphere or exclude it from their above-ground tissues by keeping it in their roots (Blaylock and Huang, 2000; Khan, 2001). Unfortunately, root analyses could not be performed at that stage of the experiment. In spite of their low or inexistent ability to extract metals from soil to their above-ground tissues, which does not allow them to be used in phytoextraction, they may be useful in phytostabilisation. Additionally, all the plant species used here are pioneer plants known to be able to adapt and survive in harsh environments and, thus, are interesting in rehabilitating contaminated soils that have also extreme chemical and physical characteristics.

The general low mobility and availability of the metals present in the soil is probably due to the soil characteristics: high pH (7.4), high organic matter content (56 mg kg^{-1} C) and clay texture (430 mg kg^{-1}). This would explain the lower concentrations measured here in the above-ground tissues of *Salix viminalis* compared to other soils (Kayser et al., 2000; Keller et al., 1999). This would also apply to the other species. On the other hand, together with the vegetative cover, the low mobility of heavy metals due to the physico-chemical characteristics of this soil seems to be quite useful for stabilising and immobilising the metals in the soil of the landfill 'Les Abattes' and to reduce their toxicity.

In a phytoremediation approach of soil contaminated by heavy metals, the plants must be chosen among the species that are able to grow on contaminated soils. This is true for phytoextraction and phytostabilisation. When they can also take up large quantities of heavy metals through high concentrations in their shoots and/or a high biomass production, then phytoextraction can be considered. Ultimately, the option to be taken will depend on the physico-chemical characteristics of the soil to be decontaminated as well as on local laws and further use of the soil.

Long term uptake and uptake correlation with soil contamination are being currently investigated on these species as well as other species in order to offer a general answer for the future of this contaminated soil and the further possible use of these plants in other situations.

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